ICRS2023 TORONTO

33RD ANNUAL
SYMPOSIUM
OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

TORONTO
ONTARIO, CANADA

JUNE 24-29, 2023

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LAND ACKNOWLEDGEMENT

Toronto is situated in one of the world's most diverse cities and we acknowledge that where we meet has, for thousands of years, been the traditional land of the Huron-Wendat, the Haudenosaunee, and most recently, the Mississaugas of the Credit. Today, this meeting place is still the home to many Indigenous people from across Turtle Island and we are grateful to have the opportunity to meet on this land.

Symposium Programming by Cortical Systematics LLC

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USA

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2023 Symposium on the Cannabinoids

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ICRS Pre-Conference Activities

Marriott Downtown at CF Eaton Centre
Toronto, Ontario, CANADA

Saturday, June 24th

12.00 – 16.00	**ICRS Pediatric Special Interest Group Meeting** Hosted by Canadian Childhood Cannabinoid Clinical Trials (C4T) Conference Level: York A/B
	Introduction to Session
	Keynote
	Global Perspectives on Cannabinoid Therapeutics A series of short talks followed by a facilitated round table discussion on cannabinoids used for medical purposes in children, with perspectives from physicians, pharmacists, parents, youth and researchers.
	Farewell

Notes:

ICRS Registration: June 24th, 2023 (16.00 – 18.00)

Conference Level

Welcome Reception: 18.00 – 20.00

Conference Level Foyer

Day 1
Sunday, June 25th

8:30	Land Acknowledgement		
8.40	Welcome and Opening Remarks		
Oral Session 1. Phytocannabinoids: Routes of Administration, Pharmacokinetics, Interactions and Driving *Chairs:* Saoirse O'Sullivan and Ryan Vandrey			
8.45	Hayley Thorpe*, Anne Canella, Hakan Kayir, M. Asfandyaar Talhat, Larissa Kouroukis, Yu Gu, Ron Johnson, Boyer Winters, Sandra Sanchez-Roige, Abraham Palmer, Craig Bailey and Jibran Khokhar	CELL ADHESION MOLECULE 2 MODERATES CANNABIS EDIBLE INTAKE, Δ^9-TETRAHYDROCANNABINOL RESPONSE, AND THE ELECTROPHYSIOLOGICAL PROPERTIES OF PRELIMBIC CORTICAL NEURONS	1
9.00	Ryan Vandrey*, Carlos Austin Zamarripa, Ashley Dowd, Tory Spindle, Elise Weerts, Ed Cone, Ruth Winecker and Ron Flegal	DOSE EFFECTS OF ORAL AND VAPORIZED DELTA-8-THC AND COMPARISON TO DELTA-9-THC IN HEALTHY ADULTS	2
9.15	Saoirse O'Sullivan*, Matthew Jones, Taygun Uzuneser, Steven Laviolette and Andrew Yates	A NOVEL CANNABIDIOL: TETRAMETHYLPYRAZINE (CBD:TMP, ART12.11) COCRYSTAL IMPROVES THE BIOAVAILABILITY AND EFFICACY OF CBD	3

9.30	Andriy Gorbenko*, Jules Heuberger, Linda Klumpers and Geert Jan Groeneveld	5-WAY CROSSOVER CLINICAL TRIAL TO ASSESS MODULATING EFFECTS OF CBD ON PSYCHOTROPIC AND ANALGESIC EFFECTS OF THC	4
9.45	Patricia Di Ciano*, Tarek Rajji, Lauren Hong, Sampson Zhao, Patrick Byrne, Yoassry Elzohairy, Jeffrey Brubacher, Michael McGrath, Bruna Brands, Sheng Chen, Wei Wang, Omer Hasan, Christine Wickens, Pamela Kaduri and Bernard Le Foll	EFFECTS OF PREFERRED CANNABIS ON DRIVING IN ADULTS OVER THE AGE OF 65	5
10.00	COFFEE BREAK		
ORAL SESSION 2. PHYTOCANNABINOIDS: MEDICAL USE *CHAIRS:* ZIVA COOPER AND BERNARD LE FOLL			
10;30	Andrea Narayan*, Luke Downey and Amie Hayley	SLEEP AND ANXIOLYTIC EFFECTS OF 150MG NIGHTLY SUPPLEMENTATION OF CANNABIDIOL (CBD) FOR PRIMARY INSOMNIA	6
10.45	Ruben van Boxel*, Shiral Gangadin, Albert Batalla and Matthijs Bossong	THE IMPACT OF CANNABIDIOL TREATMENT ON BRAIN FUNCTION AND METABOLISM OF PATIENTS WITH A PSYCHOTIC DISORDER	7
11.00	L Cinnamon Bidwell*, Renée Martin-Willett, Marco Ortiz Torres, Greg Giordano, Jonathon Lisano, Carillon Skrzynski, Kent Hutchison and Angela Bryan	SHORT-TERM ANXIOLYTIC AND HARM-REDUCING EFFECTS OF CANNABIDIOL IN CANNABIS IN FLOWER AND EDIBLE FORMS	8
11.15	Nicholas Glodosky*, Carrie Cuttler and Ryan McLaughlin	CHRONIC AND ACUTE EFFECTS OF CANNABIS USE ON DAILY CORTISOL RHYTHMS IN A NATURALISTIC ENVIRONMENT	9

11.30	Jonathan Ross*, Deepika Slawek, Joanna Starrels, Yuting Deng, Chinazo Cunningham and Julia Arnsten	IMPLEMENTING AN EVIDENCE-BASED, HARM REDUCTION-FOCUSED, MEDICAL CANNABIS PROGRAM IN AN ACADEMIC MEDICAL CENTER IN BRONX, NY	10
11.45	**NIDA Update** **Heather Kimmel, Ph.D.** Health Science Adminstrator National Institute on Drug Abuse Epidemiology Research Branch (ERB) National Institutes of Health		
12.00	**Presidential Plenary Lecture** **TRENDS IN CANNABIS PRODUCTS AND CONSUMER PATTERNS OF USE: UNDERSTANDING THE IMPACT OF CANNABIS LEGALIZATION IN CANADA** **David Hammond, Ph.D.** Professor and University Research Chair in Public Health in the School of Public Health Sciences at the University of Waterloo		
12.45	**Lunch** **NIDA-Supported Trainee Mentorship Networking Lunch**		
Oral Session 3. Phytocannabinoids: Use Patterns, Prenatal Effects, Harms, and Harm Reduction *Chairs:* Cecilia Hillard and Jibran Khokhar			
14.15	Richard Quansah Amissah*, Hakan Kayir, Malik Talhat, Ahmad Hasan, Yu Gu, Ron Johnson, Karolina Urban and Jibran Khokhar	NEURAL AND BEHAVIORAL CORRELATES OF EDIBLE CANNABIS-INDUCED POISONING: CHARACTERIZING A NOVEL PRECLINICAL MODEL	11

14.30	Kendrick Lee*, Mohammed Sarikahya, Samantha Cousineau, Ken Yeung, Steven Laviolette and Daniel Hardy	PRENATAL Δ^9-THC-INDUCED FETAL GROWTH DEFICITS AND POSTNATAL CARDIAC DYSFUNCTION ARE AMELIORATED BY MATERNAL OMEGA-3 FATTY ACID SUPPLEMENTATION IN RATS	12
14.45	César Martinez Ramirez*, Garrett Sauber, Jennifer Sterrett and Cecilia Hillard	MATERNAL CBD EXPOSURE IN MICE ALTERS SOCIAL BEHAVIOR AND BEHAVIORAL FLEXIBILITY IN ADULT OFFSPRING	13
15.00	Hilary Marusak*, Julia Evanski, Leah Gowatch, Amanpreet Bhogal, Samantha Ely and Clara Zundel	SYNERGISTIC EFFECTS OF PRENATAL CANNABIS AND CHILDHOOD TRAUMA EXPOSURE ON LARGE-SCALE FUNCTIONAL BRAIN NETWORKS IN CHILDREN: A DOUBLE HIT TO THE DEVELOPING ENDOCANNABINOID SYSTEM?	14
15.15	Justin Matheson*, Leila Daddoust, Adam Zaweel, Marcos Sanches, Ahmed Hassan, Matt Sloan, Leslie Buckley, Amy Porath, James MacKillop, Christian Hendershot, Stefan Kloiber and Bernard Le Foll	EVALUATING GENDER DIFFERENCES IN CANNABIS USE PATTERNS AND MOTIVES AND SEVERITY OF CANNABIS-RELATED HARMS AMONG ADULTS ACCESSING ADDICTION TREATMENT SERVICES	15
15.30	Amanda Doggett*, Kyla Belisario, André McDonald, Mark Ferro, James Murphy and James MacKillop	EXAMINING THE LONGITUDINAL IMPACTS OF CANNABIS LEGALIZATION IN CANADA ON A SAMPLE OF HIGH-RISK EMERGING ADULTS	16
15.45	DATABLITZ SESSION 1		DB1
16.15 – 18.15	POSTER SESSION 1 RECEPTION		P1

Day 2
Monday, June 26th

8.25	Opening Remarks		
8.30 - 9.30	**Presidential Plenary Lecture** **ASTROCYTE CB1RS LINK SOCIAL OLFACTION, STRESS TRANSMISSION AND COGNITIVE IMPAIRMENT** **Jaideep Bains, Ph.D.** Director of the Krembil Research Institute, University Health Network (UHN), Toronto, formerly Scientific Director of the Hotchkiss Brain Institute at the University of Calgary		
Oral Session 4. Cannabinoids: Signalling, Pain, Addiction, and Opioids *Chairs:* Josee Guindon and Michelle Glass			
9.30	Hayley Marie Green*, David Benjamin Finlay and Michelle Glass	POSITIVE ALLOSTERIC MODULATION OF THE TYPE 1 CANNABINOID RECEPTOR POTENTIATES ENDOCANNABINOID SIGNALLING AND CHANGES ERK1/2 PHOSPHORYLATION KINETICS	17
9.45	Dongman Chao*, Hai Tran, Quinn Hogan and Bin Pan	MONOACYLGLYCEROL LIPASE INHIBITOR ALLEVIATES PAIN TRANSIENTLY VIA PERIPHERAL MECHANISM AND PAIN-INDUCED DEPRESSION LONG-TERM VIA CENTRAL MECHANISM	18
10.00	Yuma Ortiz*, Joshua Bilbrey, Jasmine Felix, Erik Kienegger, Marco Mottinelli, Sushobhan Mukhopadhyay, Christopher McCurdy, Lance McMahon and Jenny Wilkerson	CANNABIDIOL AND MITRAGYNINE EXHIBIT DIFFERENTIAL INTERACTIVE EFFECTS IN THE ATTENUATION OF PACLITAXEL-INDUCED MECHANICAL ALLODYNIA, ACUTE ANTINOCICEPTION, AND SCHEDULE-CONTROLLED RESPONDING IN MICE	19

10.15	Idaira Oliva*, Feezan Kazi, Lucas Cantwell, Ganesh Thakur, Jonathon Crystal and Andrea Hohmann	NEGATIVE ALLOSTERIC MODULATION OF CB1 CANNABINOID RECEPTOR SIGNALING SUPPRESSES OPIOID SELF-ADMINISTRATION AND RELAPSE	20
10.30	Omar Soler-Cedeño*, Hai-Ying Zhang, Pinaki Bhattacharjee, Guo-Hua Bi, Hannah Alton, Emma Xiong, Qing-Rong Liu, Malliga Iyer and Zheng-Xiong Xi	BRAIN CB2 RECEPTOR: A NEW THERAPEUTIC TARGET FOR TREATING OPIOID USE DISORDERS, MAJOR FINDINGS FROM A NEW CB2-KO-EGFP REPORTER MOUSE LINE	21
10.45	Joshua Watkins*, Vishakh Iyer, Rachel Hahn, Petra Aradi, Christos Iliopoulos-Tsoutsouvas, Spyras Nikas, Alexandros Makriyannis, Christina Johnson, Laura Bohn, Istvan Katona and Andrea Hohmann	CANNABINOID CB1 AGONIST-INDUCED RESPIRATORY DEPRESSION IN AWAKE MICE	22
11.00	COFFEE BREAK		
ORAL SESSION 4, CONT. CANNABINOIDS: SIGNALLING, PAIN, ADDICTION, AND OPIOIDS *CHAIRS:* JOSEE GUINDON AND MICHELLE GLASS			
11.30	Shanna Babalonis*, Michelle Lofwall, Paul Nuzzo, Laura Fanucchi and Sharon Walsh	SUPRATHERAPEUTIC DOSE COMBINATIONS OF OPIOIDS AND CANNABIS: ABUSE POTENTIAL, PHYSIOLOGIC EFFECTS AND SAFETY PROFILE IN HUMANS	23
11.45	Elisa Pabon* and Ziva D. Cooper	PAIN SENSITIVITY AS A FUNCTION OF CANNABIS USE FREQUENCY	24
12.00	Stephanie Lake*, Conor Murray, Timothy Fong, Elisa Pabon, Brittany Henry and Ziva Cooper	SEX- AND DOSE-DEPENDENT ANALGESIC AND REINFORCING EFFECTS OF SMOKED CANNABIS: A PREVIEW OF DATA FROM THE CANSEX STUDY	25

ORAL SESSION 5. CANNABINOIDS: FIBROSIS *CHAIRS:* RESAT CINAR AND YANKEL GABET			
12.15	Muhammad Arif*, Abhishek Basu, Kaelin Wolf, Joshua Park, Lenny Pommerolle, Madeline Behee and Resat Cinar	WHY IS PERIPHERAL CB1R ANTAGONISM A RATIONAL THERAPEUTIC STRATEGY FOR PULMONARY FIBROSIS? EVIDENCE FROM MULTI-OMICS APPROACH FOR ESTABLISHING A TRANSLATIONAL LINK	26
12.30	Abhishek Basu*, Kaelin M. Wolf, Muhammad Arif, Madeline Behee, Charles N. Zawatsky, Malliga R. Iyer and Resat Cinar	INHALATIONAL DELIVERY OF MRI-1867 (ZEVAQUENABANT), A THIRD-GENERATION CANNABINOID RECEPTOR 1 (CB1R) ANTAGONIST, EMERGED AS A NOVEL THERAPEUTIC MODALITY IN PULMONARY FIBROSIS	27
12;45	Liana Younis*, Ana Iden, Ida Gluzamn and Yankel Gabet	A PROTECTIVE EFFECT OF THE CB2R AGONIST OSTEOGENIC GROWTH FACTOR (OGP) AGAINST BLEOMYCIN-INDUCED PULMONARY FIBROSIS IN MICE	28
13:00	**LUNCH** **TRAINEE COMMUNICATIONS WORSKHOP**		
ORAL SESSION 6. CANNABINOIDS: CANCER, SKIN, EYE, AND GASTROINTESTINAL SYSTEM *CHAIRS:* ALEX STRAIKER AND LINDA PARKER			
14.30	Ana Iden*, Aaron Naim, Tamar Liron, Marilena Vered and Yankel Gabet	THE ANTI-TUMORIGENIC ROLE OF CANNABINOID RECEPTOR 2 IN SKIN CARCINOGENESIS	29
14.45	Evandro Jose Beraldi*, Catherine MacNaughton Keenan, Laurie Wallace and Keith Alexander Sharkey	ROLE OF CB1 RECEPTORS IN THE GASTROINTESTINAL TRACT AFTER MICROBIOTA DEPLETION AND NATURAL RECOLONIZATION	30

15.00	Ronen Rosenblum*, Yara Eid Mutlak, Anat Gelfand, Nitsan Maharshak, Ayal Hirsch, Hila Novak Kotzer and David Meiri	A NOVEL SELECTIVE CANNABINOID DERIVATE SUPPRESSES MURINE AND HUMAN IBD THROUGH A STAT3-DEPENDENT MECHANISM	31
15.15	Jenna Billingsley*, Emily Richter, Heather Bradshaw, Jim Wager-Miller, Kelsey Andreis, Kyle Yust, Taryn Bosquez-Berger, Natalia Murataeva and Alex Straiker	ENDOCANNABINOID REGULATION OF TEARING	32
15.30	DATABLITZ SESSION 2		DB2
16.00 – 18.00	POSTER SESSION 2 RECEPTION		P2
18:00	BUSINESS MEETING		

Notes:

Presenting Author*

Day 3
Tuesday, June 27th

8.25	Opening Remarks		
Oral Session 7. Cannabinoids: Signalling, Behaviour, Anxiety, and CNS *Chairs:* Matt Hill and Melanie Kelly			
8.30	Khalil Eldeeb*, Sandra Leone-Kabler, Mark Scialdone and Allyn Howlett	Δ8-ISO-TETRAHYDROCANNABINOL AND NEURONAL CELL SIGNALING	33
8.45	Alexander Young*, Melanie Kelly and Eileen Denovan-Wright	MICROGLIA-MEDIATED NEURONAL DEATH CAN BE SUPPRESSED BY SELECTIVE CANNABINOID RECEPTOR AGONISTS IN VITRO	34
9.00	Ozge Gunduz-Cinar*, Laura Castillo, Maya Xia, Elise Van Leer, Emma Brockway, Gabrielle Pollack, Olena Bukalo, Aaron Limoges, Sarvar Oreizi-Esfahani, Veronika Kondev, Rita Báldi, Farhana Yasmin, Ao Dong, Judy Harvey White, Resat Cinar, George Kunos, Yulong Li, Larry Zweifel, Sachin Patel and Andrew Holmes	ENDOCANNABINOIDS IN THE CORTICO-AMYGDALA NEUROCIRCUIT MEDIATE FEAR EXTINCTION	35
9.15	Barkha Yadav-Samudrala*, Ben Gormal, Hailey Dodson, Shreya Ramineni, Diane Wallace, Michelle Peace, Justin Poklis, Wei Jiang and Sylvia Fitting	DIFFERENTIAL EFFECTS OF ACUTE THC ON BEHAVIOR AND THE ENDOCANNABINOID SYSTEM IN NEUROHIV MOUSE MODEL	36

9.30	Gabriella R. Smith*, Kathleen McCoy, Emma Grant, Saad Sualeh, Josie Everett Mayil Bhat., Blake Woods, Max Rose, Ken Mackie and Anna Kalinovsky	DEFICITS IN ENDOCANNABINOID SIGNALING IN THE CEREBELLUM DISRUPTS SOCIAL PREFERENCE	37
9.45	Alex Straiker* and Ken Mackie	MUSCARINIC CANNABINOID SUPPRESSION OF EXCITATION, A NOVEL FORM OF COINCIDENCE DETECTION	38
10:00	IN MEMORIAM		
10.15	**COFFEE BREAK**		
ORAL SESSION 8. NOVEL LIPID PATHWAYS AND ENDOCANNABINOID PROFILES *CHAIRS:* HEATHER BRADSHAW AND MARIO VAN DER STELT			
10.45	Floor Stevens*, Remco Peter, Berend Gagestein, Iakovi Tfofi, Hans Aerts and Mario van der Stelt	DISCOVERY OF A NOVEL METABOLIC PATHWAY OF N-ACYLETHANOLAMINES BY GLUCOCEREBROSIDASE-2	39
11.00	Anne-Sophie Archambault*, Francesco Tinto, Jean-Philippe Lavoie, Élizabeth Dumais, Mélissa Simard, VIncenzo Di Marzo and Nicolas Flamand	HUMAN EOSINOPHILS AND NEUTROPHILS BIOSYNTHESIZE NOVEL LIPOXYGENASE METABOLITES FROM MONOACYLGLYCEROLS AND N-ACYL-ETHANOLAMINES	40
11.15	Toru Uyama*, Mohammad Mamun Sikder, Sumire Sasaki, Zahir Hussain, S. M. Khaledur Rahman, Katsuaki Hoshino, Yoshimi Miki, Makoto Murakami and Natsuo Ueda	PLAAT5 AS A CA2+-INDEPENDENT N-ACYLTRANSFERASE PRODUCING ANANDAMIDE AND OTHER N-ACYLETHANOLAMINES IN TESTIS	41

11.30	Heather Bradshaw*, Clare Johnson, Shivani Collur, Hannah Bentz, Praveen Kulkarni and Craig Ferris	EFFECTS OF AGE ON PLASMA LEVELS OF CANNABINOIDS AND ENDOLIPIDS AFTER VAPORIZED CANNABIS IN MICE	42
11.45	**KANG TSOU MEMORIAL SPEAKER** **ENDOCANNABINOIDS DRIVE MEMORY GENERALIZATION IN THE STRESSED MOUSE BRAIN** **SHEENA JOSSELYN, PH.D.** Senior Scientist at The Hospital for Sick Children (SickKids), Professor of Psychology and Physiology at the University of Toronto, Canada Research Chair in Brain Mechanisms Underlying Memory, Senior Fellow in the Canadian Institute for Advanced Research (CIFAR), Fellow of the Royal Society of Canada		
12.30	**BOX LUNCH** A box lunch will be served and then you are free to enjoy Toronto Box lunch for all, regardless of whether going on outing or not.		
13.15 -	**FREE TIME** **OUTINGS**		

Notes:

Presenting Author*

Day 4
Wednesday, June 28th

8.45	Opening Remarks	
NIH/NCCIH Symposium **Looking Beyond Δ^9-THC: A Translational Perspective on the Analgesic Properties of Minor Cannabinoids and Terpenes** Chair: Inna Belfer		
9.00	David Shurtleff	PROGRAMMATIC PRIORITIES IN SUPPORTING CANNABIS RESEARCH
9.05	Aditi Das	MINOR CANNABINOID METABOLITES GENERATED BY HUMAN CYTOCHROME P450S ARE BIOACTIVE
9.15	Sara Jane Ward	ANTI-ALLODYNIC EFFECTS OF CANNABIGEROL (CBG) IN RODENT MODELS OF NEUROPATHIC PAIN
9.25	Ziva Cooper	ASSESSING THE ANALGESIC AND SUBJECTIVE EFFECTS OF BETA-CARYOPHYLLENE ADMINISTERED ALONE AND COMBINED WITH DELTA-9-THC: A PLACEBO-CONTROLLED INVESTIGATION IN VOLUNTEERS
9.35	Hance Clarke	REAL WORLD EVIDENCE - HAVE WE LEARNED ANYTHING ABOUT THE ROLE OF MINOR CANNABINOIDS?

9.45	Inna Belfer	MODERATED Q & A / DISCUSSION SESSION
10.00	COFFEE BREAK	

'ON THE SHOULDERS OF GIANTS':
RAPHI MECHOULAM MEMORIAL SYMPOSIUM

CHAIR: YOSSI TAM

10:30	Mechoulam Family	
10.45	Allyn Howlett	FROM STEREOCHEMISTRY TO PHARMACOLOGY: KNOWING YOUR LEFT FROM YOUR RIGHT
11:00	Esther Shohami	THE ENDOCANNABINOID SYSTEM IS NEUROPROTECTIVE AFTER TRAUMATIC BRAIN INJURY IN MICE: MY 30 YEARS TRIP WITH RAPHI IN THE CANNABIS FIELDS
11.15	George Kunos	HOW RAPHAEL MECHOULAM GOT ME HOOKED ON (ENDO)CANNABINOIDS
11.30	Linda Parker	A QUARTER OF A CENTURY COLLABORATING WITH RAPHI
11:45	Aron Lichtman	WALKING THROUGH THE CANNABIS FIELD WITH RAPHI: HIS CURIOUS MIND, KIND HEART, GENEROUS SPIRIT, AND LOVE OF LEARNING AND PEOPLE MADE THE WORLD A BETTER PLACE
12:00	Ethan Russo	RAPHI AND ADVENTURES IN PHYTOMEDICINE

12:15	**2023 ICRS Mechoulam Awardee** **IMPROVING OUR UNDERSTANDING OF CANNABINOID PHARMACOLOGY AND MEDICATION DEVELOPMENT THROUGH THREE DECADES OF CONTROLLED HUMAN CANNABINOID ADMINISTRATION STUDIES** Marilyn Huestis, Ph.D. Adjunct Professor in the Department of Epidemiology and Preventive Medicine, School of Medicine, University of Maryland Baltimore and recently retired as a tenured senior investigator and Chief, Chemistry and Drug Metabolism Section, IRP, National Institute on Drug Abuse and National Institutes of Health		
13:00	**Lunch**		
Oral Session 9. Minor Cannabinoids *Chairs:* Steve Kinsey and Carrie Cuttler			
14.00	Sarah Olivia Vanegas*, Thomas Gamage, Jonathan Maturano, David Sarlah and Steven Kinsey	*IN VIVO* EFFECTS OF MINOR CANNABINOIDS CANNABINOL, CANNABICHROMENE, AND CANNABICYCLOL OCCUR VIA MULTIPLE RECEPTOR MECHANISMS	43
14.15	Vered Cohen*, Elazar Besser, Shiraz Schiller and David Meiri	A NEWLY-IDENTIFIED PHYTOCANNABINOID MODULATES THE ESTROGEN RECEPTOR AND REDUCES TUMOR PROGRESSION IN A MOUSE MODEL OF BREAST CANCER	44
14.30	John Jackson, Claudia Dietrich, Ali Shademani, Manisha Dosanjh, Mark Pryjma, Dana Lambert* and Charles Thompson	CANNABICHROMENE (CBC), CANNABIGEROL (CBG) AND SILVER NANO-PARTICLES DELIVER BROAD SPECTRUM ANTIMICROBIAL SYNERGY IN HYDROGEL-FORMING WOUND CARE DRESSINGS	45
14.45	Carrie Cuttler*, Aria Petrucci, Ethan Russo and Ziva Cooper	ACUTE EFFECTS OF CANNABIGEROL IN HUMANS: A DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSSOVER, FIELD TRIAL	46

15.00	ICRS William A. Devane Early Career Awardee **SEX DIFFERENCES IN ENDOCANNABINOID SIGNALING PATHWAYS INVOLVED IN CHRONIC PAIN AND CANCER: PRESENT AND INSIGHTS INTO THE FUTURE** Josee Guindon, Ph.D. Associate Professor in the Department of Pharmacology and Neuroscience at Texas Tech University Health Sciences Center	
15.30	DATABLITZ SESSION 3	DB3
16.00 – 18.00	Poster Session 3 Reception	P3
19.00	AWARDS CEREMONY AND ICRS BANQUET	

Notes:

Presenting Author*

DEPARTURE: **THURSDAY, JUNE 29TH**

DATABLITZ SESSION 1

DAY 1, SUNDAY, JUNE 25TH: 15:45 - 16:15

Christine M. Wickens*, Robyn D. Robertson, Jan G. Ramaekers, Tom R. Arkell and Eef L. Theunissen	CANNABIS AND DRIVING: RECENT EPIDEMIOLOGICAL EVIDENCE	DB1-1 [P1-5]
Marta De Felice*, Hanna J. Szkudlarek, Mar Rodríguez-Ruiz, Taygun C. Uzuneser, Mohammed H. Sarikahya, Mathusha Pusparajah, Juan Pablo Galindo Lazo, Shawn N. Whitehead, Ken K.-C. Yeung, Walter J. Rushlow and Steven R. Laviolette	ADMINISTRATION OF N-ACETYLCYSTEINE PREVENTS THE LONG-LASTING DEPRESSIVE-LIKE PHENOTYPES INDUCED BY ADOLESCENT THC EXPOSURE	DB1-2 [P1-9]
Catherine Hume*, Samantha Baglot, Lucia Javorcikova, Savannah Lightfoot, Jessica Scheufen and Matthew Hill	EFFECTS OF PRENATAL TETRAHYDROCANNABINOL (THC) VAPOUR EXPOSURE AND HIGH-FAT DIET ON RAT FEEDING PATTERNS, ADIPOSITY, AND GLUCOSE METABOLISM	DB1-3 [P1-10]
Lindsay Lo*, Justin Strickland, Ryan Vandrey and Caroline MacCallum	A SYSTEMATIC REVIEW AND META-ANALYSIS OF CLINICAL TRIALS INVESTIGATING ACUTE CANNABIDIOL (CBD) IMPAIRMENT: COMPARISON WITH PLACEBO AND DELTA-9-TETRAHYDROCANNABINOL (THC)	DB1-4 [P1-19]
Samantha Johnstone*, Maryam Sorkhou, Molly Zhang, Sarah Dermody, Rachel Rabin and Tony George	CANNABIS CRAVINGS PREDICT COMPENSATORY INCREASES IN CIGARETTE USE IN SCHIZOPHRENIA: FINDINGS FROM CANNABIS ABSTINENCE STUDIES	DB1-5 [P1-21]

Conor Murray*, Alexa Torrens, Stephanie Lake, Timothy Fong, Elisa Pabon, Brittany Henry, Daniele Piomelli and Ziva Cooper	PHARMACOKINETICS OF THC AND CANNABIS ABUSE LIABILITY IN MEN AS A FUNCTION OF FREQUENCY OF CANNABIS USE	DB1-6 [P1-25]
Marieka DeVuono*, Mina Nashed, Mohammed Sarikahya, Andrea Korcsis, Roger Hudson, Kendrick Lee, Sebastian Vanin, Daniel Hardy and Steven Laviolette	PRENATAL EXPOSURE TO THC AND CBD PRODUCES SEX-SPECIFIC EFFECTS ON ADOLESCENT NEUROPSYCHIATRIC OUTCOMES	DB1-7 [P1-51]
DATABLITZ presenters present BOTH a 4-minute presentation AND a POSTER at ICRS2023. DATABLITZ abstract page numbers are indicated [IN BRACKETS].		

Notes:

Presenting Author*

DATABLITZ SESSION 2

DAY 2, MONDAY, JUNE 26TH: 15:30 - 16:00

Lucy Rose Thomsen*, David Benjamin Finlay, Michelle Glass and Rhonda Joy Rosengren	CLINICALLY RELEVANT DRUGS MODULATE THE EFFECT OF AMB-FUBINACA IN MALE AND FEMALE MICE	DB2-1 [P2-2]
Angela Henderson-Redmond*, Courtney Lulek, Malabika Maulik, Mary Piscura, Kayla DeSchepper, Josee Guindon and Daniel Morgan	MUTANT MICE EXPRESSSING AN INTERNALIZATION-RESISTANT FORM OF CB1R DISPLAY ENHANCED TOLERANCE TO CANNABINOIDS	DB2-2 [P2-3]
Daniel Barrus*, Kyle Rehrauer, Sara Kearney, Anghelo Gangano, Primali Navaratne, Terry-Elinor Reid, Adrian Roitberg, Ion Ghirviriga, Alexander Grenning, Christopher Cunningham and Thomas Gamage	AXIALLY CHIRAL CANNABINOIDS DISPLAY ENHANCED CB2 SELECTIVITY	DB2-3 [P2-5]
Jonah Wirt*, Maria Gerasi, Edward Stahl, Christina Brust, Laura Bohn, Alexandros Makriyannis and Andrea Hohmann	POSITIVE ALLOSTERIC MODULATION OF CB1 CANNABINOID SIGNALING REVERSES PATHOLOGICAL PAIN IN A PROBE-DEPENDENT MANNER WITHOUT PRODUCING TOLERANCE OR UNWANTED SIDE EFFECTS	DB2-4 [P2-13]
Szabolcs Dvorácskó*, Resat Cinar and Malliga Iyer	TARGETING CANNABINOID RECEPTOR 1 (CB1R) WITH NOVEL FOUR-ARM BITOPIC ANTAGONISTS	DB2-5 [P2-14]
Carlos Henrique Alves Jesus*, Jonah Wirt, Paniz Azizi, John Hailine, Hasaan Kazi and Andrea Hohmann	ACETAMINOPHEN PRODUCES ANTINOCICEPTION THROUGH A DIACYLGLYCEROL LIPASE-DEPENDENT MECHANISM IN RODENT MODELS OF INFLAMMATORY AND POST-SURGICAL PAIN	DB2-6 [P2-19]

Deepika Slawek*, Chenshu Zhang, Yuting Deng, Yuval Zolotov, Giovanna Calderon DiFrancesca, Stephen Dahmer, Joanna Starrels, Chinazo Cunningham and Julia Arnsten	MEDICAL CANNABIS AND OPIOID USE AMONG ADULTS WITH CHRONIC PAIN: PRELIMINARY RESULTS FROM AN 18-MONTH LONGITUDINAL STUDY	DB2-7 [P2-38]

DATABLITZ presenters present BOTH a 4-minute presentation AND a POSTER at ICRS2023.

DATABLITZ abstract page numbers are indicated [IN BRACKETS].

Notes:

Presenting Author*

DATABLITZ SESSION 3

DAY 4, WEDNESDAY, JUNE 28TH: 15:30 - 16:00

Savannah Lightfoot*, Andrei Nastase, Gabriela Costa Lenz Cesar and Matthew Hill	INVESTIGATING THE EFFECTS OF ACUTE THC VAPOUR EXPOSURE ON AUDITORY FEAR CONDITIONING AND FEAR EXTINCTION	DB3-1 [P3-1]
Camila Alvarez*, Brandon Laurence Oliver, Natalie Elizabeth Zlebnik and Nicholas Vincent DiPatrizio	ROLE OF THE GUT-BRAIN ENDOCANNABINOID SYSTEM IN FOOD REWARD	DB3-2 [P3-3]
Mohammed Sarikahya*, Karen Wong, Samantha Cousineau, Marta De Felice, Matthew Jones, Anubha Dembla, Amanda Alcaide, Ken Yeung, Daniel Hardy, Walter Rushlow and Steven Laviolette	TARGETING THE BASOLATERAL AMYGDALA GLUTAMATERGIC SYSTEM TO REVERSE THE LONG-TERM IMPACTS OF PRE-NATAL THC EXPOSURE ON ANXIETY AND AFFECTIVE PATHOPHENOTYPES	DB3-3 [P3-4]
Annamaria Tisi, Giulia Carozza, Lucia Di Re, Giacomo Giacovazzo, Lucia Scipioni, Sergio Oddi, Rita Maccarone and Mauro Maccarrone *	UP-REGULATION OF CANNABINOID RECEPTOR 2 (CB2) AND FATTY ACID AMIDE HYDROLASE (FAAH) IN THE RETINA OF A MOUSE MODEL OF ALZHEIMER'S DISEASE	DB3-4 [P3-13]
Maria Teresa Grande*, Samuel Ruiz de Martin Esteban, Maria Andrea Arnanz, Ana Maria Martinez Relimpio, Laura Martin Perez, Almudena Lopez Escobar, Ricardo Mostany, Cecilia Hillard and Julian Romero	CANNABINOID TYPE 2 RECEPTORS MODULATE MICROGLIA FUNCTIONS IN A MOUSE MODEL OF ALZHEIMER DISEASE	DB3-5 [P3-29]

Ariel Rothner*, Tom Gov, Liad Hinden, Alina Nemirovski, Joseph Tam and Barak Rosenzweig	SYSTEMIC CHANGES IN ENDOCANNABINOIDS AND ENDOCANNABINOID-LIKE MOLECULES IN RESPONSE TO PARTIAL NEPHRECTOMY-INDUCED ISCHEMIA IN HUMANS	DB3-6 [P3-30]
Riley Gournay*, Parker Williams, Danielle Fernandez, Mia Bingaman, Chloe Martinez, Jordyn Moore, Morgan Ferretti, Anna Marie Nguyen and Ellen Leen-Feldner	THE ACUTE VERSES REPEATED EFFECTS OF CANNABIDIOL ON WORRY AND ANXIETY: A DOUBLE-BLIND, RANDOMIZED PLACEBO CONTROLLED TRIAL	DB3-7 [P3-42]

DATABLITZ presenters present BOTH a 4-minute presentation AND a POSTER at ICRS2023.

DATABLITZ abstract page numbers are indicated [IN BRACKETS].

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POSTER SESSION 2: 65 POSTERS
DAY 2, MONDAY, JUNE 26TH: 16:00 - 18:00

POSTER SESSION 3: 48 POSTERS
DAY 4, WEDNESDAY, JUNE 28TH: 16:00 - 18:00

POSTER SESSION 1
DAY 1, SUNDAY, JUNE 25TH: 16:15 - 18:15

Alexandru Zabara*, Christiane Schweiggert, Thomas Zwick, Jimmy Doucoure, Milena Aldag, Thomas Lindemann, Nathalie Richard, Richard Goessl, Bianca Nibali and Elodie Chenal	A JOURNEY THROUGH THE DEVELOPMENT OF CANNABIDIOL SOLID ORAL DOSAGE FORMS: BALANCING API LOADING, PHYSICO-CHEMICAL STABILITY, AND *IN-VIVO* PHARMACOKINETIC PERFORMANCE	P1-1
Joseph J Wakshlag*, Wayne Schwark and Robert Davis	TWENTY FOUR HOUR PHARMACOKINETICS OF A FULL SPECTRUM CANNABIDIOL AND CANNABIDIOLIC ACID PRODUCT: COMPARATIVE ASSESSMENT IN HUMANS AND DOMESTIC ANIMALS	P1-2
Thomas Arkell*, Jan Ramaekers, Robyn Robertson, Eef Theunissen and Christine Wickens	CANNABIS AND DRIVING: RECENT EXPERIMENTAL EVIDENCE	P1-3
Johannes Ramaekers*, Eef Theunissen, Thomas Arkell, Christine Wickens and Robyn Robertson	CANNABIS AND DRIVING: RECENT TOXICOLOGICAL ISSUES	P1-4
Christine M. Wickens*, Robyn D. Robertson, Jan G. Ramaekers, Thomas R. Arkell and Eef L. Theunissen	CANNABIS AND DRIVING: RECENT EPIDEMIOLOGICAL EVIDENCE	P1-5
Alisha K. Holloway* and Erica G. Bakker	GENETIC BASIS FOR PRODUCTION OF THCV, A RARE AND POTENTIALLY MEDICALLY VALUABLE CANNABINOID	P1-6
Sara L. MacPhail*, Katelyn Walker, Eric Sparkes, Iain S. McGregor, Adam Ametovski and Elizabeth A. Cairns	PHYTOCANNABINOIDS AS INHIBITORS OF OXIDOREDUCTASES: NOT JUST NAD(P)H BINDING SITE INHIBITORS	P1-7
Sebastian Vanin*, Kendrick Lee, Brennan Tse, Sukham Brar, Mina Nashed, Steven Laviolette, Edith Arany and Daniel Hardy	IN UTERO EXPOSURE TO CANNABIDIOL LEADS TO GLUCOSE INTOLERANCE AND ALTERED HEPATIC TRANSCRIPTOME EXCLUSIVELY IN MALE RAT OFFSPRING AT 3-MONTHS OF AGE	P1-8

Marta De Felice*, Hanna J. Szkudlarek, Mar Rodríguez-Ruiz, Taygun C. Uzuneser, Mohammed H. Sarikahya, Mathusha Pusparajah, Juan Pablo Galindo Lazo, Shawn N. Whitehead, Ken K.-C. Yeung, Walter J. Rushlow and Steven R. Laviolette	ADMINISTRATION OF N-ACETYLCYSTEINE PREVENTS THE LONG-LASTING DEPRESSIVE-LIKE PHENOTYPES INDUCED BY ADOLESCENT THC EXPOSURE	P1-9
Catherine Hume*, Samantha Baglot, Lucia Javorcikova, Savannah Lightfoot, Jessica Scheufen and Matthew Hill	EFFECTS OF PRENATAL TETRAHYDROCANNABINOL (THC) VAPOUR EXPOSURE AND HIGH-FAT DIET ON RAT FEEDING PATTERNS, ADIPOSITY AND GLUCOSE METABOLISM	P1-10
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POSTER SESSION 2
DAY 2, MONDAY, JUNE 26TH: 16:00 - 18:00

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Angela Henderson-Redmond*, Courtney Lulek, Malabika Maulik, Mary Piscura, Kayla DeSchepper, Josee Guindon and Daniel Morgan	MUTANT MICE EXPRESSSING AN INTERNALIZATION-RESISTANT FORM OF CB1R DISPLAY ENHANCED TOLERANCE TO CANNABINOIDS	P2-3
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Kathleen McCoy*, Brynna Webb, Ken Mackie and Anna Kalinovsky	EXPRESSION AND FUNCTION OF DAGLa IN CEREBELLAR PURKINJE CELLS	P3-9
Bogna Ignatowska-Jankowska*, Aysen Gurkan-Ozer, Alexander Kuck, Micah Niphakis, Daisuke Ogasawara, Benjamin Cravatt and Marylka Yoe Uusisaari	ENDOCANNABINOID SYSTEM MODULATION AFFECTS LOCOMOTORY EPISODE STRUCTURE	P3-10
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ICRS2023 PRESIDENTIAL PLENARY: 11:45AM Sunday, June 25th, 2023

TRENDS IN CANNABIS PRODUCTS AND CONSUMER PATTERNS OF USE: UNDERSTANDING THE IMPACT OF CANNABIS LEGALIZATION IN CANADA

DAVID HAMMOND, Ph.D.

Professor and University Research Chair in Public Health
School of Public Health Sciences
University of Waterloo

Biography

David Hammond is a Professor and University Research Chair in Public Health in the School of Public Health Sciences at the University of Waterloo.

Professor Hammond's research focuses on population-level interventions to reduce chronic disease, primarily in the areas of tobacco control, obesity prevention, and substance use policy.

Professor Hammond has served as an Advisor to the World Health Organization, as well as regulatory agencies and governments around the world on tobacco control policy. He has also served as an Expert Witness on behalf of governments around the world in litigation in the areas of tobacco, nutrition, and cannabis. Professor Hammond's work has been recognized by awards from the World Health Organization, the Canadian Cancer Society, the Royal Statistical Society of Canada, the Canadian Institutes of Health Research, the Governor General of Canada.

ICRS2023 PRESIDENTIAL PLENARY: 8:30AM Monday, June 26th, 2023

ASTROCYTE CB1RS LINK SOCIAL OLFACTION, STRESS TRANSMISSION AND COGNITIVE IMPAIRMENT

JAIDEEP BAINS, Ph.D.

Director of the Krembil Research Institute,
University Health Network (UHN), Toronto,
formerly Scientific Director of the Hotchkiss Brain Institute
at the University of Calgary

Biography

Dr. Bains, an internationally recognized expert in brain research, was Scientific Director of the Hotchkiss Brain Institute at the University of Calgary. He has recently been appointed University Health Network (UHN) as the new Director of the Krembil Research Institute.

For the past two decades, Dr. Bains has led a successful research team at the University of Calgary, making many important discoveries that have provided insights into how the brain adapts to stress. These studies have shed light on links between neurotransmission, synaptic plasticity and brain network states, with important behavioural and physiological responses in different models of stress. More recently, Dr. Bains has pioneered approaches that use advanced technology platforms to measure changes in brain circuits in response to stress. His team developed a novel system for behavioural assessment and combined this with optogenetic and circuit mapping, to link activity in the brain's stress command neurons to the transmission of stress/fear from one individual to another. He has also been active in the potential for clinical application of this research through commercialization efforts.

ICRS2023 KANG TSOU MEMORIAL LECTURE: 11:45AM Tuesday, June 27th, 2023

ENDOCANNABINOIDS DRIVE MEMORY GENERALIZATION IN THE STRESSED MOUSE BRAIN

SHEENA JOSSELYN, Ph.D.

Senior Scientist at The Hospital for Sick Children (SickKids),
Professor of Psychology and Physiology at the University of Toronto,
Canada Research Chair in Brain Mechanisms Underlying Memory,
Senior Fellow in the Canadian Institute for Advanced Research (CIFAR),
Fellow of the Royal Society of Canada.

Biography

Sheena Josselyn is a Senior Scientist at The Hospital for Sick Children (SickKids) and a Professor in the departments of Psychology and Physiology at the University of Toronto in Canada. She holds a Canada Research Chair in Brain Mechanisms underlying Memory, is a Senior Fellow in the Canadian Institute for Advanced Research (CIFAR), and is a Fellow of the Royal Society of Canada.

Her undergraduate degrees and a master's degree in Clinical Psychology were granted by Queen's University in Kingston (Canada). Sheena received a PhD in Neuroscience/Psychology from the University of Toronto with Dr. Franco Vaccarino as her supervisor. She conducted post-doctoral work with Dr. Mike Davis (Yale University) and Dr. Alcino Silva (UCLA). Dr. Josselyn received several awards, including the Innovations in Psychopharmacology Award from the Canadian College of Neuropsychopharmacology (CCNP) and the Effron Award from the American College of Neuropsychopharmacology (ACNP).

Dr. Josselyn is interested in understanding how the brain encodes, stores and uses information. Several human disorders (ranging from autism spectrum disorder to Alzheimer's disease) may stem from disrupted information processing. Therefore, this basic knowledge is not only critical for understanding normal brain function, but also vital for the development of new treatment strategies for these disorders.

ICRS2023 MECHOULAM AWARDEE: 12:15 AM, Wednesday, June 28th, 2023

IMPROVING OUR UNDERSTANDING OF CANNABINOID PHARMACOLOGY AND MEDICATION DEVELOPMENT THROUGH THREE DECADES OF CONTROLLED HUMAN CANNABINOID ADMINISTRATION STUDIES

MARILYN HUESTIS, Ph.D.

Adjunct Professor, Department of Epidemiology and Preventive Medicine,
School of Medicine, University of Maryland Baltimore
Retired Tenured Senior Investigator and Chief,
Chemistry and Drug Metabolism Section, IRP,
National Institute on Drug Abuse and National Institutes of Health

Biography

Professor Dr. Marilyn A. Huestis recently retired as a tenured senior investigator and Chief, Chemistry and Drug Metabolism Section, IRP, National Institute on Drug Abuse, National Institutes of Health, after 23 years of conducting controlled drug administration studies. She is an Adjunct Professor in the Department of Epidemiology and Preventive Medicine, School of Medicine, University of Maryland Baltimore. She thoroughly enjoys mentoring doctoral students in Toxicology, has to date directly overseen the research of 16 distinguished new toxicologists, and currently has 2 students pursuing their dissertation research. Her research program focused on discovering mechanisms of action of cannabinoid agonists and antagonists, effects of *in utero* drug exposure, and the neurobiology and pharmacokinetics of novel psychoactive substances, the emerging face of drug abuse. She has published 408 peer-reviewed manuscripts and book chapters and more than 490 abstracts were presented at national and international meetings. Professor Huestis received a bachelor's degree in biochemistry from Mount Holyoke College (cum laude), a master's degree in clinical chemistry from the University of New Mexico (with honors), and a doctoral degree in toxicology from the University of Maryland (with honors). Professor Huestis received a Doctor Honoris Causa from the Faculty of Medicine, University of Helsinki in Finland in 2010. Other important awards include, 2016 Marian W. Fischman Lectureship Award from the College on Problems of Drug Dependence, 2016 Saferstein Memorial Distinguished Lecturer at Northeastern University to be awarded April 2016, Excellence in Scientific Research, Women Scientist Advisory NIDA Investigator Award March 27, 2015, Norman P. Kubasik Lectureship Award, AACC Upstate New York Section May 7, 2015, Distinguished Fellow Award from the American Academy of Forensic Sciences (AAFS) in 2015, The International Association of Forensic Toxicologists (TIAFT) Alan Curry Award in 2010, the American Association for Clinical Chemistry Outstanding Contributions in a Selected Area of Research Award in 2008, the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) Irving Sunshine in 2007, the AAFS Rolla N. Harger Award in 2005, and the Irving Sunshine Award for Outstanding Research in Forensic Toxicology in 1992. The journal *Clinical Chemistry* featured her as an "Inspiring Mind". She currently serves on the new National Commission on Forensic Sciences, and the Organization of Scientific Area Committee on Toxicology, World Anti-doping Agency's Prohibited List Committee, the Scientific Working Group on Toxicology (SWG-TOX), Transportation Research Board Committee on Alcohol and Other Drugs, and the National Safety Council's Alcohol, Drugs and Impairment Division Executive Board. Professor Huestis is past president of the Society of Forensic Toxicologists, past Chair of the Toxicology Section of the American Academy of Forensic Sciences, and the first woman president of The International Association of Forensic Toxicologists.

2023 WILLIAM A DEVANE EARLY CAREER INVESTIGATOR AWARDEE LECTURE:
3:00PM, Wednesday, June 29th, 2023

SEX DIFFERENCES IN ENDOCANNABINOID SIGNALING PATHWAYS INVOLVED IN CHRONIC PAIN AND CANCER: PRESENT AND INSIGHTS INTO THE FUTURE

JOSEE GUINDON, Ph.D.

Associate Professor
Department of Pharmacology and Neuroscience
Texas Tech University Health Sciences Center

Biography

Dr Josée Guindon is a Tenured Associate Professor in the Department of Pharmacology and Neuroscience and Graduate Advisor in the Translational Neuroscience and Pharmacology Graduate Concentration at Texas Tech University Health Sciences Center (TTUHSC). She has obtained her Doctorate in Veterinary Medicine in 2000, Master in 2002 and Ph.D. in 2007 from Université de Montréal. She did her post-doctoral training at University of Georgia with Dr. Andrea G. Hohmann for which she received a substantial post-doctoral fellowship from the Fonds de la Recherche du Québec en Santé (FRQS). Since Dr. Guindon started her laboratory at TTUHSC in 2014, she has been continuously funded by The CH Foundation, Garrison Institute on Aging, Texas Center for Comparative Cancer Research, and NIH. Dr Guindon got awarded her first R01 from NIDA to study mechanisms of cannabinoid tolerance. Dr. Guindon won several career awards. Indeed, in 2019, she won the TTUHSC President Early Career Investigator Award and, in 2016, the Unsung Hero Award honored by the Provost and Dean of TTUHSC. She has already published more than 47 manuscripts and 7 first-authored book chapters as well as giving more than 50 invited seminars for local and international conferences. Dr. Guindon is an expert in the behavioral, pharmacological, biochemical, and transgenic analysis of pain mechanisms, using various pain models and investigating sex differences. She has greatly contributed and pioneered, neural and brain endocannabinoid mechanisms that regulate and modulate pain and how they influence sex hormones. Therefore, opening new and novel understandings of the ways pain is transmitted through the nervous system and interacting with the endocannabinoid system. Dr Guindon has also been developing new translational models and discovering novel approaches to study the pharmacological management of chronic pain and cancer.

NIH/NCCIH Symposium
Wednesday, June 28th, 9-10am

National Center for Complementary and Integrative Health

LOOKING BEYOND Δ^9-THC: A TRANSLATIONAL PERSPECTIVE ON THE ANALGESIC PROPERTIES OF MINOR CANNABINOIDS AND TERPENES

Participants

David Shurtleff, PhD (Chair; Speaker), Deputy Director, Acting Scientific Director, National Center for Complementary and Integrative Health (NCCIH), National Institutes of Health; Bethesda, MD (David.Shurtleff@nih.gov)
Inna Belfer, MD, PhD (Co-Chair; Moderator), Program Director, NCCIH, Bethesda, MD (inna.belfer@nih.gov)
Aditi Das, PhD (Speaker), Lab Chief, Georgia Institute of Technology, Atlanta, GA (aditi.das@chemistry.gatech.edu)
Sara Jane Ward, PhD (Speaker), Center for Substance Abuse Research, Temple University, Philadelphia, PA (sara.ward@temple.edu)
Ziva Cooper, PhD (Speaker), Director, UCLA Center for Cannabis and Cannabinoids, Los Angeles, CA (ZCooper@mednet.ucla.edu)
Hance Clarke MD, FRCPC, PhD (Speaker), Director of Pain Services; Toronto General Hospital, Toronto, Ontario Canada (hance.clarke@utoronto.ca)

Agenda

1. Programmatic priorities in supporting cannabinoid research (Presenter: Dr. David Shurtleff, 5 min)
2. Minor cannabinoid metabolites generated by human cytochrome P450s are bioactive (Presenter: Dr. Aditi Das, 10 min)
3. Anti-allodynic effects of cannabigerol (CBG) in rodent models of neuropathic pain (Presenter: Dr. Sara Jane Ward, 10 min)
4. Assessing the analgesic and subjective effects beta-caryophylline administered alone and combined with delta-9-THC: A placebo controlled investigation in volunteers (Presenter: Dr. Ziva Cooper, 10 min)
5. Real-world evidence - Have we learned anything about the role of minor cannabinoids? (Presenter: Dr. Hance Clarke, 10 min)
6. Moderated Q & A / discussion session (Moderator: Dr. Inna Belfer, 15 min)

Abstract

Pain is one of the primary conditions for which people report using cannabis for medical purposes. In addition to its major psychoactive constituent [D^9-tetrahydrocannabinol (Δ^9-THC)], cannabis contains over 120 minor cannabinoids as well as over 500 other chemicals, including the terpenes that give cannabis its distinctive flavoring and aroma. The degree to which these minor plant constituents contribute to the potential medicinal effects of cannabis, and specifically to its analgesic effects, is under active investigation. This translational symposium will provide an overview of studies investigating the potential pain-relieving effects of selected minor cannabinoids and/or terpenes, from molecular pharmacology to human studies. The symposium will begin by showcasing NCCIH programmatic priorities and funding opportunities for minor cannabinoid / terpene research. Next, molecular research exploring the mechanism by which select minor cannabinoids (e.g., cannabigerol, cannabicitran, cannabicyclol, and cannabichromene) reduce inflammation associated with pain via their actions on pain receptors (e.g., vanilloid/transient receptor potential cation channel subfamily V (TRPV), subfamily A (TRPA), subfamily M (TRPM)) will be presented, followed by a presentation assessing the effects of selected cannabis constituents, including the terpene beta-caryophyllene, in preclinical models of pain and inflammation. A translational viewpoint will follow, linking these preclinical findings to a clinical study with research volunteers probing the potential pain-relieving effects of beta-caryophyllene. A final presentation will focus on results of analysis of cannabinoid constituent profiles of products from medical cannabis patients enrolled in a longitudinal, observational study exploring their impact on clinical outcomes related to pain. The session will end with moderated Q&A and discussion involving all participants. Together, these presentations will provide the audience with a broad overview of the pharmacology and potential pain-relieving effects of cannabis constituents beyond Δ^9-THC.

CELL ADHESION MOLECULE 2 MODERATES CANNABIS EDIBLE INTAKE, Δ^9-TETRAHYDROCANNABINOL RESPONSE, AND THE ELECTRO-PHYSIOLOGICAL PROPERTIES OF PRELIMBIC CORTICAL NEURONS

Hayley H. A. Thorpe[1], Anna E. Canella[1], Hakan Kayir[1], M. Asfandyaar Talhat[1], Larissa A. Kouroukis[1], Samiha Kazi[1], Yu Gu[1], Ron Johnson[1], Boyer D. Winters[2], Sandra Sanchez-Roige[3], Abraham A. Palmer[3,4], Craig D. C. Bailey[1] and Jibran Y. Khokhar[1,5]

[1]Department of Biomedical Sciences, University of Guelph, ON, Canada.
[2]Department of Psychology, University of Guelph, ON, Canada.
[3]Department of Psychiatry, University of California San Diego, La Jolla, CA, United States. [4]Institute for Genomic Medicine, University of California San Diego, San Diego, CA, USA. [5]Department of Anatomy and Cell Biology, Western University, London, ON, Canada

Introduction: Genome-wide association studies identify *Cell Adhesion Molecule 2* (*CADM2*) as a candidate risk gene for lifetime cannabis use and posit its involvement in neurobiological processes that overlap with cannabinoid activity. We therefore assessed the role of mouse *Cadm2* in behavioral, physiological, and neurobiological features relevant to cannabis vulnerability.

Methods: Male and female *Cadm2* WT (WT), heterozygous (HT), and knockout (KO) mice were used to assess cannabis-relevant phenotypes. Preference for cannabis oil and Δ^9-tetrahydrocannabinol (THC) was determined with a two-edible choice paradigm. THC (3 or 10mg/kg, i.p.) injection response was evaluated with the cannabinoid tetrad assay. Blood serum THC and 11-hydroxy-THC levels were assessed 30min and 2h after 10mg/kg injection. Cognitive performance after 2mg/kg THC challenge was assessed with the 5-choice serial reaction time task. Glutamate and GABA receptor subunit expression in the frontal cortex and striatum were quantified with western blotting. Layer V pyramidal neuron activity at baseline and in response to 1μM THC was assessed via whole-cell patch clamp recordings in the prelimbic cortex.

Results: THC and cannabis oil-containing edible preference was reduced in *Cadm2* KO mice. Whereas expected THC-induced hypolocomotion and hypothermia was recorded in WT and HT groups, KO ablated hypothermia and evoked hyperlocomotion. We found no differences in THC or 11-hydroxy-THC serum levels. THC did not affect cognitive performance in any genotype and there was no evidence that *Cadm2* KO altered glutamate and GABA receptor subunits levels previously correlated with human *CADM2*. Electrophysiological recordings revealed elevated layer V prelimbic pyramidal neuron membrane resistance and attenuated resting membrane potential and afterhyperpolarization amplitude in brains from *Cadm2* KO mice, but there were no genotype differences in postsynaptic currents elicited by THC bath application.

Conclusion: Complementing human correlational evidence, *Cadm2* expression modifies voluntary cannabinoid intake and pharmacological drug response and may thus inform how *CADM2* polymorphisms may be a causal genetic contributor to cannabis use vulnerability.

Acknowledgements: Funded by the Natural Science and Engineering Research Council PGS-D scholarship, California Tobacco-Related Disease Research Program (Grant Number T29KT0526 and 28IR-0070), and Canadian Institutes of Health Research Project Grant (PJT-173442).

DOSE EFFECTS OF ORAL AND VAPORIZED DELTA-8-THC AND COMPARISON TO DELTA-9-THC IN HEALTHY ADULTS

Ryan Vandrey [1], Carlos Austin Zamarripa [1], Ashley Dowd [1], Tory Spindle [1], Elise Weerts [1], Ed Cone [1], Ruth Winecker [2] and Ron Flegel [3]

[1] Johns Hopkins University School of Medicine
[2] RTI International
[3] Substance Abuse and Mental Health Services Administration

Introduction. Due to language in the U.S. Agriculture Improvement Act of 2018, there has been a proliferation of retail cannabis products in the U.S. predominantly containing delta-8-THC. Little controlled research on delta-8-THC has been conducted since the 1970s. The aim of the current study was to characterize the acute effects of oral and inhaled delta-8-THC, compared with a positive control dose of delta-9-THC and placebo, on subjective drug effects, cardiovascular effects, cognitive performance, and pharmacokinetics.

Methods: Healthy adults were recruited to participate in 2 studies, one that evaluated the acute effects of oral delta-8-THC (0 mg, 10 mg, 20 mg and 40 mg) and oral delta-9-THC (20 mg) and another that evaluated the same doses of delta-8-THC and delta-9-THC administered via vaporization. The experiments were run concurrently with most participants opting to complete both studies in sequential order. In those cases, the order of study completion (i.e., oral then vaporized or vice versa) was counterbalanced. Both studies used a within-subject crossover design, dose order within each study was randomized, and a minimum of 6 days separated each test session to allow for drug washout. Vital signs, self-reported drug effects, performance on a battery of cognitive tasks, and blood, urine and oral fluid samples were obtained from participants at baseline and repeatedly for 8 hours after dosing.

Results: To date, 9 of 20 participants have completed the oral dosing study and 6 of 20 have completed the vaporization study. In the oral dosing study, pharmacodynamic assessments show a dose-orderly effect of delta-8-THC on all assessments. The 20 mg delta-9-THC dose showed qualitatively stronger drug effects than all 3 doses of delta-8-THC on most measures, though the 40 mg delta-8-THC dose was comparable. In the vaporized dose study, pharmacodynamic outcomes showed stronger peak drug effects compared with the oral dose study, which is consistent with prior cannabis research. There was a lack of dose-orderliness on subjective drug effects for delta-8-THC in the vaporized dose study as many outcomes were rated similarly across the 3 doses of delta-8-THC. Puff topography data showed that puff volume was lower for the 20 mg and 40 mg dose conditions compared with the 10 mg dose and placebo, suggesting participants were titrating inhalation behavior. Delta-9-THC produced qualitatively higher effects on several subjective drug effect outcomes compared with delta-8-THC, but the 40 mg delta-8-THC dose produced similar or stronger effects on heart rate and cognitive performance compared with 20 mg delta-9-THC in this study. Pharmacokinetic testing of biological samples is pending.

Conclusions: Acute doses of oral and vaporized delta-8-THC produced pharmacodynamic drug effects that fully overlap in distinguishing characteristics with the acute effects of delta-9-THC. However, delta-8-THC produced a lower magnitude of effects compared with delta-9-THC at the same dose, which is consistent with prior research on binding affinity between the 2 isomers. The 40 mg dose of delta-8-THC resulted in equivalence or near equivalence in the magnitude of drug effect observed at the 20 mg dose of delta-9-THC on most outcomes.

Acknowledgements: This study was funded by the Substance Abuse and Mental Health Services Administration.

A NOVEL CANNABIDIOL: TETRAMETHYLPYRAZINE (CBD:TMP, ART12.11) COCRYSTAL IMPROVES THE BIOAVAILABILITY AND EFFICACY OF CBD

Saoirse O'Sullivan[1], Matthew Jones[2], Taygun Uzuneser[2], Steven Laviolette[2] and Andrew Yates[1]

[1]Artelo Biosciences, USA, [2] University of Western Ontario, Canada

The therapeutic utility of cannabidiol (CBD) is hampered by its physical and pharmacokinetic properties, including high lipophilicity, poor solubility and stability, and poor oral bioavailability. Cocrystallisation is a useful method for overcoming problematic properties of drugs and is well-established in drug development. Cocrystals consist of the drug substance (i.e. CBD) and a co-former molecule which modify the physicochemical properties whilst retaining the intrinsic pharmacological drug activity. Artelo Biosciences has developed a patented cocrystal of CBD with the coforming agent tetramethylpyrazine (TMP; also called ligustrazine), a plant-derived compound from the Ligusticum species that is widely used in Chinese medicine. The cocrystal is referred to as ART12.11.

Two studies were designed to test the bioavailability of ART12.11 compared to CBD alone in fed and fasted male beagle dogs. In study one, in the fasted state, the mean values for C_{max}/Dose and AUC/Dose were 15-fold and 35-fold higher, respectively, after administration of ART12.11 capsules compared to CBD alone. In the fed state, the exposure of CBD was comparable. A second, repeated dose study confirmed the initial findings, showing enhanced CBD (Fig 1A) or CBD-7COOH (Fig 1C) plasma levels after dose 1 in the fasted state. CBD and CBD-7COOH levels were also increased after the seventh administration of ART12.11 in the fasted state. In the fed state, CBD levels were comparable on day 1 and 7 (Fig 1B), but CBD-7COOH levels were higher on day 1 in the fed state (Fig 1D).

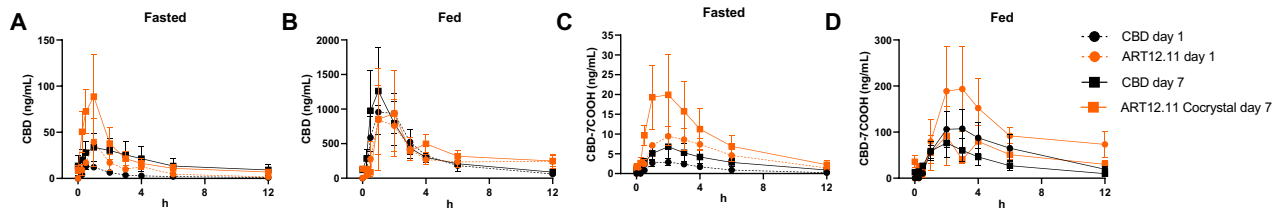

Figure 1. Pharmacokinetic profiles of ART12.11 and CBD (both 10 mg/kg) along in fed and fasted beagle dogs taken after the first (day 1) and seventh (day 7) dose of test article (n=3). CBD levels are presented in A and B, and CBD metabolite (CBD-7COOH) levels in C and D.

Behavioural pharmacological studies were performed to test the efficacy of ART12.11 (5 mg/kg, PO equivalent to 3.5 mg/kg CBD and 1.5 mg TMP) versus CBD alone (10 mg/kg, PO) in rats exposed to a chronic unpredictable stress protocol for 2 weeks. Behaviour testing was done 2 hours after the drug was administered. ART12.11 significantly outperformed CBD in the light-dark box (more time spent in the light chamber, greater % of time in the light versus dark chamber and greater number of entries into the light chamber), social interaction test (more time spent with a stranger animal, more time spent interacting with a stranger animal and greater % time interacting with the stranger animal versus empty cage) and the sucrose preference test. In all cases, ART12.11 (5 mg/kg PO) reversed the effects of chronic stress on anxiety and depression behaviour to levels equal to or even less than the non-stressed controls. In contrast, CBD alone was not efficacious in these tests at the dose tested (10 mg/kg, PO).

In summary, we have shown that ART12.11 improves the oral bioavailability of CBD. In conjunction, behaviour studies show that oral administration of ART12.11 has a strong anxiolytic effect, increases social behaviour in stressed rats, and alleviates anhedonia-like symptoms in stressed rats. The effects of ART12.11 were significantly greater than observed with CBD alone. These data support the continued drug development of ART12.11 as a proprietary compound with superior pharmaceutical properties and promising pharmacological differentiation (Home - CBD Cocrystal).

5-WAY CROSSOVER CLINICAL TRIAL TO ASSESS MODULATING EFFECTS OF CBD ON PSYCHOTROPIC AND ANALGESIC EFFECTS OF THC

Andriy A. Gorbenko[1,2], Jules A.A.C. Heuberger[1], Linda E. Klumpers[3,4,5] and Geert J. Groeneveld[1,2]

1: Centre for Human Drug Research, Leiden, The Netherlands;
2: Leiden University Medical Centre, Leiden, The Netherlands;
3: Verdient Science, Denver, CO, US;
4: Tomori Pharmacology, Denver, CO, US;
5: Larner College of Medicine, University of Vermont, VT, US

Introduction: Δ^9-tetrahydrocannabinol (THC) is the main psychoactive and analgesic component of cannabis. Cannabidiol (CBD) is the main non-intoxicating component. This clinical trial investigated the hypothesis that CBD counteracts negative THC effects, potentially improving clinical usability of cannabis as an analgesic.

Methods: This was a randomized, double blind, placebo-controlled, 5-way cross-over trial in 26 healthy volunteers. On each visit participants received one of the following oral treatments: placebo; THC; THC+CBD 10mg; THC+CBD 30mg; THC+CBD 450mg. The THC dose was 9mg in all THC-treatments. Psychoactive and analgesic effects were quantified using standardised assessments. Nociceptive thresholds and post-test pain rating were measured for electrical pain, cold pain, heat pain and pressure pain. Pharmacokinetic (PK) sampling was performed for parent compounds and metabolites. Data was analysed using mixed-model ANCOVA.

Results: There were no significant differences in visual analogue scale (VAS) Feeling High, postural stability and heart rate between THC alone and THC + 10mg or 30mg CBD, whereas THC+CBD 450mg resulted in significantly more increased VAS Feeling High compared to THC alone (estimate of difference (EoD): 60.5%, 95%CI: 12.7 – 128.5%, **p<0.01**). Postural stability was significantly more impaired after treatment with THC+CBD 450mg, compared to THC alone (EoD: 23.0%, 95%CI: 7.4 – 40.9%, **p<0.01**), and heart rate was significantly more increased after treatment with THC+CBD 450mg, compared to THC alone (EoD: 4.2 bpm, 95%CI: 1.7 – 6.8, **p<0.01**). THC treatments (with or without CBD) did not produce significant analgesic effects on nociceptive thresholds compared to placebo. Post-test pain VAS was significantly reduced after treatment with THC alone compared to placebo for heat pain (EoD: -5.8, 95%CI: -5.8 – -0.1, **p=0.04**), cold pain (EoD: -3.9, 95%CI: -7.1 – -0.7, p=**0.02**) and electrical pain (EoD: -4.3, 95%CI: -7.3 – -1.4, **p<0.01**), although not pressure pain, where only a trend was observed (EoD: -2.9, 95%CI: -6.1 – 0.4, p=0.09). There were no significant differences in post-test pain VAS between the different THC-containing treatments. Finally, markedly higher THC concentrations were measured after coadministration with 450mg CBD compared to THC alone (2.2x mean Cmax increase, 2.7x mean AUC24h increase).

Conclusion: CBD 10mg and 30mg did not reduce psychotropic effects of THC. CBD 450mg increased psychotropic effects of THC, likely by increasing THC exposure by way of PK interaction. THC-induced reduction in post-test pain rating in the absence of analgesic effects on nociceptive thresholds is consistent with THC acting on pain experience rather than nociception. The observed analgesic effects did not differ between THC-treatments, regardless of CBD dose.

Acknowledgements: This research was funded by ZonMw (grant no. 848120001).

EFFECTS OF PREFERRED CANNABIS ON DRIVING IN ADULTS OVER THE AGE OF 65

Patricia Di Ciano[1], Tarek Rajji[1], Lauren Hong[2], Sampson Zhao[1], Patrick Byrne[3], Yoassry Elzohairy[3], Jeffrey Brubacher, Michael McGrath[3], Bruna Brands[4], Sheng Chen[1], Wei Wang[1], Omer Hasan[1], Christine Wickens[1], Pamela Kaduri[1] and Bernard Le Foll[1]

[1]Centre for Addiction and Mental Health, Toronto, Canada;
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Aim: Laboratory studies demonstrate that cannabis increases standard deviation of lateral position (SDLP; 'weaving') and reaction time, with compensatory changes in speed and following distance. However, there are no investigations into the impact of cannabis on driving performance in adults over 65 years of age.

Methods: 31 adults (mean age: 69 ± 3.5; 21M; 10F) smoked their preferred cannabis to their usual level of intoxication. Driving was assessed, and blood collected, before smoking cannabis and then again at 30 minutes and 180 minutes afterwards; in a control session they did not smoke cannabis. Data were analyzed with mixed effects models with Time and Treatment as fixed effects, controlling for session, baseline blood THC and the outcome measure at baseline.

Results: Most participants (n=23) used cannabis more than once a week for non-medical purposes (n=25). Comparison of the least square mean of the cannabis to the non-cannabis condition revealed that 'weaving' was increased 30 minutes after smoked cannabis ($p=0.013$), while mean speed decreased ($p=<0.001$). Blood THC was increased 30 minutes after cannabis (mean THC = 25.56 ± 5.06 ng/mL; $p<0.0001$), but THC concentration was not correlated with driving. Blood THC at the time of the drive was above the legal limit of 5 ng/ml in most participants (n=25).

Conclusion: These results indicate that older drivers have significant impairment after smoking their usual dose of recreational cannabis.

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SLEEP AND ANXIOLYTIC EFFECTS OF 150MG NIGHTLY SUPPLEMENTATION OF CANNABIDIOL (CBD) FOR PRIMARY INSOMNIA

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Introduction: Cannabidiol (CBD) may have sleep and anxiolytic benefits in healthy and clinical populations; yet there is little consensus on effective dosage and treatment periods. This study examined whether the permitted Australian daily dose of over-the-counter CBD oil (150mg) improved sleep and anxiety symptomology in those with primary insomnia over a 2-week period.

Methods: A total of 30 participants with moderate-severe primary insomnia first completed a single-blind placebo-run week, followed by a 2-week double blind, parallel, randomised placebo-controlled dosing period (ACTRN- 12620000070932). Participants attended four in-person assessments (V0-V4). Daily subjective and objective total sleep time (TST), sleep onset latency (SOL), wake after sleep onset (WASO), number of awakenings after sleep and sleep efficiency (SE) were measured using daily sleep diary entries and wrist-mounted actigraphy. Weekly Leeds Sleep Evaluation Questionnaires (LSEQ) & Glasgow Sleep Effort Scale (GSES) were used to measure sleep quality and effort. Separate linear mixed effect models were applied to assess differences in subjective and objective sleep measures. The State-Trait Anxiety Index was used to measure trait anxiety at V0 and V4 only. T-tests were performed to examine differences between groups.

Results: Daily supplementation of 150mg CBD improved subjective sleep quality over time [LSEQ (V0 – V4 mean difference = 26.59, SE = 4.18, $p < 0.001$)] but was not superior to placebo at V4 ($p > 0.05$). Sleep effort scores also improved over time (GSES mean difference = -4.07, SE = 0.89, $p < 0.001$); however, CBD was not superior to placebo at study conclusion ($p > 0.05$). CBD improved daily sleep quality during the first week of active dosing only, relative to placebo (mean difference = 0.36, SE = 0.18, $p = 0.044$). As measured by Actigraphy, CBD improved objective TST relative to placebo at trial conclusion relative to placebo (mean difference = 0.86, SE = 0.29, $p = 0.003$), but did not change any other objective or subjective sleep measures (all $p > 0.05$). Over time, both CBD (mean difference V1-V4 = 1.47, SD = 2.70, $p = 0.054$), and placebo increased trait anxiety (mean difference V1= V4 = 2.80, SD = 4.62, $p = 0.034$), but treatment groups did not differ in efficacy at study conclusion ($p > 0.05$).

Conclusions: Daily supplementation of 150mg CBD for 2-weeks produces select anxiolytic, and subjective sleep benefits in unmedicated adults with primary insomnia. At these doses, the beneficial effects of CBD may be masked by peripheral improvement in sleep hygiene. A larger, crossover trial with varying CBD doses and active control group will help confirm sleep and anxiolytic benefits in those with primary insomnia.

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THE IMPACT OF CANNABIDIOL TREATMENT ON BRAIN FUNCTION AND METABOLISM OF PATIENTS WITH A PSYCHOTIC DISORDER

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Introduction: Psychosis is a chronic and serious mental disorder with an urgent need for novel and more effective treatments. A promising potential new antipsychotic agent is the non-intoxicating cannabinoid compound cannabidiol (CBD). The first clinical trials with CBD treatment of psychosis patients have shown its ability as an effective, safe and well-tolerated antipsychotic agent. However, the neurobiological mechanisms underlying the antipsychotic profile of CBD are currently unknown.

Methods: In this randomised, double-blind, placebo-controlled, parallel-group intervention study, we investigated the impact of 28-day adjunctive CBD or placebo treatment (600 mg daily) on brain function and metabolism of 31 recent-onset psychosis patients (<5 years after diagnosis). Before and after treatment, patients underwent a Magnetic Resonance Imaging (MRI) session, which provided the opportunity to examine the impact of CBD treatment on three robust pathophysiological features of psychosis patients: 1) resting state connectivity in functional brain networks, 2) prefrontal metabolite concentrations including glutamate and N-acetyl-aspartate (NAA; neuronal integrity marker) levels, and 3) brain activity during reward processing. Symptomatology was also determined.

Results: CBD treatment significantly changed functional connectivity in the default mode network (time x treatment interaction $p=0.037$), with increased connectivity in the CBD (from 0.59 ± 0.39 to 0.80 ± 0.32) and reduced connectivity in the placebo group (from 0.77 ± 0.37 to 0.62 ± 0.33). Although there were no significant treatment effects on prefrontal metabolite concentrations, we showed that decreased psychotic symptom severity over time was associated with both diminishing glutamate ($p=0.029$) and NAA levels ($p=0.019$) in the CBD, but not the placebo group. CBD treatment did not affect brain activity patterns during reward anticipation and receipt or functional connectivity in executive and salience networks.

Conclusions: Adjunctive CBD treatment of recent-onset psychosis patients induced changes in default mode functional connectivity, but not in prefrontal metabolite concentrations or brain activity during reward processing. Abnormal default mode connectivity likely contributes to psychosis vulnerability through its role in internal modes of cognition, such as examining one's own thoughts, emotions and perceptions. Our findings support the notion that CBD treatment attenuates impaired default mode connectivity of psychosis patients, which may be involved in the therapeutic effects of CBD.

SHORT-TERM ANXIOLYTIC AND HARM-REDUCING EFFECTS OF CANNABIDIOL IN CANNABIS IN FLOWER AND EDIBLE FORMS

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Introduction: Cannabis is increasingly used to self-treat anxiety; however, data are mixed on short-term anxiolytic effects. Additionally, the primary cannabinoids Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have varying pharmacological actions that may render different short-term effects on anxiety and other cannabis related-effects.

Methods: These are the first data on the primary hypotheses from a fully powered NIDA-funded R01(DA044131; PI Bidwell; ClinicalTrials.gov Identifier: NCT03491384) that uses experimental methodologies to examine the impact of different cannabinoids in individuals with anxiety. Using an at-home administration procedure compliant with federal law, the present study examined the acute effects of three cannabis chemovars with different THC to CBD ratios to test whether chemovars with a higher CBD content produce different effects in anxious cannabis users. Participants who reported at least mild to moderate anxiety (Score of ≥ 5 on the Generalized Anxiety Disorder-7 (GAD-7) scale) and current use of (or intent to use) cannabis to cope with anxiety were recruited for the study [N=201 adults (Male = 91, Female = 110); Mean cannabis use days past 14 days = 6.36 (SD = 5.24)]. Participants were randomly assigned to *ad libitum* administration of one of three chemovars of cannabis flower or edibles: Flower users were randomized to purchase either Strain A (THC dominant: 24% THC; <1% CBD), Strain B (THC+CBD: 14% THC; 9% CBD), or Strain C (CBD dominant: 24% CBD; <1% THC). Edible users were randomized to purchase either Edible A (THC dominant: 10 mg THC; 0 mg CBD), Edible B (THC+CBD: 10 mg THC; 10 mg CBD), or Edible C (CBD dominant: 10 mg CBD; .17 mg THC). We note that the amount of THC and CBD varied across the flower and edible products and thus were not directly compared, but broadly used to assess the impact of cannabinoid profiles commonly found on the legal market. Participants were ssessed in a mobile pharmacology lab before (pre-use) and immediately after (post-use) *ad libitum* administration of their assigned chemovar. A total of 491 participants were analyzed (flower=308, edible=136, non-user=43); 149 participants in the THC-dominant user group, 159 participants in the CBD-dominant user group; 136 participants in the THC + CBD group, and 43 participants in the non-user (control) group. Using a mixed model ANOVA design, with four groups, two assessment points (pre- and post-use, and an average ICC = .5 between assessment points, a total n=240 (60 per group) allowed us to detect a groupXtime interaction effect as small as f=.12.

Results. Plasma cannabinoids as well as subjective mood and intoxication effects were assessed at each time point. Participants who used the CBD-dominant and THC+CBD chemovars had less THC and more CBD in plasma after cannabis use compared to participants who used the THC-dominant chemovar. The CBD dominant chemovar was associated with acute reductions in anxiety and tension as compared to the THC dominant and the THC+CBD chemovars. In addition, the use of all three strains was associated with intoxication and positive subjective effects. Results pointed to graduated drug reward effects across strains, with the highest levels of intoxication and positive mood being present in the THC dominant chemovar, the next highest in the THC+CBD chemovar, and the lowest levels present in the CBD dominant chemovar. Similarly, adverse effects, including paranoia, demonstrated a graduated effect across strain groups, with the highest levels in the THC dominant chemovar, the THC+CBD chemovar falling in the middle, and the CBD dominant showing significantly lower levels than the other two strain groups. Non-users did not show significant change in either objective or subjective mood and intoxication effects between the study time points.

Conclusions. In one of the first studies to examine the differential effects of *ad libitum* use of cannabinoids on measures relevant to individuals with anxiety who use cannabis, the CBD dominant chemovar was associated with short-term anxiolytic effects as compared to a THC+CBD and a THC dominant chemovar. Participants using the CBD dominant and THC+CBD chemovars also reported lower THC plasma levels and yet still some level of intoxication and positive drug effects, which is intriguing from a harm reduction perspective. Our study contributes novel data on the peak intoxication effects of various THC to CBD ratios using chemovars that are widely available in state-regulated markets, with an emphasis on the effects of CBD. Further research is needed to clarify the anxiolytic and harm reduction potential of CBD in cannabis products.

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CHRONIC AND ACUTE EFFECTS OF CANNABIS USE ON DAILY CORTISOL RHYTHMS IN A NATURALISTIC ENVIRONMENT

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Introduction: Stress relief is the most cited reason for habitual cannabis use. Our published research has revealed blunted cortisol reactivity to acute stress in human chronic cannabis users as well as blunted corticosterone reactivity in female rodents trained to self-administer vaporized cannabis. Cannabis users may also have a disrupted cortisol awakening response, as two studies conducted in adolescents and patients experiencing psychosis indicate a flattened diurnal cortisol slope (DCS) in cannabis users. However, no published studies have examined whether healthy adult cannabis users also demonstrate flattened DCS. Thus, the primary purpose of this study was to examine whether healthy adult cannabis users demonstrate flattened DCS relative to non-users. As dysregulations in cortisol are also associated with depression, we further sought to examine whether dysregulations in DCS predict the heightened levels of depression typically reported by chronic cannabis users. Finally, we explored acute effects of cannabis use on subjective stress ratings and changes in cortisol concentrations in a naturalistic environment.

Methods: A target sample of n = 80 participants (40 cannabis users and 40 non-users) is currently being recruited to address these objectives. Participants are asked to collect multiple saliva samples and rate their subjective stress levels repeatedly throughout the day, including immediately upon awakening, 30 min after waking, and immediately before bed, in their natural environments. Cannabis users are asked to collect additional saliva samples and provide subjective stress ratings immediately before and after using cannabis. Enzyme-linked immunosorbent assays are used to quantify salivary cortisol concentrations throughout the day as well as before and after cannabis use. The Beck Depression Inventory is also administered to assess recent levels of depression.

Results: Results from 15 cannabis users and 25 non-users indicate that cannabis users have flatter DCS relative to non-users (t = -1.88, p = .034 [one-tailed], d = 0.18). As expected, cannabis users also reported higher average subjective stress ratings (t = 1.96, p = .028 [one-tailed], d = 1.19) and depression scores (t = 1.94, p = .030 [one-tailed], d = 0.39) compared to non-users. However, neither depression (r = -.09, p =.567) nor subjective stress (r = -.22, p =.177) were significantly correlated with DCS. Finally, we found that stress ratings (t = 4.68, p < .001, d = 0.84) and cortisol concentrations (t = 2.69, p = .023, d = 0.05) were significantly decreased following cannabis use.

Conclusions: These results extend prior findings by demonstrating flattened DCS in healthy chronic cannabis users. However, these were unrelated to cannabis users' heightened levels of negative affect. Contrary to laboratory studies indicating increased cortisol concentrations following cannabis use, we found a significant decrease in cortisol concentrations following acute cannabis use. These findings corroborate cannabis users' self-reported experience of decreased stress following cannabis use and likely differ from laboratory findings due to the stress associated with using cannabis in a foreign laboratory, rather than a comfortable home environment.

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IMPLEMENTING AN EVIDENCE-BASED, HARM REDUCTION-FOCUSED, MEDICAL CANNABIS PROGRAM IN AN ACADEMIC MEDICAL CENTER IN BRONX, NY

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Background: Demand for medical cannabis is increasing rapidly. Academic medical centers are well-positioned to deliver evidence-based patient care and clinician training in this area, yet few have developed coordinated medical cannabis programs. Working closely with hospital leaders, we established the Montefiore Medical Cannabis Program (MMCP) to provide access to medical cannabis certification for patients in a rigorous and safe manner, and to serve a population disproportionately impacted by the criminal justice enforcement of cannabis.

Methods: Montefiore Medical Center is a large academic medical center located in the Bronx, a borough of New York City with high rates of poverty, opioid overdose deaths, and chronic diseases including diabetes, cardiovascular disease and HIV. In 2016, our team of internal medicine physicians began developing a program to provide evidence-based, non-judgmental medical cannabis care in primary care settings familiar to patients. After meeting with key institutional stakeholders to discuss the scope of the program and obtain their support, we launched the MMCP in 3 primary care medicine practices. We developed a set of best practices for clinical visits to ensure that patients were rigorously evaluated and safely certified and that were informed by harm reduction principles, including: eliciting motivation for medical cannabis use, determining relative risks and benefits of medical cannabis, providing specific recommendations on cannabis formulation and dosing, and counseling patients on potential adverse effects and how to use cannabis within the scope of state and federal regulations. Regular medical provider meetings were held to review patient cases, discuss new or complex issues that arose during certifications, and create standardized tools to guide medical cannabis evaluations and care.

Results: Over 6 years, the MMCP has expanded from 5 internal medicine providers in 3 primary care practices to 13 providers in 6 clinics. As of March 2023, 1805 unique patients have been certified, most for chronic pain. Among patients with available demographic and clinical data, 1142 (63%) are women, 679 (38%) Black and 852 (47%) Hispanic, median age is 57 years, 765 (42%) are enrolled in Medicaid, 286 (16%) have opioid use disorder, and 261 (14%) are living with HIV and 81 (4%) with sickle cell disease. Since the MMCP's founding, we have added a robust extramurally funded research and evaluation program. Despite successful scale-up of the MMCP, we have encountered barriers impacting patients' ability to obtain medical cannabis, including long wait times for certification appointments because of high demand, limited resources to help patients navigate the on-line patient self-registration process, and high out-of-pocket (unreimbursed) costs to purchase medical cannabis.

Conclusions: Implementing an academic medical center-based medical cannabis program has been a complex and iterative effort. Nonetheless, our experience indicates that such programs can play a leading role in delivering rigorous, evidence-based, and equitable treatment for patients seeking medical cannabis, and in expanding the cannabis evidence base. Institutional, local and national policies should recognize that patients need accurate information about cannabis, should provide guidance for practitioners to advise patients about safe medical cannabis use, and should advocate for fair access to well-regulated medical cannabis products.

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NEURAL AND BEHAVIORAL CORRELATES OF EDIBLE CANNABIS-INDUCED POISONING: CHARACTERIZING A NOVEL PRECLINICAL MODEL

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Introduction: Accidental exposure to Δ^9-tetrahydrocannabinol (THC) cannabis edibles, leading to cannabis poisoning, is common in children and pets; however, the neural and behavioral correlates of cannabis poisoning are unknown. Therefore, we examined the effects of acute edible cannabis-induced poisoning on behavior in juvenile and adult male and female rats and on neural activity adult male and female rats.

Methods: Adult Sprague-Dawley rats (6 males, 7 females) were implanted with electrodes in the prefrontal cortex (PFC), dorsal hippocampus (dHipp), cingulate cortex (Cg), and nucleus accumbens (NAc). Cannabis poisoning was then induced by exposing rats to a mixture of Nutella (6 g/kg) and THC-containing cannabis oil (20 mg/kg). Subsequently, cannabis tetrad and neural oscillations were examined 2, 4, 8, and 24 h after THC exposure. In another cohort of adult rats (16 males, 15 females), we examined the effects of cannabis poisoning on learning (active avoidance) and prepulse inhibition (acoustic startle reflex), and the plasma and brain THC and 11-hydroxy-THC concentrations. In a third cohort of juvenile (16 males, 16 females) rats, cannabis poisoning was induced and the cannabis tetrad as well as prepulse inhibition were examined.

Results: Cannabis poisoning caused gamma power suppression (decreased neural activity) in all regions in a time-dependent manner. Moreover, in the Cg, dHipp, and NAc, there were sex differences in the extent of gamma power suppression. Cannabis poisoning also resulted in hypolocomotion, hypothermia, and analgesia in a time-dependent manner in juvenile and adult rats of both sexes. Compared to control adult rats, THC adult rats were unable to learn to avoid foot shock in an active avoidance task. Additionally, both juvenile and adult THC rats had reduced prepulse inhibition compared to controls. There were also sex differences in brain and plasma THC and 11-OH-THC levels following cannabis poisoning.

Conclusions: Our results suggest that cannabis poisoning causes impairments in learning and information processing, possibly due to decreased gamma power in the dHipp and PFC. Additionally, most of the changes in neural activity and behavior appear 2 hours after ingestion, suggesting that interventions at or before this time might be effective in reversing the effects of cannabis poisoning.

PRENATAL Δ⁹-THC-INDUCED FETAL GROWTH DEFICITS AND POSTNATAL CARDIAC DYSFUNCTION ARE AMELIORATED BY MATERNAL OMEGA-3 FATTY ACID SUPPLEMENTATION IN RATS

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Introduction: Reports suggest that ~3-20% of pregnant women in North America consume cannabis in pregnancy. Clinical studies suggest that cannabis use in pregnancy leads to fetal growth restriction, and fetal growth deficits are associated with an increased risk of developing cardiovascular disease in postnatal life. We have previously demonstrated that maternal exposure to 3 mg/kg/day of Δ9-tetrahydrocannabinol (THC) from gestational day 6 to term results in fetal growth restriction along with impaired cardiac function and cardiac remodeling (e.g., increase in collagen type 1 and 3) in 3-week-old rat offspring. Given omega-3 fatty acids (*i.e.*, DHA and EPA) have been shown to play an important role for both development and cardiovascular health, we hypothesize that supplementation with an omega-3 enriched maternal diet will ameliorate the cardiac deficits in offspring exposed to THC *in utero*.

Methods: Pregnant Wistar rats were administered vehicle saline (VEH) or 3 mg/kg THC i.p daily from gestational day (GD) 6 to term. For each treatment, dams were either fed with control diet or omega-3 enriched diets from GD5 to postnatal day (PD) 21. LC-MS was used to quantify cardiac fatty acid content at PD21. Offspring were followed up with echocardiography to assess cardiac function at PD21. All data were analyzed using one-way ANOVA with post-hoc Tukey's test.

Results: Throughout gestation, there were no significant changes in maternal outcomes (i.e., maternal weight gain, food intake and gestation length) and litter size between all groups. We confirmed that THC results in significant ($p<0.05$) decreases in birthweight and cardiac function (*e.g.,* cardiac output, stroke volume, ejection fraction) in PD21 offspring. This was also associated with a significant 70% decrease in cardiac DHA. However, in offspring where dams were fed with the omega-3 enriched diet, THC-exposed offspring did not exhibit fetal growth restriction. Moreover, at PD21, maternal supplementation with the omega-3 diet ameliorated THC-induced deficits in cardiac function and recovered DHA content in hearts relative to the control group.

Conclusions: These data suggest that maternal omega-3 supplementation may replenish cardiac DHA and reduce the fetal and postnatal cardiac deficits associated with prenatal THC exposure. Considering that omega-3 fatty acids are important for development and have potential cardioprotective effects, we speculate it improves both placental efficiency and reduces the incidence of postnatal cardiac remodeling. Future studies are warranted to determine if shorter windows of intervention (i.e., lactation only) rescues cardiac deficits in offspring.

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MATERNAL CBD EXPOSURE IN MICE ALTERS SOCIAL BEHAVIOR AND BEHAVIORAL FLEXIBILITY IN ADULT OFFSPRING

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Introduction: Cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) are the major cannabinoids in the cannabis plant. CBD is currently FDA approved for the treatment of severe childhood epilepsies. In addition, CBD is added to food and drink and sold over the counter; sales in 2022 reached $1.8 billion. CBD is considered safe by the general public and is widely used, including during pregnancy. Use of CBD products during pregnancy is concerning given that CBD has been detected in breast milk and umbilical cords of cannabis using mothers, indicating that the developing child is exposed to CBD. Few preclinical studies suggest prenatal CBD exposure in mice may alter hippocampal neurogenesis.

Methods: Mouse dams were provided with CBD-containing (1 mg/kg/day or 3 mg/kg/day) or vehicle cereal at the beginning of pregnancy and continuing until litter was born. More than 90% of the cereal was consumed within 1 hour throughout the treatment period. Morris water maze behavioral assay was used to evaluate spatial learning (Day 1-4), spatial memory (Day 5), and cognitive flexibility (Day 6). Data were analyzed using an unpaired t-test ($p<0.05$ considered significant). Three-chamber social preference behavioral assay was used to assess preference for social stimulus (unfamiliar mouse) over a non-social stimulus (novel object). Interaction time data was analyzed using two-way ANOVA followed by post hoc Bonferroni tests for multiple comparisons within and between groups. Social preference index data was analyzed using one-way ANOVA.

Results: In the Morris water maze, CBD-exposed (3 mg/kg/day), adult male and female mice significantly took less time to reach platform location and significantly spent more time searching for platform location in correct quadrant on Day 5 when compared to vehicle-exposed offspring, evidence of enhanced spatial memory. CBD-exposed (3 mg/kg/day), adult male and female offspring significantly took more time to learn new platform location on Day 6 when compared to vehicle-exposed offspring, evidence of impaired cognitive flexibility. CBD-exposed (1 mg/kg/day), adult female mice had a significant decrease in the social preference index when compared to vehicle-exposed offspring in the three-chamber social preference assay; CBD-exposed, adult female mice had no difference in interaction time between a social stimulus and a non-social stimulus. Both findings suggest prenatal CBD caused a significant social impairment.

Conclusion: We have identified a significant deficit in social behavior on the three-chamber social preference behavioral assay in adult females following *in utero* CBD exposure. Similar social deficits are also found in several transgenic mouse models of autism. These findings suggest the hypothesis that prenatal CBD exposure can have detrimental effects on brain development and support further studies into the mechanisms involved.

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SYNERGISTIC EFFECTS OF PRENATAL CANNABIS AND CHILDHOOD TRAUMA EXPOSURE ON LARGE-SCALE FUNCTIONAL BRAIN NETWORKS IN CHILDREN: A DOUBLE HIT TO THE DEVELOPING ENDOCANNABINOID SYSTEM?

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Introduction: In recent years, there has been an increase in the use of cannabis during pregnancy, and the potency of cannabis has rapidly risen. These trends have raised concerns about the potential harm to brain development in individuals who were exposed to cannabis in utero. Prenatal cannabis exposure (PCE) can disrupt the endocannabinoid system, which plays a crucial role in shaping pre- and post-natal brain development, including the fine-tuning of neuronal networks. Additionally, childhood trauma exposure (CTE) is believed to further disturb endocannabinoid signaling, contributing to differences in brain structure and function in youth, as well as an increased risk of psychiatric disorders. Here, we examine the interactive effects of PCE and CTE on organization of large-scale functional brain networks in children. Our focus was on the salience network (SN), which plays a crucial role in supporting higher-order cognitive and emotion-related processing. We also investigated the connectivity of the SN with two other large-scale neurocognitive networks: the default mode network (DMN) and the frontoparietal network (FPN). These networks change dynamically across development and may be sensitive to early exposures (e.g., PCE, CTE).

Methods: Here, we report on developmental history and resting-state functional magnetic resonance imaging scans collected from 9,862 children ($M \pm SD = 10.93 \pm 0.64$ years, 48% female; 66% White) from the Adolescent Brain Cognitive Development (ABCD) study. Data were analyzed with linear mixed models with PCE, CTE, and PCE x CTE as predictors of within- and between-network connectivity of the SN (i.e., SN-SN, SN-FPN, SN-DMN).

Results: Thirty-five percent of youth in the sample reported CTE and 3.8% of caregivers reported using cannabis during pregnancy. There was a significant interaction between PCE and CTE in predicting SN-FPN connectivity in youth, such that PCE was associated with lower connectivity only within trauma-exposed youth. PCE exposure was not associated with SN-FPN connectivity in those without CTE. There were no interactive effects of PCE and CTE on SN-SN or SN-DMN connectivity.

Conclusions: The findings suggest that PCE may make children more vulnerable to the effects of CTE on the organization of large-scale neural networks that are linked to higher-order cognitive and emotion-related functioning. More detailed assessments of the potential sensitizing and/or synergistic effects of PCE and CTE on the endocannabinoid system are necessary for a comprehensive understanding of their impact on the developing brain. These findings provide insight into the connection between early exposures and later psychiatric risk.

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EVALUATING GENDER DIFFERENCES IN CANNABIS USE PATTERNS AND MOTIVES AND SEVERITY OF CANNABIS-RELATED HARMS AMONG ADULTS ACCESSING ADDICTION TREATMENT SERVICES

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Introduction: Cisgender men are more likely than cisgender women to use cannabis and meet criteria for cannabis use disorder (CUD), yet women may escalate their use of cannabis faster than men and experience more frequent or severe mood symptoms (e.g., suicidality and psychological distress). Furthermore, little is known about cannabis use and related harms among gender minorities (i.e., people whose gender identity and/or expression differs from their sex assigned at birth). Our goal was to examine relationships between gender identity and cannabis-related outcomes among patients accessing addiction treatment centers.

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=324 cisgender men, n=205 cisgender women, and n=16 identified as gender minority [GM]). All participants self-reported psychiatric and substance use history and completed a scale measuring patterns of medical and non-medical (recreational) cannabis use. The Marijuana Motives Measure (MMM) was used to capture motives for cannabis use (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).

Results: The majority of the sample self-reported an alcohol use disorder (55.6%), followed by CUD (25.3%), opioid use disorder (20.2%), and cocaine use disorder (19.8%). There were few gender differences in self-reported substance use disorder diagnosis, except that GM were more likely to self-report CUD than men or women. Women and GM were more likely than men to self-report diagnosis of generalized anxiety disorder, panic disorder, post-traumatic stress disorder (PTSD), and major depressive disorder. GM were more likely than both men and women to self-report diagnosis of any anxiety disorder and social anxiety disorder. Women were less likely than men to receive a recommendation or authorization to use cannabis for medical purposes. GM were more likely than men to be using cannabis to treat PTSD symptoms, while women were more likely than men to be using cannabis to treat pain. There were virtually no gender differences in cannabis product use (including THC/CBD content), source of products, or sources of product information, for either medical or non-medical purposes. There were no significant gender differences in motives for cannabis use (MMM) or presence/severity of cannabis-related harms (CUDIT-R and MPS).

Conclusions: Despite some gender differences in self-reported psychiatric diagnoses (women and GM were more likely than men to report anxiety disorders, PTSD, and depression), there were strikingly few gender differences in cannabis use for medical or non-medical purposes, motives for using cannabis, or severity of cannabis-related harms. In patients accessing addiction treatment services, gender may have less of an influence on cannabis use, motives, and harms than in the general population. Future work should consider how other factors such as race, poverty, and trauma intersect with gender to impact cannabis use and harms.

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EXAMINING THE LONGITUDINAL IMPACTS OF CANNABIS LEGALIZATION IN CANADA ON A SAMPLE OF HIGH-RISK EMERGING ADULTS

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Introduction: In 2018, recreational cannabis use was legalized in Canada. A key concern prior to and throughout legalization was that the increase in access to cannabis could, in-turn, lead to increased use in the population. Emerging adults tend to show the greatest consumption of recreational cannabis, making them a key group for monitoring the impacts of legalization. However, nearly five years after legalization, there remains a gap in the literature of robust longitudinal examinations of how legalization may have changed patterns of cannabis consumption in emerging adults. The purpose of this study was to examine the impacts of recreational cannabis legalization in emerging adults, including their frequency of cannabis use as well as their experiences of cannabis-related consequences.

Methods: Using data from the longitudinal cohort study BETA-H, a sample of young adults aged 19.5-23 in Hamilton, Ontario who reported regular substance use at enrollment were followed every four months for three years (n=619). Between February 2017 and February 2020, 7 time points represented pre- and post-legalization periods, with the first post-legalization time point (October 2018-February 2019) considered a transition period. Linear mixed models were used to examine how cannabis use frequency and experiences of cannabis-related consequences changed over time. Interactions terms were used to test if those with higher cannabis use frequencies pre-legalization experienced different changes over time compared to those who used cannabis less frequently or not at all.

Results: Models demonstrated that there were significant decreases in cannabis use frequency ($F=2.25$, $p<0.001$), as well as a reduction in experiences of cannabis-related consequences ($F=9.95$, $p<0.001$) in this sample over time. Post-hoc testing indicated individuals who used cannabis more frequently pre-legalization significantly decreased their use and saw a decrease in experiences of cannabis-related consequences over time. Those who did not use cannabis pre-legalization experienced a small increase in use over time, but showed no significant changes in their experiences of cannabis-related consequences.

Conclusions: Findings of this robust longitudinal study were consistent with expected patterns of use in emerging adults, who tend to decrease their substance use over time, sometimes referred to as "aging out." Cannabis legalization did not appear to impact frequency of cannabis use nor experiences of cannabis-related consequences in this sample. Instead, aging out patterns of cannabis use occurred despite legalization, indicating that this federal policy change did not substantially impact the emerging adults in this sample.

POSITIVE ALLOSTERIC MODULATION OF THE TYPE 1 CANNABINOID RECEPTOR POTENTIATES ENDOCANNABINOID SIGNALLING AND CHANGES ERK1/2 PHOSPHORYLATION KINETICS

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Introduction: Orthosteric activation of CB1 by exogenous agonists is known to cause a plethora of adverse side effects in vivo. Positive allosteric modulation is an interesting therapeutic approach that is hoped to offer improved therapeutic potential (antinociception/reducing intraocular pressure, among other indications) and a reduced on-target side effect profile compared to orthosteric agonists. Endocannabinoids 2-AG and AEA orthosterically activate CB1 under physiological conditions, though it is not clear how positive allosteric modulators would affect this paradigm.

Methods: Bioluminescence resonance energy transfer assays in HEK293 cells were preformed to investigate G protein dissociation, ERK1/2 phosphorylation and β-arrestin 2 translocation paradigms, while immunocytochemistry was performed to measure internalisation of CB1 in response to the PAMs ZCZ011, GAT229, and ABD1236 alone and in combination with the orthosteric agonists AEA, 2-AG, and AMB-FUBINACA.

Results: ZCZ011, GAT229, and ABD1236 were found to be allosteric agonists in all pathways tested. Interestingly, the PAM ZCZ011 (alone) induced a different ERK1/2 phosphorylation time course compared to that of orthosteric agonists, entailing a biphasic response which contrasts to a more classical single, transient activation. In combination with 2-AG but not AEA or AMB-FUBINACA, ZCZ011 and ABD1236 caused the transient peak of ERK1/2 phosphorylation to become a sustained response. In combination with AEA, all PAMs increased the potency and efficacy of AEA-induced G protein dissociation, ERK1/2 phosphorylation, β-arrestin 2 translocation, and internalisation. However, in combination with full agonists 2-AG and AMB-FUBINACA, the PAMs were found to increase efficacy of agonist-induced G protein dissociation, cause no change in ERK1/2 phosphorylation, increase the potency of β-arrestin 2 translocation, and internalisation of CB1.

Conclusions: PAMs can potentiate endocannabinoid CB1 agonism by AEA to a larger extent than in combination with higher efficacy orthosteric agonists. PAM effects may therefore retain physiologically regulated spatiotemporal signalling of endocannabinoids in vivo. Compared to the global receptor activation following exogenous orthosteric agonist administration, this may underpin the more favourable adverse effect profile of PAMs.

MONOACYLGLYCEROL LIPASE INHIBITOR ALLEVIATES PAIN TRANSIENTLY VIA PERIPHERAL MECHANISM AND PAIN-INDUCED DEPRESSION LONG-TERM VIA CENTRAL MECHANISM

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Introduction: Many patients with chronic pain suffer from depression. The endocannabinoid (eCB) system is involved in pain and depression after pain. The endocannabinoid 2-arachidonoylglycerol (2-AG) is metabolized by monoacylglycerol lipase (MAGL), and inhibitors of MAGL could increase the concentration of 2-AG. The effect of MAGL inhibitors on pain-induced depression was not explored.

Methods: In this study, we tested the effects of the MAGL blocker, MJN110, on pain-like and depression-like behaviors in rats with spared nerve injury (SNI), and in vivo dorsal root single-unit recordings were employed to evaluate the effects of MJN110 on the excitability of dorsal root ganglion (DRG) neurons.

Results: Higher doses of MJN110 (1 and 5mg/kg, intraperitoneal injection) for 14 days could transiently alleviate pain-like behaviors, and long-term alleviate depression-like behaviors, while a low dose of MJN110 (0.3mg/kg) could alleviate depression-like behaviors while having no effects on pain-like behaviors. With electrophysiological recordings, we found that higher doses (1 and 5mg/kg), not 0.3mg/Kg, of MJN110 could block the spontaneous firing of DRG neurons after SNI by targeting cannabinoid type 1 receptors (CB1Rs), not CB2Rs. However, MJN110 (0.3mg/kg) could still modify the activity of the medial prefrontal cortex, which is a key brain area in pain and pain-induced depression.

Conclusions: These data show that a lower dose of MJN110 can alleviate pain-induced depression by targeting CB1Rs in the central nervous system, even though it is insufficient to alleviate pain-like behaviors and the hypersensitivity of DRG neurons. Our study also suggests that the sensory and affective components of pain can be separable dimensions and differentially modified.

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CANNABIDIOL AND MITRAGYNINE EXHIBIT DIFFERENTIAL INTERACTIVE EFFECTS IN THE ATTENUATION OF PACLITAXEL-INDUCED MECHANICAL ALLODYNIA, ACUTE ANTINOCICEPTION, AND SCHEDULE-CONTROLLED RESPONDING IN MICE

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Introduction: For many chemotherapy patients, peripheral neuropathy is a debilitating side effect. Mitragyna speciosa (kratom) contains the alkaloid mitragynine (MG), which produces analgesia in multiple preclinical pain models. In humans, anecdotal reports suggest cannabidiol (CBD) may enhance kratom-related analgesia. We examined the interactive activity of MG and CBD in a mouse chemotherapy-induced peripheral neuropathy (CIPN) model. We also examined MG+CBD in acute antinociception and schedule-controlled responding assays, as well as examined underlying receptor mechanisms.

Methods: Male and female C57BL/6 mice received a cycle of intraperitoneal (*ip*) paclitaxel injections (cumulative dose 32 mg/kg). The von Frey assay was utilized to assess CIPN allodynia. In paclitaxel-naïve mice, schedule-controlled responding for food was conducted under a fixed ratio (FR)-10, and hot plate antinociception was examined.

Results: MG dose-relatedly attenuated CIPN allodynia (ED_{50} 102.96 mg/kg, *ip*), reduced schedule-controlled responding (ED_{50} 46.04 mg/kg, *ip*), and produced antinociception (ED_{50} 68.83 mg/kg, *ip*). CBD attenuated allodynia (ED_{50} 85.14 mg/kg, *ip*) but did not decrease schedule-controlled responding or produce antinociception. Isobolographic analysis revealed 1:1, 3:1 MG+CBD mixture ratios additively attenuated CIPN allodynia. All combinations decreased schedule-controlled responding and produced antinociception. WAY-100635 pretreatment (0.01 mg/kg, *ip*) antagonized CBD anti-allodynia. Naltrexone pretreatment (0.032 mg/kg, *ip*) antagonized MG anti-allodynia and acute antinociception but produced no change in MG-induced decreased schedule-controlled behavior. Yohimbine pretreatment (3.2 mg/kg, *ip*) antagonized MG anti-allodynia and produced no change in MG-induced acute antinociception or decreased schedule-controlled behavior.

Conclusions: Although more optimization is needed, these data suggest CBD combined with MG may be useful as a novel CIPN therapeutic.

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NEGATIVE ALLOSTERIC MODULATION OF CB1 CANNABINOID RECEPTOR SIGNALING SUPPRESSES OPIOID SELF-ADMINISTRATION AND RELAPSE

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Introduction: The endocannabinoid system interacts with the reward system to modulate natural reinforcers, as well as drugs of abuse. Previous preclinical studies showed that direct blockade of CB1 receptors could be employed to treat substance use disorder, but this strategy failed during clinical trials due to severe psychiatric side effects, including anxiety and depression. Alternative strategies have emerged to circumvent the side effects of direct CB1 binding by developing allosteric modulators. We hypothesized that pharmacological inhibition of CB1 cannabinoid receptor signaling though negative allosteric modulation (NAM) would reduce the reinforcing properties of morphine and decrease opioid addictive behaviors.

Methods: By employing i.v. self-administration in mice, we studied the effects of the CB1 biased NAM GAT358 in morphine intake, relapse-like behavior, and motivation to work for morphine infusions.

Results: Our data revealed that GAT358 reduced morphine infusion intake during the maintenance phase of morphine self-administration under fixed ratio 1 schedule of reinforcement. GAT358 also decreased morphine seeking behavior after forced abstinence. Furthermore, GAT358 dose-dependently decreased the motivation to obtain morphine infusions in a progressive ratio schedule of reinforcement. Strikingly, GAT358 did not alter the motivation to obtain food rewards in an identical progressive ratio task.

Conclusion: Our results suggest that CB1 NAMs reduced the reinforcing properties of morphine and could represent a viable therapeutic route to decrease opioid addicted behaviors and relapse. Furthermore, the effect of CB1 NAMs on decreasing opioid self-administration is reward-specific not affecting the self-administration of natural rewards.

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BRAIN CB2 RECEPTOR: A NEW THERAPEUTIC TARGET FOR TREATING OPIOID USE DISORDERS, MAJOR FINDINGS FROM A NEW CB2-KO-EGFP REPORTER MOUSE LINE

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Introduction: Brain CB2 receptors (CB2R) are thought to be mainly expressed in microglia. However, direct evidence demonstrating CB2R expression in microglia is still limited, and growing evidence indicates neuronal CB2R expression in rats and mice. There are two CB2-reporter mouse lines (BAC-based CB2R-GFPTg and CB2-eGFP-flox-5xFAD) in which the CB2-GFP signal is detected in some microglia. However, concerns regarding whether GFP expression truly reveals endogenous CB2R expression in these two mouse lines complicate data interpretation.

Methods: Here, we report a new CB2-KO-eGFP reporter mouse line in which the eGFP gene sequence replaces the endogenous CB2R-coding region. In this new mouse line, we detected a high-density CB2-eGFP signal in midbrain dopamine (DA) neurons and cortical and subcortical glutamate neurons, whereas a very weak CB2-eGFP signal was detected in microglia. Given the critical role of DA neurons in drug reward and addiction, we explored the role of CB2R in opioid reward and addiction-like behaviors in rodents.

Results: We found that CB2-KO-eGFP mice showed higher basal levels of locomotion than their wildtype littermates; however, oxycodone-induced hyperlocomotion was unaffected. Systemic administration of MRI-2594, a novel CB2R agonist, produced dose-dependent analgesia (hot-plate test) in wildtype mice but not in CB2-KO-eGFP mice and inhibited intracranial self-stimulation (ICSS) maintained by optical stimulation of midbrain DA neurons in DAT-Cre mice. Pretreatment with MRI-2594 dose-dependently inhibited intravenous heroin self-administration in rats and wildtype mice but not in CB2-KO-eGFP mice, enhanced oxycodone-induced analgesia in wildtype mice, and inhibited heroin-triggered reinstatement of drug seeking in rats.

Conclusion: Together, these findings suggest that brain CB2Rs are expressed mainly in DA and glutamate neurons, and that the novel CB2R agonist MRI-2594 deserves further research as a new pharmacotherapy for opioid use disorders.

CANNABINOID CB1 AGONIST-INDUCED RESPIRATORY DEPRESSION IN AWAKE MICE

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Introduction: Illicit synthetic cannabinoids, commonly known as spice compounds, can induce acute respiratory depression requiring emergency medical care in humans. These compounds, which target the CB1 receptor and represent the largest class of novel psychoactive substances in the United States, are used recreationally to mimic the psychoactive effects of cannabis. These respiratory effects in humans call into question convention that CB1 agonists are not detrimental to respiration and highlight the need for effective therapeutic interventions. In the present study, we investigated the respiratory effects of the classical synthetic cannabinoids CP55,940 and WIN55,212-2, as well as the novel arrestin- and G protein-biased CB1 agonists AM11250 and AM12059, in awake mice. We also use RNAScope *in situ* hybridization to determine the distribution of cells expressing CB1 mRNA in brain regions that control respiration.

Methods: Whole-body plethysmography was used to assess the respiratory effects of CP55,940, WIN55,212-2, AM11250, and AM12059 on respiratory minute volume, tidal volume, and frequency. Cannabimimetic effects were evaluated via tetrad tests. All compounds were delivered via IP injection. Initial dose response was acquired via within-subjects dosing, with escalating half-log doses administered between 0.01 mg/kg and 3 mg/kg in 20-minute increments. The effects of specific doses of AM11250 and AM12059 were evaluated in separate groups of mice with a recording duration of two hours. RNAScope *in-situ* hybridization was used to determine the distribution of cells expressing CB1 mRNA; histology was evaluated by confocal microscopy.

Results: All CB1 agonists induced respiratory depression at strikingly low doses in wild type mice, affecting both respiratory frequency and average tidal volume. Respiratory effects of all agonists were fully blocked by coadministration of the CB1 antagonist AM251 and was entirely absent in CB1 knockout mice. Respiratory depression was unaffected by coadministration of the CB2 antagonist AM630, or the peripherally restricted CB1 antagonist AM6545. *In situ* hybridization revealed expression of CB1 mRNA in the rostral ventrolateral medulla, including in the ventral respiratory column and pre-Botzinger Complex.

Conclusions: Our data supports the hypothesis that CB1 agonists can suppress respiratory activity in rodents. Pharmacological and histological results are consistent with a site of action in the central nervous system.

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SUPRATHERAPEUTIC DOSE COMBINATIONS OF OPIOIDS AND CANNABIS: ABUSE POTENTIAL, PHYSIOLOGIC EFFECTS AND SAFETY PROFILE IN HUMANS

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Introduction: Opioid misuse is a global epidemic, with an estimated 33 million people worldwide misusing opioids. Medical and recreational use of cannabis is also rapidly increasing. However, there are no controlled data available on the safety and abuse potential of supratherapeutic opioid-cannabinoid drug combinations (i.e., doses/routes that occur with misuse); data is also lacking on the effects of cannabis in those with opioid use disorder.

Methods: Participants with mild to moderate opioid use disorder (but without physical dependence on opioids) and a history of cannabis use were enrolled into this within-subject, randomized, double-blind, placebo-controlled, inpatient study (n=8). During each session, an inhaled vaporized cannabis dose (0, 10, 30 mg THC) was administered 15 min prior to an intranasal oxycodone dose (0, 15, 30 mg). Participants received all dose combinations across 9 experimental sessions. Data were collected prior to (baseline) and in regular intervals for 6 hours after dose administration. Primary outcomes include safety/physiologic outcomes (e.g., oxygen saturation, end tidal carbon dioxide concentration [$EtCO_2$]), respiration rate) and subjective measures of abuse potential (e.g., drug liking, feeling high, take drug again).

Previous human laboratory work suggests large effect sizes can be expected (given controlled dosing, inpatient research setting, supratherapeutic dose range; d=1.55 – 4.73 across outcomes); power analyses suggest n<9 with a power=0.8 (alpha=0.05). We have completed n=8 participants, suggesting a fully statistically powered data set, although additional participants (n=2) are currently being enrolled.

Results: When administered separately, oxycodone and cannabis produced prototypical, dose-related effects; for example, dose-related increases in abuse-related subjective effects (e.g., drug liking, high) relative to placebo (p<.05). When active doses were administered in combination, 1) peak subjective ratings increased in magnitude and 2) the duration of effects was longer, relative to either drug alone (i.e., overall greater AUC with drug combination). Cannabis alone did not alter breathing outcomes; however, the high dose of cannabis (30 mg) potentiated oxycodone-induced (30 mg) respiratory depression ($EtCO_2$; p<.05).

Conclusions: At supratherapeutic doses, cannabis appears to potentiate abuse-related subjective effects of oxycodone. Cannabis also increased the physiological risk of opioids (i.e., worsened breathing) when high dose combinations (30 mg THC, 30 mg oxycodone) were administered. Overall, these data suggest that caution should be used when combining these agents, particularly in those with a history of misusing opioids, as cannabis may 1) increase the abuse liability of opioids, 2) further depress opioid-related decreases in breathing and 3) change the overall risk profile of high doses of opioids.

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PAIN SENSITIVITY AS A FUNCTION OF CANNABIS USE FREQUENCY

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Introduction: Pain is one of the most common indications for medical cannabis use. Acute cannabis and cannabinoid administration reduces pain response in healthy volunteers and in some patient populations. However, the extent to which cannabis use frequency influences sensitivity to pain in the absence of acute administration, and how this may differ between males and females, has not been systematically examined. In the present analysis, we investigated the association between frequency of cannabis use and behavioral and subjective measures of pain in a sample of healthy participants, using a Cold Pressor Test (CPT), and compared responses between males and females.

Methods: After biochemical confirmation of cannabis abstinence for at least twelve hours, 52 healthy adults who use cannabis (19F, 33M; 29.1 ± 7.1 years) completed a CPT, an experimental pain test that has predictive validity for therapeutics used to treat chronic pain. During the CPT, participants immersed their hand in cold water (4°C) and time to report pain (pain threshold) and time of hand withdrawal (pain tolerance) were recorded. Subjective pain ratings of the cold-water stimulus were measured using the Short-Form McGill Pain Questionnaire (SF-MPQ) and the 'Painfulness' and 'Bothersomeness' scale. Outcome measures were compared between individuals who reported using cannabis frequently (≥ 5 days /week; $N_{frequent}$ = 29; 10F, 19M) and individuals who reported using cannabis occasionally (≤ 3 days /week; $N_{occasional}$ = 23; 9F, 14M). Data were analyzed using regression modeling (α = 0.05).

Results: On average, individuals who used cannabis frequently reported using 6.1 ± 1.3 days/week, and individuals who used cannabis occasionally reported using 2.4 ± 1.4 days/week. Individuals who used cannabis frequently exhibited lower pain tolerance than individuals who used cannabis occasionally ($\beta_{frequency}$ = -16.4, $p < 0$ .005). Pain threshold and subjective measures of pain did not vary as a function of cannabis use frequency. Male cannabis users exhibited higher pain threshold (β_{sex} = 3.2, $p < 0.05$) and tolerance (β_{sex} = 20.0, $p < 0$ .005), than female cannabis users. There were no sex differences in subjective measures of pain, and there was no significant interaction between frequency of cannabis use and sex on any outcome measures.

Conclusions: Pain tolerance in healthy abstinent individuals who use cannabis varied as a function of frequency of cannabis use. Individuals who reported using frequently exhibited lower pain tolerance when compared to individuals who reported using occasionally. Further, male cannabis users exhibited higher pain threshold and tolerance when compared to female cannabis users. These findings suggest cannabis use frequency and sex may impact sensitivity to painful stimuli, a factor that should be considered when considering the clinical use, and study of, cannabinoid as potential analgesic therapies.

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SEX- AND DOSE-DEPENDENT ANALGESIC AND REINFORCING EFFECTS OF SMOKED CANNABIS: A PREVIEW OF DATA FROM THE CANSEX STUDY

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Introduction: Cannabis use is increasing among women. We have previously reported on heightened sensitivity to the subjective reinforcing effects of cannabis with delta-9-tetrahydrocannabinol (THC) in women relative to men, consistent with observations from preclinical research. Sex hormones—particularly estradiol—are proposed as a potential driver of these differences. We sought to build on our prior work on sex differences by testing two active cannabis strengths (doses) against a placebo and controlling for female hormone levels.

Methods: Data from healthy male (n = 31) and female (n = 14) volunteers who use cannabis (males: 4.2 days/week; females: 4.9 days/week) were extracted from an ongoing placebo-controlled, within-subject study. Participants smoked 560 mg of 0% (0 mg), 4% (~20 mg), and 10% (~60 mg) THC cannabis in randomized order across three outpatient study sessions. Female participants were regularly cycling, not on hormonal contraceptives, and completed study sessions during their mid-follicular phase (corresponding with rising estradiol and low progesterone). Subjective mood- and drug-related effects were assessed with the Mood and Physical Symptoms Visual Analog Scale (MPS-VAS) and the Smoked Cannabis Rating Form (SC-RF), respectively. Pain threshold and tolerance were measured in response to the Cold Pressor Test (CPT), an experimental pain test with predictive validity for therapeutics used to treat chronic pain. We also measured cannabis' reinforcing effects through a self-administration task. Generalized linear mixed effects models were used to test dose-, sex-, and time-dependent effects.

Results: Significant main effects of cannabis dose were noted for ratings of intoxication (increases), stimulation (increases) and cannabis craving (decreases; $p<0.001$). Sex-dependent effects were noted for stimulation and craving (M>F at high dose). Low dose significantly reduced ratings of anxiety in females and both doses increased ratings in males ($p<0.05$), producing a significant sex difference (M>F) at low dose ($p<0.05$). Significant main effects of cannabis dose were found for all SC-RF drug ratings (strength, liking, desire to take again, good effect, bad effect). Under the low dose, males exhibited significantly greater ratings of bad drug effect at peak intoxication ($p<0.05$). All other drug ratings were similar across sexes. Proportionally more females self-administered the low cannabis dose (31% vs. 23%), but there was not a significant sex-dependent dose effect. There was a significant main effect of cannabis dose on pain tolerance and threshold (increases; $p<0.05$). While a higher pain threshold under both cannabis doses was visually apparent for males relative to females, the sex difference did not reach statistical significance ($p>0.10$). Contrastingly, at the high dose, females tended towards greater pain tolerance relative to males ($p<0.10$).

Conclusions: Early data from this ongoing outpatient study that controls for circulating levels of estradiol in female participants suggests that they may be less prone to anxiogenic and bad drug effects of a lower dose of cannabis, whereas males were more responsive to the stimulating effects of a higher dose of cannabis. However, no sex differences were apparent in self-administration of cannabis nor in subjective ratings of drug likability, good drug effects, or desire to take the drug again. A signal of complex sex-dependent effects was found for analgesic endpoints, but our understanding is limited until more data is generated. This data, representing <40% of planned study observations, provides an early signal into possible sex differences in the acute therapeutic and adverse effects of cannabis, setting the stage for future analyses incorporating pharmacokinetics, hormonal, and tolerance effects.

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WHY IS PERIPHERAL CB1R ANTAGONISM A RATIONAL THERAPEUTIC STRATEGY FOR PULMONARY FIBROSIS? EVIDENCE FROM MULTI-OMICS APPROACH FOR ESTABLISHING A TRANSLATIONAL LINK

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Introduction: Pulmonary fibrosis (PF) is a progressive disease with a poor prognosis and a lack of effective treatments. Therefore, identifying effective therapeutic modalities is required to improve outcomes. However, the lack of predictive progressive biomarkers in the clinic causes a significant gap in the preclinical to clinical translational process. This study presents a multiomics framework for bridging translational gaps between the common animal PF model and human IPF to facilitate target identification and prioritization for IPF drug discovery. This could facilitate translational strategies from the bench to the clinic for PF treatment.

Methods: We generated transcriptomics and metabolomics data from lung tissue and pulmonary function tests (PFT) in bleomycin-induced PF at 7, 14, 21, and 28 days after a single dose of oropharyngeal bleomycin. We developed and employed systems biology analyses to elucidate the key temporal molecular and physiological alterations, including differential expression, co-expression network (CN), and transcriptional regulatory network (TRN) reconstruction.

Results: Using CN analysis, we identified two gene subnetworks (G-1 and G-2), out of five subnetworks, having a central role in multiple changes in the transcriptome, metabolome, and pulmonary function. The two identified gene subnetworks and their relevant biological pathways in mice also exist in IPF patients' lungs, which helped to establish clinical relevance. Furthermore, we performed a TRN analysis that revealed two (out of 13) gene expression progression tracks (T-1 and T-2) as major regulators of G-1 and G-2 in the CN. Moreover, genes in T-1 and T-2 are associated with fibrosis-related pathways, including extracellular matrix receptor interactions and PI3K-Akt signaling pathways, and pathways associated with inflammatory responses. Thus, we hypothesized that an ideal therapeutic candidate for PF should be able to attenuate fibrotic changes in the G-1 and G-2 subnetworks of GCN. We selected cannabinoid receptor 1 (CB_1R) as it was part of G-2 and known to be associated with IRF5 and IRF7, two common regulators of T-1 and T-2. We demonstrated that CB_1R overactivation could contribute to the PF pathology by affecting three subnetworks (10722 genes) out of five in GCN, including central clusters G-1 and G-2, which are the major drivers of the transcriptomics and metabolomics changes in PF.

Conclusions: Our study endorsed the therapeutic potential of peripheral CB1R antagonism for clinical translation and demonstrated the benefit of using the multi-omics-based framework to identify druggable targets for pharmacological intervention and developing rational combination therapies using the bleomycin-induced PF model. Mouse Lung Fibrosis Atlas can be accessed freely at https://niaaa.nih.gov/mouselungfibrosisatlas.

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INHALATIONAL DELIVERY OF MRI-1867 (ZEVAQUENABANT), A THIRD-GENERATION CANNABINOID RECEPTOR 1 (CB_1R) ANTAGONIST, EMERGED AS A NOVEL THERAPEUTIC MODALITY IN PULMONARY FIBROSIS

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a life-threatening disease. Among different pathogenic mechanisms, overactivity of the endocannabinoids/cannabinoid receptor 1 (CB_1R) system plays a central role in the development of IPF in humans, which makes CB_1R inhibition a potential therapeutic option (*Cinar, JCI Insight 2017 2(8):e92281, unpublished observations*). Accordingly, we have developed MRI-1867 (Zevaquenabant) as a peripherally restricted hybrid CB_1R/iNOS inhibitor. Systemic administration of MRI-1867 is effective in mitigating experimental pulmonary fibrosis (PF) in mice, and it has completed a Phase 1 clinical trial. We also found that alveolar macrophages (AMs) and epithelial cells expressing CB_1R in the local fibrotic environment are likely responsible for the progression of PF (*unpublished observations*). Since alveolar cells are accessible via the inhalational route, we hypothesized that pulmonary delivery of MRI-1867 may yield high exposure of critical target cells and thus could further guarantee CNS safety by limiting circulatory distribution to other tissues.

Methods: The bioavailability of MRI-1867 administered via the oropharyngeal (O.P.) or intraperitoneal (I.P.) route was measured by LC-MS/MS. PF was induced in mice by O.P. administration of bleomycin (1 U/kg b.w.). MRI-1867 treatment started 5 days later and mice were sacrificed on day 14. Therapeutic efficacy was evaluated *in vivo* by pulmonary function tests, and *ex vivo* by Masson-trichrome staining and hydroxyproline measurement. In addition, phenotypic and genotypic characterization of the lungs from different treatment groups was carried out to study the potential pathways modulated by MRI-1867.

Results: O.P. delivery of MRI-1867 at the dose of 0.5 mg/kg/day achieved the same therapeutic lung concentration of ~20 μM as achieved by an I.P. dose of 10 mg/kg/day. In the PF model, mice treated with MRI-1867 via both O.P. and I.P. dosage showed improvement in pulmonary functions, reduced collagen deposition, and preservation of alveolar space. Phenotypic characterization of lung macrophages revealed that the treatment with MRI-1867 significantly reduced monocyte-derived AMs, particularly M2 macrophages. MRI-1867 also reduced the levels of inflammatory and chemotactic cytokines and chemokines in the BALF of fibrotic mice. Transcriptomics analyses of the lungs revealed that MRI-1867 treatment attenuated the gene signatures involved in fibrosis initiation and modification pathways, fibroblast proliferation, as well as inflammatory pathways.

Conclusions: Pulmonary delivery of MRI-1867 is as effective as systemic delivery in mitigating PF, and it offers a more targeted therapeutic modality that could reinforce better CNS safety.

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A PROTECTIVE EFFECT OF THE CB2R AGONIST OSTEOGENIC GROWTH FACTOR (OGP) AGAINST BLEOMYCIN-INDUCED PULMONARY FIBROSIS IN MICE

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Introduction: Fibrosis is a progressive accumulation of extracellular matrix (ECM) components and may affect a wide spectrum of organs such as the lungs, accounts for an increase in mortality worldwide, and to date has few therapeutic options. Lung fibrosis is characterized by activation, proliferation, differentiation, and migration of fibroblasts, followed by deposition of ECM proteins that leads to a long-term build-up of scar tissue, severe alteration of lung architecture, and organ dysfunction. Few drugs have been approved for the treatment of lung fibrosis. The cannabinoid receptor 2 (CB2R) is widely expressed in immune and stromal cells. Recent studies support the notion that CB2R activation may attenuate the development of fibrosis. Recently, we identified an endogenous peptidic CB2 selective agonist with highly potent anti-inflammatory actions. This 14 amino-acid peptide was first named Osteogenic Growth Peptide (OGP). Here, we hypothesized that activation of CB2R by OGP prevents the onset of BLM-induced pulmonary fibrosis. Our general aim of this study was to investigate the therapeutic potential of OGP in Bleomycin-induced lung fibrosis in mice.

Methods: We calibrated the BLM-induced fibrosis model of male mice, 8 to 10-week-old, by testing different doses of BLM instilled Intratracheally in 50 µl of saline, and performed a preliminary dose-response of OGP to select one administration regimen. In the final experiment, one dose of BLM and one treatment mode of OGP was tested against a negative (saline) and a positive control (hydrocortisone, H.C.). Fibrosis analysis included survival, weight changes, live imaging of the lung using microcomputed tomography (µCT), histology (Masson's Trichome staining, Ashcroft pathology score, relative ECM area and collagen area), immunohistochemistry for fibrogenic molecules, FACS on lung homogenates and RTqPCR on RNA extracted from the lungs.

Results: In our last experiment, we therefore selected 4 study groups. (i) BLM (0.75 mg/kg), (ii) saline group (no BLM, no treatment), (iii) BLM + OGP 700ng/mouse on day 5, then 100ng/mouse/day, (iv) and BLM + Hydrocortisone (H.C) 10 mg/kg/day starting on day 5. Our data show that OGP improved the survival, weight loss and many fibrosis-related properties, i.e. OGP treatment reduced the alveolar wall ECM volume, lowered the Ashcroft histopathological score as well as the alveolar/air area fraction and collagen gene expression in the lung. Notably, the number of inflammatory cells in the lung were not affected by the OGP treatment that started on the 5th day (after the inflammatory phase).

Conclusion: These promising results demonstrate the high potential of CB2 activation by the selective agonist OGP to not only increase the survival rate of mice with severe pulmonary fibrosis, but also to decrease the fibrotic symptoms in lungs. Interestingly, this anti-fibrotic effect of OGP seems to be distinct and independent of its well-established anti-inflammatory actions. Because of the very low dose of OGP and its perfect homology between mice and humans, OGP may offer a safe and efficient treatment for fibrosis.

THE ANTI-TUMORIGENIC ROLE OF CANNABINOID RECEPTOR 2 IN SKIN CARCINOGENESIS

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Introduction: Over five million non-melanoma skin cancers (NMSC) are diagnosed globally each year. NMSC is the most frequent acquired cancer, and one of the most common malignant cancers. The endocannabinoid system (ECS) plays an active role in skin carcinogenesis wherein dysregulation of the ECS is implicated in cancer development, progression, and metastasis. Cannabinoid receptor 2 (CB2) in particular has been shown to have seemingly opposing effects in skin cancer development depending on the model, *i.e.* activation of the receptor leads to tumor cell death or promotes tumor growth.

Methods: Comparing wildtype (WT) to systemic CB2 knockout ($CB2^{-/-}$) mice, we performed a spontaneous cancer study in one-year old mice with a histopathological analysis of the skin. We subsequently used the multi-stage chemical carcinogenesis model, wherein cancer is initiated by topical application of 7,12-dimethylbenz[a]anthracene (DMBA) and promoted repeatedly by 12-O-tetradecanoylphorbol-13-acetate (TPA) for 27 weeks. Papilloma formation and regression was recorded weekly. Upon sacrifice, immune cells were quantified in skin, papillomas, and splenocytes via flow cytometry, and CB2 and IL-6 expression was determined with qPCR. Data were analyzed by Student's t-test or Mann-Whitney U test for continuous variables, Chi-Square test for categorical variables, and 2-way ANOVA for repeated measures.

Results: Using a histopathological analysis, we found that aging $CB2^{-/-}$ mice have an increased incidence of spontaneous cancerous and precancerous skin lesions compared to their wildtype (WT) counterparts. In the DMBA/TPA model, $CB2^{-/-}$ mice developed significantly more and larger papillomas overall. While the WT mice were more resistant to the chemical-induction model and experienced constant spontaneous tumor regression (remission), the $CB2^{-/-}$ mice exhibited enhanced new papilloma formation and significantly less spontaneous regression. The $CB2^{-/-}$ mice displayed an altered systemic immune profile, consisting of upregulated splenic CD4+ T cells and dendritic cells and downregulated anti-tumor eosinophils and CD8+ T cells compared to WT mice. Flow cytometry analysis of papillomas showed generally low immune cell infiltration in both genotypes; however pro-tumor myeloid-derived suppressor cells trended higher in the knockout mice, which was corroborated by higher IL-6 (produced by myeloid cells) mRNA expression in $CB2^{-/-}$ papillomas. Immune cell infiltration into papilloma-free carcinogen-exposed skin was also minimal in both groups, averaging five percent, a comparable level to the non-exposed skin. We measured CB2 expression and found that it is significantly higher in carcinogen-exposed skin compared to naïve skin in WT mice, thus suggesting a role of endogenous CB2 activation in keratinocytes in enhanced tumor regression.

Conclusions: Taken together, our data show that endogenous CB2 activation plays an anti-tumorigenic role and enhances tumor regression in non-melanoma skin carcinogenesis, potentially via a systemic immune-mediated response involving the alteration of the balance between pro- and anti-tumor immune cells, coupled with the modulation of localized keratinocyte activity.

ROLE OF CB_1 RECEPTORS IN THE GASTROINTESTINAL TRACT AFTER MICROBIOTA DEPLETION AND NATURAL RECOLONIZATION

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Introduction: The gut microbiome is being increasingly recognized to play important roles in the regulation of gastrointestinal (GI) function. A key element in the control of the GI function is the enteric nervous system (ENS). Previously, we examined the ENS and aspects of GI physiology using a mouse model of antibiotic (Abx)-induced bacterial depletion. Abx-treated mice have altered GI structure and function, including slower transit time and loss of enteric neurons in both the small intestine and colon: effects that can be reversed by natural bacterial recolonization, implying that the microbiota is essential for ENS homeostasis. There are important bidirectional interactions between the endocannabinoid system, cannabinoid (CB) receptors and the gut microbiota. CB_1 receptors are found throughout the ENS, where they modulate GI motility by providing inhibitory control in neurotransmission, slowing GI transit. We investigated if improvements in GI structure and function observed during natural recovery of the microbiota following Abx-induced depletion are dependant on CB_1 receptor signaling.

Methods: Mixed-sex wild type (WT) or CB_1 receptor-deficient (CB_1-KO) mice were maintained in groups (n = 4-9) that received: water for 4 weeks (controls); antibiotic treatment for 4 weeks; or antibiotics for 2 weeks, followed by 2 or 4 weeks of spontaneous bacterial recolonization (Abx-withdraw). GI function was assessed over the course of 1 week at the end of treatment using a number of different intestinal motility tests. After *in vivo* experiments, the ileum and colon were collected for whole-mount preparations, followed by immunofluorescence labeling and quantification of myenteric neurons. Differences between treatment group and genotype were determined using two-way ANOVA with Sidak post-hoc test.

Results: CB_1-KO mice lost significantly more body weight than WTs during the Abx treatment. Whole gut transit time was slower in Abx-treated mice of both genotypes, and partially recovered in WT mice 2 or 4 weeks after cessation of Abx treatment. In contrast, in CB_1-KO mice, recovery was complete 4 weeks after withdrawal of Abx and virtually so at 2 weeks. Small intestinal transit
was also reduced during Abx treatment in both genotypes, with partial recovery in CB_1-KO mice after 2 weeks of Abx withdrawal and full recovery by 4 weeks. In both genotypes, the Abx treatment or the subsequent microbiota recovery had no significant influence on distal colonic motility, fecal pellet output or intestinal permeability. The numbers of myenteric neurons (determined using HuC/D+ labelling) in the ileal and colonic ENS of CB_1-KO mice are virtually identical to WT mice. In Abx-treated mice of both genotypes, the numbers of myenteric neurons in the ileal and colonic ENS were significantly reduced, with full recovery after microbiota recolonization.

Conclusions: Activation of CB_1 receptors during microbiota recolonization in wild type mice significantly inhibits intestinal motility but does not limit neuroregeneration. These studies reveal further evidence of bidirectional interactions between the gut microbiota and the endocannabinoid system of the GI tract.

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A NOVEL SELECTIVE CANNABINOID DERIVATE SUPPRESSES MURINE AND HUMAN IBD THROUGH A STAT3-DEPENDENT MECHANISM

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Introduction: The endocannabinoid (EC) system consists of cannabinoid (CB) receptors and endogenous ligands for these receptors, called endocannabinoids. The profound influence of endocannabinoids on modulating immune responses has been greatly appreciated. Expression of the cannabinoid receptors on immune cells and accumulating data on the immunomodulatory effects of endocannabinoids and phytocannabinoids through these receptors have increased interest in studying the roles of these ligands in innate and adaptive immunity, specifically in inflammation associated with autoimmune diseases and chronic inflammations. Crohn's disease (CD) and ulcerative colitis (UC) related inflammation are marked by elevated production of IL-6 through STAT3 activation made by pathogenic T helper 17 (Th17) cells. Since the use of cannabis as a treatment for symptomatic control of IBD patients is increasing, understanding the mechanisms by which cannabinoids regulate the inflammation response becomes essential.

Methods: The effect of a Cannabis extract on human and mouse CD4 and Th17 cells were investigated under normal and inflammatory conditions. Freshly isolated CD4 T cells from the peripheral blood of healthy donors and IBD patients were activated in the presence of the Cannabis extract. STAT3 activation was evaluated by western blot. Chromatin immunoprecipitation (CHIP) assays were performed to examine STAT3 binding to the promoters of STAT3, IRF4, BCL2 and IL17A to evaluate downstream effects. Specific inhibitors were used for four different cannabinoid receptors to elucidate the relevant receptor. Calcium flux assays and flow cytometry were used to evaluate TRPV1 activity. A single cannabinoid was isolated from the extract by preparative HPLC/UV. We screened the effect of 13 synthetic derivates of the isolated cannabinoid on STAT3 activation. A DSS-induced UC murine model of IBD was utilized to assess the *in-vivo* effects of the derivate.

Results: In our work, we found that the Cannabis extract down-regulated STAT3 activation and Th17 differentiation. We revealed that TRPV1 is a key player in mediating these effects. In the DSS-induced UC murine model of IBD, Cannabis extract reduced disease severity. Treated mice lost less weight and showed improvement in clinical scores. We identified the cannabinoid in the Cannabis extract that was responsible for the observed effects. Since this cannabinoid is unstable, we identified a derivative termed, 331-18a-A-Me-Ester, that is more stable and potent.

Conclusions: These data suggest the ability of 331-18a-A-Me-Ester to regulate STAT3 signaling in human and mouse CD4+ T cells and participate in controlling the development of Th17 cells induced by inflammatory conditions, highlighting its potential for IBD and other autoimmune disorders therapy.

ENDOCANNABINOID REGULATION OF TEARING

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Introduction: Cannabis users frequently report dry mouth and dry eye. We recently showed that cannabinoid CB1 receptors sex-dependently regulate tearing in mice: activation of CB1 receptors reduces tearing in males, but increases tearing in females. Two endogenous cannabinoids, N-acyl ethanolamines (NAEs), including anandamide, or 2-arachidonoyl glycerol (2-AG) likely mediate these effects. NAEs are synthesized enzymatically by NAPE-PLD and metabolized by FAAH or NAAA. 2-AG is synthesized by diacylglycerol lipases, and metabolized chiefly by monoacylglycerol lipase (MAGL).

Methods: To explore which endocannabinoid regulates tearing we tested tearing, examined protein and mRNA expression of key cannabinoid genes, and measured the effect of FAAH deletion on endocannabinoid levels using lipidomics.

Results: We find evidence that both anandamide- and 2-AG-degrading enzymes contribute to regulation of tearing. Deletion and block of FAAH increased tearing in females, but not males, partly mirroring the sex-dependence of CB1. Blocking NAAA had no effect on tearing. NAEs may therefore mediate the CB1-dependent increase in tearing via FAAH. Acutely blocking MAGL had no effect in either sex but deletion of MAGL impaired tearing in males, but not females. MAGL deletion appears to also contribute to degradation of the lacrimal gland. FAAH protein is seen within restricted to the acinar cells while NAPE-PLD is enriched in myoepithelial cells. We do not detect a difference in mRNA expression for FAAH, NAPE-PLD, or MAGL by sex. In lipidomic experiments comparing males vs. females, female mice have higher levels of several NAEs, though not anandamide. In contrast males have higher levels of several acylglycerols, including 2-AG. Female FAAH knockouts have higher levels of two NAEs including anandamide than males.

Conclusion: Our results suggest that some of the sex-dependence of effects on lacrimation that we have reported for CB1 activation may extend to the endocannabinoid messengers. FAAH appears to mediate the increase in tearing seen in females while MAGL function appears to be critical for the viability of the lacrimal gland in males.

Δ8-ISO-TETRAHYDROCANNABINOL AND NEURONAL CELL SIGNALING

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Introduction: Delta-8-tetrahydrocannabinol (Δ8-THC) and delta-9-tetrahydrocannabinol (Δ9-THC) have similar chemical and functional properties. With recent changes in hemp legalization status, the chemical conversion of cannabidiol (CBD) into Δ8-THC products has increased tremendously. In this chemical reaction, numerous additional THC isomers are formed with unknown pharmacological and safety profiles in humans. Δ8-Iso-tetrahydrocannabinol (Δ8-iso-THC) is a non-natural isomer of THC formed as a byproduct in this reaction with a different tricyclic ring structure (Geci et al., Cann Can Res 2022). While limited research on Δ8-THC's pharmacological properties has been reported, even fewer studies on the minor isomers formed in the conversion reaction exist in the literature. This study investigates the effects of Δ8-iso-THC on cell signaling in cells expressing the endogenous CB1 cannabinoid receptors (CB1R).

Methods: Δ8-iso-THC was obtained from Cayman Chemical Company. N18TG2 mouse neuroblastoma cell membranes or intact cells were used for GTPγS binding, cAMP accumulation, and Extracellular Signal-Regulated Kinase (ERK) phosphorylation assays, as reported in earlier studies. Data were analyzed using GraphPad Prism.

Results: Utilizing N18TG2 cell membranes, Δ8-iso-THC (100pM-1μM) activated G proteins by stimulating [^{35}S] GTPγS binding in a dose-dependent manner with EC50=26nM. Co-incubation with CB1R antagonist/inverse agonist SR141716 (1μM) reduced the baseline GTPγS binding, but that decrease was seen without a shift in potency for Δ8-iso-THC in the log-dose response for G protein stimulation (EC50=30nM). In intact N18TG2 cells, Δ8-iso-THC (100pM-1μM) inhibited initial-rate (four minutes) forskolin (FSK)-stimulated cAMP accumulation in a dose-dependent fashion (EC50=32nM). Co-incubation with SR141716 (1μM) did not completely reverse the Δ8-iso-THC inhibition of the FSK-stimulated cAMP accumulation (EC50=24nM). In N18TG2 cells treated with the diacylglycerol lipase inhibitor, tetrahydrolipstatin (THL, Orlistat; 1μM for two hours), ERK phosphorylation was assessed after four minutes of incubation with the compounds using the in-cell western immuno-assay. Δ8-iso-THC promoted a rapid stimulation of ERK phosphorylation, consistent with stimulation that occurs via a G protein mechanism (as opposed to beta-arrestin-mediated). The rapid stimulation of ERK phosphorylation was not inhibited by co-incubation with SR141716 (1μM). Δ8-iso-THC failed to synergize or inhibit ERK phosphorylation stimulated by the CB1R partial agonist Δ9-THC or the full agonist CP55940.

Conclusion: Cell signaling by Δ8-iso-THC behaves similarly to Δ9-THC in activating G proteins, inhibiting cAMP production, and early onset ERK phosphorylation. However, this stimulation may not be produced by the CB1R alone but might result from alternative Gi-coupled receptor(s) or a biased signaling mechanism. These studies emphasize the need to investigate the non-natural isomers formed in synthesizing Δ8-THC from CBD and other potential targets for this compound.

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MICROGLIA-MEDIATED NEURONAL DEATH CAN BE SUPPRESSED BY SELECTIVE CANNABINOID RECEPTOR AGONISTS *IN VITRO*

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Introduction: Neuroinflammation is a hallmark of damage to the brain, and is primarily propagated by microglia, the resident immune cells of the brain. Pro-inflammatory microglia mediate host defense but sustained inflammatory activity can lead to impairment of neurons via accumulation of inflammatory mediators (e.g cytokines) and reactive species. The endocannabinoid system modulates immune responses and has been reported to suppress neuroinflammation via actions on microglia which express both cannabinoid type 1 (CB_1) and type 2 (CB_2) receptors. The purpose of this work was to investigate the mechanisms of microglia-mediated neuronal apoptosis and determine whether agonists of CB_1 or CB_2 receptors could improve the survival of ST*Hdh*$^{Q7/Q7}$ cells which model spiny projection neurons.

Methods: Cultured SIM-A9 microglia were stimulated with lipopolysaccharide (LPS) and interferon-γ (IFNγ) to induce a pro-inflammatory phenotype. Release of pro-inflammatory cytokines was measured using ELISAs and ProteomeProfiler antibody arrays. Conditioned media was collected from microglia and applied to cultured ST*Hdh*$^{Q7/Q7}$ neuronal cells for up to 24 h. Neuronal viability was determined using orthogonal assays (CellTiter Glo and CellTiter Blue). ACEA (2 nM – 2 μM) was used to activate microglial and neuronal CB_1 receptors. HU-308 and its enantiomer, HU-433 (2 nM – 2 μM), were used to activate microglial CB_2 receptors. Neuronal and microglial signaling in response to pro-inflammatory stimuli was monitored using in-cell western assays with antibodies specific for phospho-proteins.

Results: Microglial cells stimulated with LPS and IFNγ exhibited a phenotypic shift characterized by release of pro-inflammatory cytokines such as TNFα and IL-6. Conditioned media from pro-inflammatory microglia rapidly induced caspase-3 cleavage in ST*Hdh*$^{Q7/Q7}$ neuronal cells which culminated in a 57 ± 4% loss in cell viability within 24 h. This was largely mediated by TNFα as a 1:100 dilution of neutralizing antibodies reduced neuronal death by 60 ± 3%. When neuronal CB_1 receptors were stimulated, the cells exhibited suppression of pro-apoptotic signaling and enhanced survivability in response to the pro-inflammatory stimulus. Furthermore, stimulation of microglial CB_2 receptors with HU-308 and HU-433 suppressed the LPS-mediated induction of multiple signaling pathways including ERK and JNK and subsequent induction of the pro-inflammatory phenotype. These data demonstrate that activation of microglial CB_2 receptors suppressed the pro-inflammatory activity of microglia and neuronal CB_1 receptors suppressed the pro-death response to microglial-derived TNFα.

Conclusions: Microglia in a pro-inflammatory phenotype exhibited the capacity to kill cultured neurons in a primarily TNFα-dependent manner. Improved neuronal survival was achieved using three strategies: i) suppression of microglial inflammation by CB_2 activation, ii) neutralization of TNFα using polyclonal antibodies, and iii) suppression of neuronal pro-death signaling through CB_1 activation. These data indicate that agonists of CB_1 or CB_2 receptors have potential for therapeutic use in neuroinflammation.

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ENDOCANNABINOIDS IN THE CORTICO-AMYGDALA NEUROCIRCUIT MEDIATE FEAR EXTINCTION

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Introduction: Extinction of fearful memories is impaired in anxiety and stress related disorders such as posttraumatic stress disorder. The endocannabinoid system has a key role in facilitation of extinction as evidenced by preclinical and clinical studies. Here, we extend our previous findings to investigate the neurocircuitry that underlies this effect. We focused on the basolateral amygdala (BLA) projecting medial prefrontal cortex (mPFC) neurons as this circuit is implicated in fear extinction.

Methods: First, we used *in vivo* optogenetics and pharmacology to show the involvement of the endocannabinoid system in extinction learning by activating the BLA projecting mPFC neurons and measuring the endocannabinoids by mass spectrometry. Next, we used eCB biosensor imaging via fiber photometry to monitor real time dynamic changes in the endocannabinoids during extinction learning. We used *ex vivo* electrophysiology and CRISPR-Cas9 gene editing in combination with biochemical techniques to examine whether BLA projecting medial mPFC neurons represent a neural substrate for the effects of eCBs on extinction.

Results: First, we found that optogenetic activation of these afferents during extinction CS (conditioned stimulus) presentation elevates eCBs in BLA. Next, we showed the dynamic changes of the eCBs at these afferents using the eCB biosensor. During the extinction training eCBs were increased after CS presentation when the aversive unconditioned stimulus (shock, US) is most expected. Finally, we showed that disrupting the function of the CB1Rs expressed on these afferents via CRISPR-Cas9 gene editing impairs extinction.

Conclusions: Our results reveal the temporal dynamics of the endocannabinoids and provide a mechanistic understanding of how the endocannabinoid system at prefrontal-amygdala neurons during extinction work to facilitate extinction learning. These findings highlight targeting the eCB system as a therapeutic approach for anxiety and stress related disorders.

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DIFFERENTIAL EFFECTS OF ACUTE THC ON BEHAVIOR AND THE ENDOCANNABINOID SYSTEM IN NEUROHIV MOUSE MODEL

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Introduction: Due to the anti-inflammatory and neuroprotective properties of the endocannabinoid system (eCB), it has attracted interest as a therapeutic target for various neurodegenerative disorders, including HIV-associated neurocognitive disorders (HAND). Cannabis is the most commonly used drug among people living with HIV (PLWH) and has been reported to be used 2-3 times higher in PLWH than in the general US population. Acute and chronic cannabis use in the general US population has been shown to profoundly impair attention, learning, memory, and various other domains of cognitive function; however, its effects on neurocognition and the CNS in PLWH are divided. In the present study, we aimed to investigate the role of acute THC in the HIV-1 Tat transgenic mouse model.

Methods: HIV-1 IIIB $Tat_{1\text{-}86}$ transgenic female and male mice were assessed [~ 9-10 months of age, $N = 38(20f)$] in various behavioral paradigms, including pain sensitivity, motor activity, motor coordination, anxiety, and object recognition memory. Acute THC effects (1, 3, 10 mg/kg) on the endocannabinoid levels and related lipids in the brain were quantified via mass spectrometry, and expression of CBRs and eCB degradative enzymes being assessed by Western blot analyses.

Results: THC significantly decreased heat-evoked nociception in a dose-dependent manner and was genotype-specific, where Tat expression enhanced antinociception in the tail-flick task at higher doses. No effects were noted for motor coordination; however, a slight upregulation of locomotor activity was explicitly seen in female mice after THC administration. THC increased anxiety in females and Tat(-) animals in the elevated plus maze task. For the novel object recognition, THC administration decreased object exploration time in males and females, specifically for the Tat(−) groups. Object recognition memory was negatively altered by acute THC (10 mg/kg) injections in Tat(−) but not Tat(+) females. No alterations in the endocannabinoids or related lipid molecules in any CNS region were observed. However, female mice showed higher AEA and/or AA expression levels in almost all CNS regions compared to males, except for AA levels in the spinal cord. Tat expression affected AEA and AA levels differently for males and females in some CNS regions. Western blot analysis revealed that females had higher CB_2R, MAGL, and FAAH expression in the PFC than males. Moreover, Tat expression upregulated CB_1R expression in Tat(+) females compared to Tat(−) females, and FAAH levels were upregulated in THC-exposed Tat(+) males and females.

Conclusion: Acute THC has differential effects on various behavioral outcomes in the context of HIV, with potential sex-dependent protective effects on recognition memory.

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DEFICITS IN ENDOCANNABINOID SIGNALING IN THE CEREBELLUM DISRUPTS SOCIAL PREFERENCE

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Introduction: Autism Spectrum Disorder (ASD) is one of the most prevalent neurodevelopmental disorders, affecting 1:44 children in the United States according to the latest CDC estimates. ASD is characterized by a wide range in prevalence and severity of cognitive, social, and emotional symptoms; however, deficits in social interactions, increased social avoidance and anxiety are at the core of this neurological condition. Endocannabinoid signaling system (ECS) is implicated in emotional and social regulation. Mutations in cannabinoid receptor 1 (CB1) and endocannabinoid synthesizing enzyme diacylglycerol lipase alpha (DAGLa) are linked to increased predisposition to ASD with high confidence (ranks 1-2 in SFARI gene scoring). Families and researchers are actively seeking effective pharmacological treatments for ASD, and some have begun to experiment with cannabis to alleviate the symptoms of ASD, including social anxiety. Yet neither safety nor efficacy and specific neurological substrates of this treatment in ASD are clearly understood. This study will advance our understanding of the neurological substrates through which ECS-targeting therapies affect social preference by investigating its role in cerebellar circuits regulating social approach.

Methods: To elucidate the role of endocannabinoid signaling in the regulation of cerebellar circuits' development and activity, we generated cerebellar Purkinje cell specific conditional knockout mouse line (PC-Dagla-cKO). This mouse line allows us to evaluate cerebellar-specific requirements for 2-arachidonoylglycerol (2-AG) in regulation of social approach, the core behavioral domain affected in ASD. Behavior of PC-Dagla-cKOs was assessed in motor and social tasks. Molecular and morphological profiles of Dagla-null PCs were assessed by immunohistochemistry followed by confocal microscopy to elucidate cellular and molecular consequences of endocannabinoid signaling attenuation in the cerebellum.

Results: Dagla-null PCs exhibit altered molecular profile, suggesting that PC-derived 2-AG regulates intrinsic molecular properties of PCs, likely affecting their excitability. In addition, PC-Dagla-cKOs exhibit decreased social preference when compared to WT.

Conclusions: Our results suggest that cerebellar-specific attenuation of endocannabinoid signaling disrupts social preference, advancing our understanding of cerebellar role in the regulation of social approach, as well as highlighting novel neurological substrates through which endocannabinoid signaling regulates sociability. This work has pre-clinical significance, laying down the foundation for future development of targeted pharmacological and diagnostic tools.

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).

MUSCARINIC CANNABINOID SUPPRESSION OF EXCITATION, A NOVEL FORM OF COINCIDENCE DETECTION

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Introduction: Cannabinoid CB1 receptors have been implicated in several forms of synaptic plasticity in the brain. Depolarization-induced suppression of excitation (DSE), metabotropic suppression of excitation (MSE), long term depression (LTD) and activation-dependent desensitization each alter synaptic function, over different time scales ranging from tens of seconds to hours or even longer.

Methods: Autaptic hippocampal neurons express all of these, illustrating the rich functional and temporal heterogeneity of CB1 at a single set of synapses.

Results: Here we report that coincident activation of muscarinic acetylcholine receptors and DSE results in a substantial inhibition of excitatory transmission (~40%) lasting 10 minutes. The induction is blocked by CB1 and muscarinic M3/M5 receptor antagonists and is absent in CB1 receptor knockout cultures. Notably, once it is established, this inhibition is reversed by a CB1, but not a muscarinic, antagonist, suggesting that the inhibition occurs via persistent activation of CB1 receptors. We refer to this inhibition as muscarinic cannabinoid suppression of excitation (MCSE). MCSE can be mimicked by coapplication of oxo-M and either 2-AG or WIN55212 and requires Ca2+-release from internal stores.

Conclusions: MCSE represents a novel form of coincidence detection – important for many models of learning and memory -- between cannabinoid and muscarinic signaling systems that is expressed as a medium-term depression of synaptic signaling. Given the known roles for muscarinic and cannabinoid receptors in the hippocampus, MCSE may be important in the modulation of hippocampal signaling at the site of septal inputs, with potential implications for learning and memory, epilepsy and addiction.

DISCOVERY OF A NOVEL METABOLIC PATHWAY OF N-ACYLETHANOLAMINES BY GLUCOCEREBROSIDASE-2

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Introduction: N-acylethanolamines (NAEs) are degraded by fatty acid amide hydrolase (FAAH) and N-acylethanolamine-hydrolyzing acid amidase (NAAA). Alterations in endogenous NAE levels may affect various biological processes, such as (neuro)inflammation. Gaucher's disease is a lysosomal storage disorder that is caused by mutations in the β-Glucocerebrosidase (GBA1) gene, resulting in the accumulation of glucosylceramide (GlcCer), and neuroinflammation in a subgroup of patients. Notably, GBA1 and its congener GBA2 hydrolyze glucosylceramide, but are also able to transfer the resulting glucose (Glc) to other lipids containing a hydroxyl group. Since NAEs contain a primary hydroxyl group, the aim of this study was to investigate whether NAEs can be glycosylated by GBA.

Methods: To this end, we synthesized β-Glc-NAEs and 13C6-isotope-labeled β-Glc-NAEs and developed an LC-MS/MS method for the quantitative detection of endogenous β-glycosylated-NAEs in different biological systems using the isotopically labeled standards.

Results: We found that anandamide (AEA), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) could serve as donor for transglycosylation by recombinant GBA1 and HEK293 lysates overexpressing GBA2. Furthermore, we found that β-Glc-NAEs were predominantly synthesized by GBA2 and hydrolyzed by GBA1 in Neuro2a, HEK293 and RAW cells. β-Glc-NAEs were detected in various mouse tissues, including brain and intestine. Importantly, elevated levels of β-Glc-PEA were found in blood plasma and elevated levels of β-Glc-AEA and β-Glc-PEA were found in spleen tissue samples of Gaucher patients.

Conclusion: we have identified a novel metabolic pathway of NAEs, which may provide more mechanistic insight into Gaucher's disease and endocannabinoid signaling under (neuro)inflammatory conditions.

HUMAN EOSINOPHILS AND NEUTROPHILS BIOSYNTHESIZE NOVEL LIPOXYGENASE METABOLITES FROM MONOACYLGLYCEROLS AND *N*-ACYL-ETHANOLAMINES

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INTRODUCTION. The endocannabinoids 2-arachidonoyl-glycerol (2-AG) and *N*-arachidonoyl-ethanolamine (AEA) are lipid mediators regulating many physiological processes, notably inflammation. 2-AG and AEA are respectively part of the monoacyglycerol (MAG) and *N*-acyl-ethanolamine (NAE) families. Thus, MAGs and NAEs are considered as part of the endocannabinoidome. Endocannabinoid hydrolysis inhibitors are being investigated as potential treatment in numerous conditions. This strategy will not only increase the levels of 2-AG and/or AEA, but also those of other MAGs and/or NAEs. Increasing MAG and/or NAE levels will likely increase the levels of their metabolites. Herein we investigated whether MAGs and NAEs were substrates for the 15-lipoxygenase pathway, which is strongly involved in asthma and its severity. We thus assessed if human eosinophils and neutrophils biosynthesized the 15-lipoxygenase metabolites of MAGs and NAEs derived from linoleic acid (LA), eicosapentaenoic acid (EPA), docosapentaenoic acid n-3 (DPA) and docosahexaenoic acid (DHA).

METHODS. We synthesized the putative 15-lipoxygenase metabolites of MAGs and NAEs containing LA, EPA, DPA and DHA and optimized their detection by LC-MS/MS. Human eosinophils and neutrophils were isolated from the blood of healthy donors and incubated with MAGs and NAEs at different concentrations and times.

RESULTS. Eosinophils, which express the 15-lipoxygenase-1, metabolized all the MAGs and NAEs to the expected 15-lipoxygenase metabolites. Human neutrophils, which might express the 15-lipoxygenase-2, also metabolized most of the MAGs and NAEs, but to a much lower extent than eosinophils. Importantly, some of the new 15-lipoxygenase metabolites we disclose were found in tissues from humans and mice.

CONCLUSIONS. We successfully showed that human eosinophils and neutrophils transform MAGs and NAEs into novel 15-lipoxygenase metabolites. How these new metabolites modulate the inflammatory cascade is now being explored as they could participate in the effects of endocannabinoid hydrolysis inhibitors *in vivo*.

PLAAT5 AS A CA^{2+}-INDEPENDENT *N*-ACYLTRANSFERASE PRODUCING ANANDAMIDE AND OTHER *N*-ACYLETHANOLAMINES IN TESTIS

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Introduction: *N*-Acylethanolamines (NAEs), including anandamide, are produced from *N*-acyl-phosphatidylethanolamines (NAPEs) and exert various receptor-mediated biological activities. NAPEs are biosynthesized from membrane phospholipids by *N*-acyltransferases, which consist of Ca^{2+}-dependent cytosolic phospholipase $A_2\varepsilon$ ($cPLA_2\varepsilon$, also known as PLA2G4E) and Ca^{2+}-independent PLAAT (phospholipase A and acyltransferase) family enzymes. While $cPLA_2\varepsilon$ has been shown to be activated during cell injury such as brain ischemia, resulting in the production of NAPEs and NAEs, the roles of the PLAAT family as *N*-acyltransferase *in vivo* have been poorly understood. Among five isoforms of PLAAT (PLAAT1–5), PLAAT5 is unique due to its specific expression in the testis. In this study, we generated PLAAT5-deficient (*Plaat5*$^{-/-}$) mice by the CRISPR/Cas9 system and examined its involvement in the biosynthesis of NAPEs and NAEs in testis.

Methods: Successful generation of *Plaat5*$^{-/-}$ mice was confirmed by DNA sequencing. Particulate and supernatant fractions were prepared from the testis homogenates of wild-type (WT) and *Plaat5*$^{-/-}$ mice and subjected to the *N*-acyltransferase assay using ^{14}C-labeled phosphatidylcholine and non-labeled phosphatidylethanolamine as substrates. Furthermore, total lipids were extracted from testis homogenates by the method of Bligh and Dyer, and molecular species of NAPEs and NAEs were analyzed by liquid chromatography-tandem mass spectrometry.

Results: *Plaat5*$^{-/-}$ mice were viable and fertile. The testis of *Plaat5*$^{-/-}$ mice lacked Ca^{2+}-independent *N*-acyltransferase activity in both the particulate and supernatant fractions in contrast to the testis of WT mice in which the activity was detected. Lipid analysis revealed that the testicular levels of NAPEs and NAEs in *Plaat5*$^{-/-}$ mice were 20% and 50% lower than those in WT mice, respectively. In particular, polyunsaturated NAEs such as anandamide and docosahexaenoylethanolamide (22:6-NAE) were reduced to less than 20% of WT mice.

Conclusions: These results strongly suggest that PLAAT5 functions as a principal Ca^{2+}-independent *N*-acyltransferase in mouse testis and is responsible for the generation of NAPEs and NAEs, especially polyunsaturated NAEs including anandamide.

EFFECTS OF AGE ON PLASMA LEVELS OF CANNABINOIDS AND ENDOLIPIDS AFTER VAPORIZED CANNABIS IN MICE

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Introduction: Recently, using small animal fMRI analyses we showed that neural connectivity changes in young and old mice as a response to inhaled Cannabis differed as a function of age, sex, and amount of THC or CBD in the Cannabis (Sadaka et al, 2023 Front of Ageing Neurosci; Coleman et al, 2022 Addict Biol). Plasma from animals from both of these studies were analyzed for THC and CBD to validate the treatment groups and levels of circulating cannabinoids. We have also shown in multiple studies htyat CB treatment drives changes in endogenous lipids (Leishman et al 2018; Johnson et al 2022). Here, we further evaluated the endolipids in the plasma samples from these fMRI studies to determine if there are differences by age and circulating CB levels in eCB and related endolipids that can be predicted by age and CB treatment.

Methods: Female mice were exposed to cannabis vapor as previously described (Sadaka 2023). In brief, Cannabis high in Δ9-THC (10.3% Δ9-THC and 0.05% CBD), high in CBD (10.4% CBD and 0.36% Δ9-THC), and placebo cannabis purported to have less than 0.01%Δ9-THC and 0.01% CBD were acquired from the NIH/NIDA, Bethesda, MD through the Research Triangle Institute. Groups of mice were placed in a 38-L exposure chamber (60 cm × 45 cm × 20 cm), that included a vapor inflow tube and several small air outflow holes. The vaporizer was preheated at approximately 210°C and loaded with 0.450 g of minced cannabis. Tubing was attached from the vaporizer to the exposure chamber and the heating fan was run for a total of 60s, filling the exposure chamber with vaporized cannabis aerosols. After 30 min of passive exposure, mice were removed from the exposure chamber and returned to their cages. This exposure protocol occurred daily for 28 consecutive days. Plasma was collected via core blood upon sacrifice after the final fMRI evaluation in the Ferris Lab then stored at -80. Plasma samples were processed in the Bradshaw lab as previously described (Bradshaw HB, Johnson CT. Methods Mol Biol. 2023;2576:21-40. doi: 10.1007/978-1-0716-2728-0_3.). In brief, methanolic extracts were partially purified on C18 solid phase extraction columns and eluants were analyzed via HPLC/MS/MS.

Results: Overall NAEs levels, including the eCB, Anandamide had similar levels in placebo groups in both age groups; however, AEA was significantly *decreased* in young animals with CB treatment, where it was increased in the older animals. Levels of 2-AG were mostly unaffected in the younger group but significantly *increased* in older animals with CB treatment. There were similar patterns for other endolipids where the direction of change was more dependent on the age of the animal than the CB level in the Cannabis with the most significant differences in levels between the CB treatment groups by age. Over 40 endolipids were datable in all groups.

Conclusions: Much of the pre-clinical animal research to date has been performed on relatively young animals (2-4 months old) and our understanding of how CB signaling functions is primarily informed from those studies. It is important to recognize that the age of an individual can dictate drug action and that this is a function of multiple signaling pathways, including the modulation of endolipids. Here, we provide important information that illustrates how age as a biological variable can significantly change pharmacological outcomes.

IN VIVO EFFECTS OF MINOR CANNABINOIDS CANNABINOL, CANNABICHROMENE, AND CANNABICYCLOL OCCUR VIA MULTIPLE RECEPTOR MECHANISMS

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Introduction: The analgesic and anti-inflammatory effects of cannabinoids are well-established. For example, the effects of the major phytocannabinoid Δ^9-tetrahydrocannabinol (THC) and, to a lesser extent, cannabidiol (CBD) have been studied for decades. Recent work has investigated other compounds produced by the plant, including minor cannabinoids and terpenoids. The goal of the present study was to determine the *in vivo* effects of three lesser-studied, minor phytocannabinoids: cannabinol (CBN), cannabichromene (CBC), and cannabicyclol (CBL).

Methods: To measure acute effects of each compound, adult male and female C57BL/6J mice were administered CBN, CBC, CBL (10-200 mg/kg, ip), or vehicle and tested repeatedly in the tetrad test battery (i.e., catalepsy bar test, tail immersion, core body temperature, and spontaneous locomotor activity). To assess potential analgesic effects in chronic pain states, separate groups of mice were subjected to chronic neuropathic injury (CCI) or lipopolysaccharide (LPS)-induced inflammatory hind paw pain and treated (ip) with CBN (25-100 mg/kg), CBC (100 mg/kg), or CBL (100 mg/kg).

Results: Mice treated with CBN (≥25 mg/kg) displayed classic cannabinoid effects in the tetrad, including acute antinociception, that were only partially blocked by pretreatment with either rimonabant (CB_1 selective antagonist; 3 mg/kg, ip) or istradefyllene (adenosine A_{2A} selective antagonist; 3.2 mg/kg, ip). Similarly, neither CB_1 nor A_{2A} antagonism had any effect on CBC-induced immobility (200 mg/kg). CBL (≥50 mg/kg) induced hypothermia that was fully blocked by istradefyllene, suggesting a cannabinoid receptor-independent mechanism. Mice subjected to CCI surgery displayed acetone-induced cold allodynia that was attenuated by either CBN (≥50 mg/kg) or CBL (100 mg/kg). In the LPS model, CBN (100 mg/kg) attenuated both paw edema and proinflammatory cytokine levels.

Conclusions: Together, these findings suggest that the minor phytocannabinoids cannabinol and cannabicyclol reduce pain and inflammation, via cannabinoid receptor-dependent and -independent pathways.

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A NEWLY-IDENTIFIED PHYTOCANNABINOID MODULATES THE ESTROGEN RECEPTOR AND REDUCES TUMOR PROGRESSION IN A MOUSE MODEL OF BREAST CANCER

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Introduction: Through their interactions with the endocannabinoid system (eCBS), cannabinoids can regulate many physiological systems, including estrogen signaling. Most breast tumors express estrogen receptor alpha (ERα) and depend on estrogen for cell proliferation. Therefore, they are commonly treated with Selective Estrogen Receptor Modulators (SERMs) such as tamoxifen. Yet, despite the obvious benefits, many patients that start this therapy do not complete the whole time-course due to adverse effects. By exploiting the crosstalk between the eCBS and estrogen signaling, combination therapy with phytocannabinoids can allow for the use of an optimal dose without intolerable side effects while still achieving the same or an even better therapeutic outcome. The vast majority of studies in the breast cancer field were conducted with pure Δ^9-tetrahydrocannabinol (THC), however, the *Cannabis* plant produces many other phytocannabinoids with possible higher aptitude in targeting this type of cancer.

Methods: Breast cancer cell lines were challenged with ~30 *Cannabis* extracts with varying phytocannabinoid profiles, with and without a combination with SERMs; and cell death was assessed via imaging, Annexin V/PI flow cytometry and Caspase activation by western blot. Modulation of ERα expression, localization and activity was assessed by confocal fluorescence imaging, subcellular fractionation, a luciferase reporter assay targeting the estrogen response element (ERE) and quantitative PCR. A single cannabinoid that can achieve the same effect as the whole extract was identified utilizing advanced biochemical and mass-spectrometry approaches, and its structure was elucidated with Nuclear Magnetic Resonance (NMR) spectroscopy. The molecular mechanism was elucidated by RNA-seq and western blot. The *in-vivo* effects were assessed using a slow-release estradiol pellets supplementation mouse model.

Results: Here, we found a unique *Cannabis* extract able to sensitize ER-positive tumors to the cytotoxic effect of SERMs. From this extract, we identified and isolated a novel phytocannabinoid, which we termed 373.15b, that can mimic the effect of the whole extract without any toxicity by itself. Phytocannabinoid 373.15b has a unique structure of a dihydroxyphenol with an acetate group attached to the aromatic ring, very different from cannabidiol (CBD) and THC. It affected estrogen signaling by decreasing the mRNA and protein expression level of ERα, and reducing its transcriptional activity via a signaling pathway involving RASSF1A and AKT1. In an *in-vivo* mice model, treatment with either the *Cannabis* extract or pure 373.15b together with low doses of tamoxifen reduced tumor volumes and weights. Treatment with 373.15b by itself reduced tumor progression, but the effect was more profound when combined with tamoxifen.

Conclusions: 373.15b is a novel cannabinoid with a unique structure that decreases the mRNA and protein expression level of ERα via RASSF1A and AKT1 signaling. Co-treatment of breast cancer patients with 373.15b and SERMs is expected to reduce the length of the treatment and lower administered doses of conventional therapy, and by that also diminish the associated adverse effects.

CANNABICHROMENE (CBC), CANNABIGEROL (CBG) AND SILVER NANO-PARTICLES DELIVER BROAD SPECTRUM ANTIMICROBIAL SYNERGY IN HYDROGEL-FORMING WOUND CARE DRESSINGS

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Introduction: Anti-bacterial effectiveness claims for wound care devices in the United States require demonstration of a ≥4-Log reduction in bacterial colony forming units (CFU) after inoculation of the product with ≥10^6 CFU and incubation over the recommended wear time. Results must be demonstrated against gram-positive and gram-negative pathogens. Anti-bacterial properties of phytocannabinoids have been reported previously, however, the spectrum of activity is limited to gram-positive species only. Matrix3™ technology is a patent-pending composition of CBC, CBG and silver nano-particles which dramatically improves the broad spectrum anti-bacterial activity of silver. This study aimed to quantify the anti-bacterial activity of the Matrix3™ composition and assess the minimum effective concentration in polyvinyl alcohol (PVA) wound dressings.

Methods: Synergy was assessed using broth microdilution in 96 well checkerboard format with a fractional inhibitory concentration index (FICI) of <0.75 taken as synergy for a three-compound combination. PVA dressings were manufactured via solvent casting and contained either vehicle, CBC, CBG, and/or silver nano-particles. Dressings were inoculated with 10^6 CFU and incubated at 37°C. Bacteria were extracted and quantified by agar plate CFU enumeration at 0.5, 24, 48 and 72 hours (Day 0, 1, 2 and 3, respectively).

Results: Matrix3™ components demonstrated three-compound synergy against Methicillin-resistant *Staphylococcus aureus* (FICI = 0.64) and *Pseudomonas aeruginosa* (FICI = 0.25). On Day 3 post MRSA inoculation, dressings containing 0.1% silver, 0.3% CBC and 0.3% CBG had 380,189,396-fold fewer CFU than 0.1% silver dressings (**Figure 1.0**) and demonstrated a 4.97-Log CFU reduction from Day 0 vehicle dressings. Day 3 post *P. aeruginosa* inoculation, dressings containing 0.2% silver, 0.8% CBC and 0.8% CBG had 30,902,954-fold fewer CFU than 0.2% silver dressings (**Figure 2.0**) and demonstrated a 4.08-Log CFU reduction from Day 0 vehicle dressings.

Conclusions: The components of Matrix3™ act synergistically against gram-positive and gram-negative bacteria and have a minimum effective concentration of 0.2% silver, 0.8% CBC and 0.8% CBG in PVA dressings. Wound dressings containing Matrix3™ technology have potential to offer anti-bacterial effectiveness with substantially less silver compared to currently approved commercial products.

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Figure 1.0

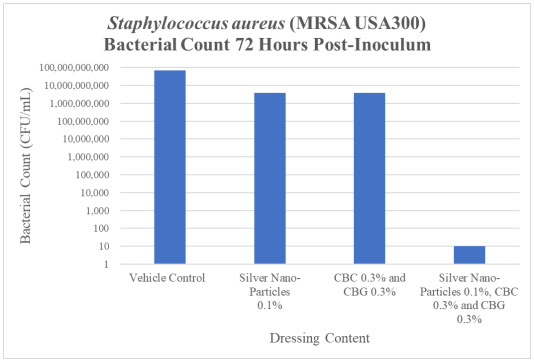
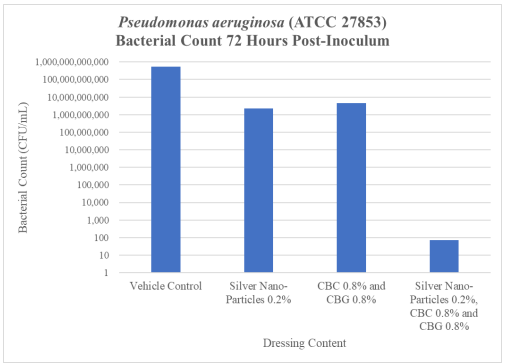


Figure 2.0



ACUTE EFFECTS OF CANNABIGEROL IN HUMANS: A DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSSOVER, FIELD TRIAL

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Introduction: Cannabigerol (CBG) is the parent compound of other cannabinoids and as such has been referred to as the "mother of all cannabinoids." Preclinical animal research suggests CBG is not intoxicating and has a range of therapeutic properties including anxiolytic effects. In a recent survey of CBG users, we found that most people reported using CBG for medicinal purposes, specifically to manage anxiety, pain, and depression. Self-reported efficacy was rated as high with minimal side effects reported. No prior research has examined acute effects of CBG on humans in an objective manner. Thus, the purpose of this study is to examine acute effects of CBG on anxiety, stress, mood, and memory, as well as potential positive and negative subjective drug effects.

Method: This double-blind, placebo-controlled, crossover, field trial examines the acute effects of CBG. Healthy cannabis-using adults complete two testing sessions via Zoom. In each session, they provide baseline subjective ratings of anxiety, stress, mood, and drug effects (e.g., intoxication, dry mouth, dry eyes, sleepiness, appetite, heart palpitations) using 0 to 10 scales prior to oral administration of 20mg hemp derived CBG or placebo tincture (T0). These subjective ratings are collected again roughly 10 mins (T1), 35 mins (T2) and 50 mins (T3) after CBG/placebo administration. Prior to T1 participants complete an online survey, prior to T2 they complete the Trier Social Stress Test, and prior to T3 they complete the immediate recall trials of the California Verbal Learning Test II and the DRUID app (a mobile app that objectively assesses impairment).

Results: To date, 13 participants (6 women, 4 men, 3 transgender/non-binary; M_{age} = 34.3) out of a targeted 34 have completed the study. Analysis of change scores in subjective ratings from T0 (baseline) to T3, revealed a large sized significant (η_p^2 = .35, p = .035) effect of CBG on stress ratings, indicating that CBG decreased subjective stress more than placebo. There was also a large-sized sized (η_p^2 = .22, p = .10) effect of CBG on anxiety ratings from T0 to T3, such that CBG produced greater reductions in anxiety than placebo. However, this effect was not statistically significant in this small preliminary sample. Results further indicate a large-sized statistically significant (η_p^2 = .34, p = .046) effect of CBG on immediate verbal memory, with significantly better test performance detected in the CBG condition. Ratings of intoxication and negative subjective drug effects remained low at all timepoints in both conditions. Moreover, there were no significant differences in any of the subjective drug effect rating change scores or DRUID app change scores in the CBG condition compared to the placebo condition.

Conclusions: Preliminary results from this clinical trial suggest potential stress relieving, anxiolytic, and memory-enhancing effects of CBG. Consistent with findings from preclinical and self-report studies, CBG does not appear to have significant intoxicating effects, impairing effects, or negative subjective drug effects.

Funding: CReDO Science

A JOURNEY THROUGH THE DEVELOPMENT OF CANNABIDIOL SOLID ORAL DOSAGE FORMS: BALANCING API LOADING, PHYSICO-CHEMICAL STABILITY, AND *IN-VIVO* PHARMACOKINETIC PERFORMANCE

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Introduction: Cannabidiol (CBD) is fast emerging as an exciting ingredient across the pharmaceutical market, powered by increasing scientific research investigating its potential benefits in a number of disease states, including central nervous system (CNS) disorders, pain management, cancer and more. While there is a remarkable opportunity for the development of CBD-based therapies in the field, formulating a highly lipophilic (logP = 6.3) and poorly soluble crystalline API can be a challenging task. In addition to physical and chemical stability challenges, the oral bioavailability of crystalline CBD has been shown to be very low in humans (approx. 6%), due to incomplete absorption in the gut and significant pre-systemic elimination in the liver.

Methods: The solid dosage forms were generated by means of high-shear homogenization and spray drying, in order to encapsulate a CBD-loaded nano-emulsion into a solid matrix capable of ensuring the chemical and physical stability whilst optimizing the bioaccessibility and bioavailability of the API. By using a combination of characterization techniques (e.g. DSC, XRD, DLS), *in-vitro* assays (Caco-2/HT 29 cell assays) and animal models (Sprague-Dawley rat model) we strived to balance the physical properties of the formulated API with its *in-vitro* bioaccessibility profile and its *in-vivo* pharmacokinetic performance.

Results: Initial use of standard oils (e.g. sesame oil, corn oil), needed to solubilize the crystalline API prior to emulsification, revealed not only a solubility limit (typically around 40%) which limits the CBD loading in the finished form, but also a tendency of the API to recrystallize after the drying process. In order to develop a patient-centric CBD formulation (API loading >>10wt%) a stable super saturated liquid solution of CBD with all-rac-α-tocopherol was developed for use in the emulsification process. Additional screening of emulsifiers and co-surfactants allowed us to develop stable, monodisperse emulsions with average particle sizes (D_{50}) below 300nm which were successfully dried in a gelatin/maltodextrin matrix without any API recrystallization (as measured by DSC). Stability data (5°C, 25°C and 40°C) revealed that the formulated API is both chemically and physically stable for at least 6 months. *In-vitro* cell data allowed us to assess the effect of particle size and choice of emulsifier/co-surfactant on CBD bioaccessibility and implicitly select the optimal candidates for *in-vivo* bioavailability assessment. Ultimately, *in-vivo* blood plasma levels showed a 6X increase in C_{max} and 5.5X increase in AUC 0-24hr for the formulated API vs. a commercially available CBD oil.

Conclusions: In order to develop more patient-centric solutions than those currently available on the market, we successfully developed a super saturated CBD solution, allowing us to generate stable nano-emulsions and ultimately solid oral dosage forms with a high API loading. By accurately controlling the size of the nano-emulsion and re-dispersibility of the solid dosage forms and by making use of specific co-surfactants we were able to modulate the bioaccessibility and bioavailability of the API and bring forward the next generation of CBD products capable of overcoming its poor oral bioavailability and allowing the industry to explore and expand upon the established therapeutic benefits of CBD more effectively.

TWENTY FOUR HOUR PHARMACOKINETICS OF A FULL SPECTRUM CANNABIDIOL AND CANNABIDIOLIC ACID PRODUCT: COMPARATIVE ASSESSMENT IN HUMANS AND DOMESTIC ANIMALS

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Introduction: Oral delivery of cannabidiol (CBD) is poor in humans. Cannabidiolic acid (CBDA) may have better bioavailability in oral preparations than CBD, which has been examined in domestic animals, yet not fully in humans at meaningful doses. A comparative aspect is presented below based on domestic animals and recent human data generated using the same CBD/CBDA blend.

Methods: A specific Cannabis sativa extract was provided to dogs, cats, horses, and humans at doses between 1-3 mg/kg of an equal mix of CBD/CBDA to assess relative 24 hr pharmacokinetics. Blood was drawn from the various species and serum or plasma was collected at 0,1,2,4,8,12, and 24 hrs. All samples were analyzed using liquid chromatography mass spectroscopy. Delivery vehicle utilized in humans (n=5; 1 mg/kg) and dogs (n=8; 2 mg/kg) was a sesame oil soft gel, horses (n= 8; 2 mg/kg) in a sesame oil, and for cats (n=8; 3 mg/kg) as dextrose based paste. In addition, the primary metabolite 7-COOH CBD was also assessed for Cmax.

Results: The pharmacokinetic profiles can be seen in table 1 below for CBD and CBDA. The CBDA/CBD ratio Cmax was 6.4, 3.5, 6.2 and 48 in dogs, cats, horses, and humans, respectively. Cmax values for 7-COOH-CBD for horses and humans peaked at approximately 4 hrs with a mean concentrations of 127 and 247 ng/mL both maintaining similar levels throughout the 24 hours PK. In dogs and cats the Cmax was lower despite receiving similar or higher doses on 24 hr PK assessment at 21 and 41 ng/mL at 2 and 4 hrs, respectively

Table 1: CBD and CBDA 24 hour pharmacokinetics - means and standard deviations

Cannabinoid	Dose (mg/kg)	Cmax (ng/mL)	Tmax (hrs)	T1/2 (hrs)	AUC (ng-h/mL)	MRT (hrs)
Dog CBD	1	268 ± 99	1.4 ± 0.5	3.4 ± 1.4	687 ± 218	4.4 ± 1.0
Cat CBD	1.4	282 ± 149.4	2.0 ± 0.8	2.1 ± 1.1	905 ± 528	3.8 ± 1.0
Horse CBD	1	6.0 ± 3.1	3.5 ± 2.1	5.7 ± 3.4	46 ± 22	6.4 ± 0.8
Human CBD	0.5	10.8 ±6.2	2.1 ± 0.5	2.4 ± 0.4	41 ± 24	4.2 ± 1.3
Dog CBDA	1	1,826 ± 1043	0.9 ± 0.5	2.3 ± 0.9	2768 ± 910	2.7 ± 0.8
Cat CBDA	1.1	1,011 ± 496	1.6 ± 1.1	2.7 ± 1.4	2639 ± 1295	3.3 ± 1.1
Horse CBDA	1	37 ± 33	0.7 ± 0.4	Not calc.	425 ± 135	12.8 ± 1.3
Human CBDA	0.5	519 ± 67	1.5 ± 0.4	1.2 ± 0.1	736 ± 132	2.0 ± 0.3

Conclusions: The Cmax and AUC for CBD in humans and horses appear to be proportionally similar regarding dosing, while dogs and cats Cmax and AUC appears to be higher suggesting that oral CBD delivery in the dog and the cat is superior. The Cmax for CBDA oral delivery is superior to CBD for all species with the greatest differential being humans with a 14-fold increased AUC compared to all other species with oral delivery in horses being least effective. When examining 7 COOH CBD Cmax concentrations in horses and humans vs. dogs and cats there is a 3-5 fold increase in this metabolite in horses and humans suggesting evolutionary differences in hepatic disposal of CBD. Recent research is suggesting that cytochrome p450 (CYP) metabolism in dogs may be primarily through CYP1A rather than typical CYP2 isomers observed in humans.

CANNABIS AND DRIVING: RECENT EXPERIMENTAL EVIDENCE

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Introduction: With cannabis use and policy evolving internationally, drug-impaired driving has become an increasingly relevant policy issue. An expert working group has developed a series of fact sheets addressing experimental evidence of THC use and driver impairment, epidemiological evidence on the association between cannabis use and crash risk, toxicology and detection methods of THC use, policy and legislation. *This presentation will review the experimental evidence regarding cannabis and its effects on driving performance.*

Methods: The scientific evidence on cannabis and driving was reviewed to provide objective information and inform the development of legislation to manage cannabis-impaired driving. The objective of the fact sheets, formulated by the interest group, is to address road safety, where the aim is to avoid traffic accidents caused by driving under the influence of cannabis. This objective demands a different approach, compared to drug control that aims to reveal illegal cannabis use. The activities of the cannabis interest group are endorsed by the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) and the EMCDDA.

Results: The extent to which cannabis impairs driving varies substantially depending on the dose and the individual. Regular cannabis users exhibit less impairment than occasional users with a given dose of THC and may attempt to compensate for impairment by driving more slowly and leaving a larger gap to the vehicle ahead. Large intra-individual variability and differences in cannabinoid pharmacokinetics depending on route of administration complicate precise determination of the impairment window following acute cannabis administration. Nonetheless, a recent meta-regression indicates that impairment is likely to subside within ~5 hours following inhalation of 20mg THC, and within ~8 hours after ingestion of 20mg THC. An important priority for future research is to establish whether patients using medicinal cannabis are similarly susceptible to driving impairment. While cannabis impacts lane keeping in a similar manner to alcohol at blood alcohol concentrations of ~.05%, cannabis and alcohol produce markedly distinct behavioral effects. There is no evidence to indicate that CBD reduces the impairing effects of THC, contrary to popular opinion.

Conclusion: Evidence to date suggests that cannabis produces modest driving impairment that may last for up to 5-8 hours depending on a multitude of factors such as dose, tolerance, and route of administration. Further research is required to better understand how driving is impacted by medicinal cannabis use.

CANNABIS AND DRIVING: RECENT TOXICOLOGICAL ISSUES

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Introduction: With cannabis use and policy evolving internationally, drug-impaired driving has become an increasingly relevant policy issue. An expert working group has developed a series of fact sheets addressing experimental evidence of THC use and driver impairment, epidemiological evidence on the association between cannabis use and crash risk, toxicology and detection methods of THC use, policy and legislation. *This presentation will review the toxicological issues regarding the association between biological and behavioral markers of THC use and their association the driving impairment.*

Methods: The scientific evidence on cannabis and driving was reviewed to provide objective information and inform the development of legislation to manage cannabis-impaired driving. The objective of the fact sheets, formulated by the interest group, is to address road safety, where the aim is to avoid traffic accidents caused by driving under the influence of cannabis. This objective demands a different approach, compared to drug control that aims to reveal illegal cannabis use. The activities of the cannabis interest group are endorsed by the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) and the EMCDDA.

Results: At the population level, there is evidence from experimental studies that the fraction of cannabis users that shows any degree of impairment increases with higher THC concentrations in blood. The correlation between THC concentration in blood and magnitude of impairment in individual cannabis users however is low. This association is clearest in occasional cannabis consumers and may differ in chronic frequent cannabis consumers who develop partial tolerance to the effects of THC. However, at the individual level, it is difficult to predict impairment in individual drivers from a THC concentration in blood. THC in oral fluid primarily represents coating of the mouth following inhalation of drug-laden smoke or vapour and is not associated with THC concentrations in blood or driver performance. Two to four hours after cannabis intake, coating of the oral fluid dissipates and then oral fluid THC concentrations parallel but are not the same concentration as blood THC concentrations. Urine concentrations of cannabis metabolites only identify past cannabis exposure and in no way identify THC impairment. Standardized field sobriety tests do not adequately detect THC induced driver impairment. Other behavioural tests to detect THC induced impairment are under active development throughout the world.

Conclusion: At present, no biological or behavioural test exists to reliably differentiate THC-impairment from an individual's non-drug driving performance

CANNABIS AND DRIVING: RECENT EPIDEMIOLOGICAL EVIDENCE

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Introduction: With cannabis use and policy evolving internationally, drug-impaired driving has become an increasingly relevant policy issue. An expert working group has developed a series of fact sheets addressing experimental evidence of THC use and driver impairment, epidemiological evidence on the association between cannabis use and crash risk, toxicology and detection methods of THC use, policy and legislation.

Methods: The scientific evidence on cannabis and driving was reviewed to provide objective information and inform the development of legislation to manage cannabis-impaired driving. The objective of the fact sheets, formulated by the interest group, is to address road safety, where the aim is to avoid traffic accidents caused by driving under the influence of cannabis. This objective demands a different approach, compared to drug control that aims to reveal illegal cannabis use. The activities of the cannabis interest group are endorsed by the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).

Results: The prevalence of THC-positive drivers varies from country to country, depending on the legal status of medical and/or recreational cannabis, the availability of cannabis, prevalence of cannabis use in the general population, traffic laws and their enforcement, and driving culture. In roadside surveys, THC is typically the most commonly detected recreational drug after alcohol. The prevalence of cannabis and of alcohol are generally higher in crash-involved drivers than in roadside surveys. Cannabis, when consumed alone, is associated with a modest increase in crash risk at the population level according to most studies which compared the presence versus the absence of cannabis. The increase in crash risk varies between studies, but the average increase is 30% to 40% in the latest meta-analysis, meaning that drivers who test positive for cannabis are approximately 1.3-1.4 times more likely to be involved in a crash than drivers who test negative for cannabis. Limited data suggest that crash risk increases for drivers with whole blood THC $\geq$ 5ng/mL, but further analyses are needed. The crash risk associated with alcohol is much higher than cannabis. Consistent with findings observed in experimental studies, epidemiological data demonstrate that drivers who combine cannabis and alcohol are at a very high risk of crashing. Limitations of epidemiological data and analysis will be discussed.

Conclusion: At a population-level, epidemiological data suggest that when consumed alone, cannabis use is associated with a modest increase in crash risk and, when consumed in combination with alcohol, is associated with much higher crash risk. Further epidemiological research exploring THC levels at which crash risk is increased are needed.

GENETIC BASIS FOR PRODUCTION OF THCV, A RARE AND POTENTIALLY MEDICALLY VALUABLE CANNABINOID

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Introduction: Tetrahydrocannabivarin (THCV) is a rare cannabinoid that has medical potential as a neuroprotectant, anti-inflammatory, and notably as a therapeutic to improve glycemic control in type 2 diabetic patients. Based on anecdotal evidence of THCV leading to increased activity levels and motivation, we initiated and will briefly discuss the preliminary results from a double blind crossover study investigating the effectiveness of THCV in this area. In order to develop botanical formulations, we have undertaken research to understand the genetic basis of THCV production and to breed plants with high levels of THCV. THCV is a homologue of THC that differs only in the length of the alkyl side chain (3C vs 5C, respectively). Determination of alkyl side chain length depends on differences in early precursor molecules, yet the same downstream cannabinoid synthase genes are utilized for synthesis of final products. While cannabis plants can produce up to one-third of their dry weight in cannabinoids, very few produce appreciable quantities of THCV (5-6% by dry weight). We identified genetic markers for genes involved in the pathway for production of propyl (3C) cannabinoids and used them to breed cultivars with >18% THCV.

Methods: We assessed cannabinoid content via HPLC in several hundred commercially available cannabis plants that were grown in a greenhouse. We genotyped all plants with a proprietary 55k SNP Illumina BeadArray. We initially performed nested association mapping based on 302 accessions (67 seed lots). We then performed nested association mapping on an additional 191 accessions (21 seed lots) which were selected to have at least one beneficial allele of a marker associated with propyl cannabinoids identified in the initial round of mapping.

Results: Two genetic markers flank the KR/FabG1 gene in the cannabis genome; both are statistically significantly associated with THCV content and were validated in additional THCV-producing germplasm. Nested association mapping of propyl cannabinoids in plants with at least one beneficial allele at KR-associated markers identified four additional markers which, in combination with the KR-associated markers, explain variation in propyl cannabinoid production. We have deployed these genetic markers in our breeding program and developed plants with >18% THCV and increased the ratio of THCV:THC to 5:1.

Conclusions: The KR gene is a strong candidate for influencing THCV production. In Arabidopsis this enzyme is integral for fatty acid biosynthesis cycles that iteratively elongate alkyl-chains by two carbon atoms per cycle. In cannabis, plastid fatty acid biosynthesis forms precursor molecules with the final alkyl chain length. In combination with KR, beneficial alleles near four additional markers significantly increase propyl cannabinoid production and the ratio of propyl to pentyl cannabinoids. Marker-assisted selection allowed us to combine beneficial alleles from diverse backgrounds and select for plants with high levels of THCV. Formulations from these plants are being used in our clinical study of THCV.

Acknowledgements: Work funded by Phylos Bioscience.

PHYTOCANNABINOIDS AS INHIBITORS OF OXIDOREDUCTASES: NOT JUST NAD(P)H BINDING SITE INHIBITORS

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Introduction: Deconvolution of phytocannabinoid actions is essential for better understanding of observed effects *in vivo*, and can help to further optimise therapeutic use. So far, most target-based phytocannabinoid screening has focused on receptors (GPCRs, ion channels, nuclear receptors, etc.), with very few enzymes explored as possible phytocannabinoid targets. Oxidoreductases are a diverse set of enzymes with broad functions, including degradation of neurotransmitters (monoamine oxidase, MAO) and innate immune responses (myeloperoxidase; MPO). The mechanism of action of several approved drugs is the inhibition of specific oxidoreductases. We previously identified oxidoreductases as possible targets of phytocannabinoids (Martin, Cairns, et al., *J Nat Prod* 2021, 84 (5)), although apart from one enzyme (lactate dehydrogenase), this hypothesis was not tested experimentally. This study sought to investigate the effects of a series of major and minor phytocannabinoids at additional selected oxidoreductases.

Methods: Fluorescent, red-shifted probes were employed to measure activity at recombinant human enzymes. Inhibition studies (10 μM) were performed using substrate concentrations at or below K_M to minimise confounds due to reversal of reaction. "Hits" were identified based on >40% inhibition, with IC_{50} then determined. Inhibition of MAO-A and MAO-B were investigated with tyramine, dopamine, and serotonin as substrates, clorgiline and pargyline as positive control inhibitors, and Ampliflu Red to measure activity. Inhibition of MPO used H_2O_2 as a substrate, AZD3241 as a positive control, and 3'-(p-aminophenyl)-fluorescein (synthesized by Eric Sparkes) to measure activity. All compounds were screened against horseradish peroxidase to confirm lack of non-specific inhibition.

Results: Neutral, varin, acid, and varinic acid forms of THC, CBD, CBG were screened, as were CBN, CBNA, CBC, CBCV, CBGO, and CBL. THC inhibited MAO-B tyramine-initiated activity (IC_{50}=1.46 μM), while CBNA inhibited MAO-A activity with either tyramine (IC_{50}=10.3 μM) or serotonin (IC_{50}=773.4 nM). No other cannabinoids had appreciable activity at either MAO. THC, CBD, and CBG significantly inhibited MPO *in vitro* (IC_{50}=8.9 μM, 10.6 μM, and 12.4 μM respectively), although less potently than the known inhibitor AZD3241 (106.1 nM).

Conclusions: Inhibition of enzymes by phytocannabinoids should not be underestimated as plausible mechanisms of actions for their observed *in vivo* effects. Our data demonstrates that phytocannabinoids may interact with several types of oxidoreductases, not limited to a single subgroup (e.g., NAD(P)H). Work investigating the implications of inhibitory actions at MAO and MPO *in vivo* is ongoing.

Acknowledgements: Supported by the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded centre for medicinal cannabis research at The University of Sydney.

IN UTERO EXPOSURE TO CANNABIDIOL LEADS TO GLUCOSE INTOLERANCE AND ALTERED HEPATIC TRANSCRIPTOME EXCLUSIVELY IN MALE RAT OFFSPRING AT 3-MONTHS OF AGE

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Introduction: With the legalization of recreational cannabis usage in Canada, there has been an increase in its use during pregnancy. This is concerning given maternal cannabis use is associated with decreased birth weights and dysregulation of glucose levels in the offspring. Specifically, the contributions of cannabidiol (CBD), the primary non-psychoactive compound in cannabis, remain elusive. Therefore, this study aimed to assess if gestational exposure to CBD alone results in postnatal dysglycemia in the offspring.

Methods: Pregnant Wistar rat dams received daily intraperitoneal (i.p.) injections of vehicle or CBD (3 mg/kg) from gestational day 6 through parturition. At 3-months of age offspring underwent an i.p. glucose tolerance test (ipGTT). At 3-weeks and 3-months offspring were sacrificed and pancreata collected for immunohistochemical analysis. Livers were also collected at 3-months for bulk RNA-sequencing (Genome Quebec), and downstream differential expression analysis, Over-representation Analysis, and Gene Set Enrichment Analysis.

Results: Exposure to 3 mg/kg CBD *in utero* led to an increased area under the ipGTT curve exclusively in 3-month-old male offspring, indicating glucose intolerance. However, the CBD exposed male offspring exhibited no deficits in pancreatic β or α cell mass, suggesting that the observed glucose intolerance may be due to dysregulation of peripheral glucose control. In the livers of CBD exposed males, bulk RNA-sequencing indicated significant changes in the expression levels of genes involved in insulin signaling, circadian regulation of gene expression, and liver development.

Conclusion: Overall, our data suggests that exposure to CBD in gestation leads to glucose intolerance in male offspring at 3 months of age, possibly through dysregulation of insulin signaling and development in the liver. Collectively, these results indicate that despite its increased popularity, exposure to CBD alone in pregnancy may be detrimental for postnatal metabolic health.

Acknowledgements: Funded by OGS and OGGS scholarships, as well as a CIHR and a Heart and Stroke Foundation grant.

ADMINISTRATION OF N-ACETYLCYSTEINE PREVENTS THE LONG-LASTING DEPRESSIVE-LIKE PHENOTYPES INDUCED BY ADOLESCENT THC EXPOSURE

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Introduction: Clinical and pre-clinical evidence has shown that youth who consume cannabis during vulnerable windows for neurodevelopment are more susceptible to developing anxiety and depression later in life. Previous studies have demonstrated that adolescent exposure to delta-9-tetrahydrocannabinol (THC) can induce profound dysregulations in nucleus accumbens (Acb), a critical brain area for depression and drug addiction. Moreover, the functional dissociation between the two Acb subregions, shell (AcbSh) and core (AcbC), may underlie differences in THC-related pathophysiology. Beside the critical need of increasing knowledge about the long-term outcomes of adolescent THC exposure, there is also the urgency of developing potential strategies to prevent the THC-induced side effects. N-acetylcysteine (NAC), an over-the-counter supplement known for its antioxidant properties and for modulating glutamate transmission, has been suggested as a safe intervention to ameliorate the symptomatology of both neuropsychiatric and cannabis use disorders. The present study aims to investigate the neuroprotective properties of NAC against the THC-induced maladaptation and explore potential mechanisms underlying these effects.

Methods: Adolescent male Sprague Dawley rats were treated from postnatal day (PND) 35 to 45 with increasing doses of THC (PND 35-37 2.5 mg/kg, PND 38-41 5 mg/kg, PND 42-45 10 mg/kg, i.p., twice a day) or vehicle. Between PND 35 and 65, a subset of vehicle and THC-treated rats had *ad libitum* access to NAC dissolved in drinking water (0.9 g/L). At adulthood (PND 75), rats underwent a battery of behavioral paradigms to assess depressive- and anxiety-like phenotypes. *In vivo* electrophysiological recordings were performed to investigate the neural activity of putative GABAergic neurons in AcbSh and AcbC. Molecular assays in AcbSh and AcbC targeting relevant neurotransmitters and biomarkers associated with cannabis-related dysregulations are currently in progress.

Results: Preliminary data revealed that adolescent THC exposure induced a longer immobility in the forced swimming test and a higher latency to feed during the novelty suppressed feeding test at adulthood. These abnormalities were fully prevented by concomitant administration of NAC and THC. On the other hand, rats treated with either THC or NAC+THC spent less time and did less entries in the open arms of the elevated plus maze as well as showed an increase in freezing behaviour during the contextual fear conditioning paradigm compared to their vehicle groups. Analysis of the neural activity of the GABA cells did not reveal any THC-induced abnormalities in both AcbSh and AcbC. However, NAC exposure decreased the firing frequency of GABAergic population in AcbC compared to the other group treatments.

Conclusions: Our findings suggest that NAC might be an effective intervention against the adolescent THC-induced depressive-like symptoms and further investigations on relevant signaling pathways are crucial to understand these protective outcomes.

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EFFECTS OF PRENATAL TETRAHYDROCANNABINOL (THC) VAPOUR EXPOSURE AND HIGH-FAT DIET ON RAT FEEDING PATTERNS, ADIPOSITY AND GLUCOSE METABOLISM

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Introduction: It is reported that between 4-20% of people use cannabis during pregnancy, particularly for managing side effects, managing pre-existing conditions, and/or for recreational purposes. At present, there are many unanswered questions surrounding the long-term effects of prenatal cannabis exposure (PCE), therefore additional research is essential to assess associated risk and develop more appropriate guidelines. Both clinical and pre-clinical evidence suggests that PCE is associated with increased risk for development of obesity and diabetes later in life. However, this association has not been fully explored. Therefore, the aim of this study was to characterise the effects of PCE on adiposity, feeding patterns, and glucose metabolism, and assess if an interaction between PCE and high-fat diet (HFD) exists, with focus on potential sex differences.

Methods: Pregnant Sprague Dawley rats were exposed to vapour from a Δ9-tetrahydrocannabinol (THC)-dominant cannabis extract (100mg/ml THC) or vehicle (polyethylene glycol) for 15-min/day across the entire gestational period. Our lab has previously characterised the maternal-fetal transmission of THC in this model. Age-matched male and female offspring from THC ('PCE', n=12 for each sex) and vehicle ('Control', n=12 for each sex) exposed pregnant dams were maintained on standard chow and subjected to testing in adulthood. *Chow Testing:* To assess adiposity, body weight and plasma leptin levels were measured; to assess energy intake, daily chow intake patterns were measured; and to assess glucose metabolism, rats were subjected to a glucose tolerance test (GTT), and blood glucose and plasma insulin levels measured over time. *HFD Access:* Following chow testing, half of the rats in each group were given high-fat diet access ('HFD', 65% fat, n=6 for each treatment & sex), and the other half given low fat diet access ('LFD', 10% fat, n=6 for each treatment & sex) for 4-months. During this time, body weight and energy intake was tracked. *HFD Testing:* Rats were re-subjected to adiposity, energy intake and glucose metabolism measurements (as described above). Upon euthanasia, white adipose tissue (WAT) was removed and weighed to directly measure adiposity.

Results: *Chow Testing:* We showed that PCE improved glucose metabolism in both sexes, demonstrated by a reduction in peak blood glucose levels during GTT. In addition, PCE increased total daily chow intake in both sexes, indicating energy balance dysfunction. However, alterations in energy intake were not accompanied by differences in body weight or plasma leptin levels, implying that PCE did not influence adiposity under these conditions. *HFD Access:* Rats were then given access to the HFD or LFD for 4-months. Rats on HFD showed the stereotypical 'diet-induced obesity' phenotype: increased energy intake, body weight gain, plasma leptin levels and WAT mass. Interestingly, irrespective of diet or sex, PCE decreased body weight gain over time, implying that PCE blunted growth trajectories during adulthood. *HFD Testing:* After 4-months of HFD or LFD access, PCE was associated with changes in glucose metabolism that were both diet and sex dependent. In males, PCE *impaired* glucose metabolism, where PCE increased GTT blood glucose levels, and this was further amplified by HFD. In females, irrespective of diet, PCE *improved* glucose metabolism, where PCE decreased GTT blood glucose levels, and was accompanied by a significant increase in plasma insulin. In contrast to 'chow testing' findings, PCE had no effect on total daily HFD or LFD intake, and PCE did not influence plasma leptin levels or WAT mass with either diet or sex, suggesting that PCE did not alter energy balance or adiposity with HFD.

Conclusions: Regardless of sex, PCE improved glucose metabolism and increased daily chow energy intake but had no effect on adiposity in chow-maintained adult rats. Further, these PCE effects were influenced by diet, as PCE *impaired* glucose metabolism in males on HFD but *improved* glucose metabolism in females on HFD diet, indicating a significant interaction between PCE, sex and HFD with respect to glucose metabolism. Moreover, PCE did not alter HFD intake, or post-HFD adiposity irrespective of sex, implying that there was no interaction between PCE, sex and HFD with respect to energy balance or adiposity. Overall, this data implies that PCE has particularly prominent effects on glucose metabolism in adulthood, enhancing our understanding of the long-term physiological effects of PCE.

A RANDOMIZED CONTROLLED TRIAL ON THE EFFECT OF A 20:1 CANNABIDIOL (CBD): Δ^9-TETRAHYDROCANNABINOL (THC) MEDICATION (CANNEPIL®) ON NEUROCOGNITION, ATTENTION AND MOOD

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Introduction: As cannabinoid-based medications gain popularity in the treatment of refractory medical conditions, it is crucial to examine the neurocognitive effects of commonly prescribed products to ensure associated safety profiles. The present study aims to investigate the acute effects of a standard 1mL sublingual dose of CannEpil®, a 20:1 cannabidiol (CBD) to Δ9-tetrahydrocannabinol (THC) medicinal cannabis oil, on neurocognition, attention, and mood.

Methods: A randomised, double-blind, placebo-controlled, within-subjects design assessed 31 healthy participants (16 female, 15 male), aged between 21 and 58 years, over a two-week experimental protocol. Neurocognitive performance outcomes were assessed using the Cambridge Neuropsychological Test Automated Battery, with the Profile of Mood States questionnaire, and the Bond-Lader Visual Analogue Scale used to assess subjective state and mood. Plasma concentrations of CBD, THC, and their metabolites were also measured.

Results: CannEpil increased Total Errors in Spatial Span and Correct Latency (median) in Pattern Recognition Memory, while also increasing Efficiency Score (lower score indicates greater efficiency) relative to placebo (all $p < .05$). Subjective Contentedness ($p < .01$) and Amicability ($p < .05$) were also increased at around 2.5 hours post dosing, relative to placebo. Drowsiness or sedative effect was reported by 23% of participants between three to six hours post CannEpil administration.

Conclusions: CannEpil impairs select aspects of visuospatial working memory and delayed pattern recognition in healthy adults, while largely preserving mood states. Intermittent reports of drowsiness and sedation underscore the inter-individual variability of medicinal cannabis effects on subjective state.

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GENDER DIFFERENCES IN CANNABIS AND ALCOHOL USE PATTERNS RELATED TO MENTAL HEALTH OUTCOMES IN A COMMUNITY SAMPLE USING CANNABIS FOR ANXIETY

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Introduction: The public is increasingly using cannabis medicinally for anxiety. However, reviews on the effects of cannabinoids on anxiety remain mixed, and the need for data on the risks or benefits of using legal market products to treat anxiety continues to grow. Meanwhile, anxiety is highly comorbid with alcohol use disorder (AUD). The presence of AUD can double the odds of having an anxiety-related disorder, with documented gender differences between men and women. Thus, this study examined associations between cannabis use, alcohol use, and mental health among a sample using legal market cannabis to treat symptoms of anxiety.

Methods: This data draws from a longitudinal study of cannabis use, anxiety symptoms, and inflammation among individuals with mild to moderate generalized anxiety. Participants completed baseline self-report measures on cannabis and alcohol use in the prior two weeks, measures of affective disturbance, and a blood draw for inflammatory markers.

Results: 182 participants completed the baseline session (M_{age}= 31.5, SD=12.6; 58.8% female and transgender). Women had significantly lower rates of affective disturbance, less use of cannabis and alcohol, and lower levels of both pro- and anti-inflammatory cytokines than men. Alcohol Use Disorder for the whole sample was positively, significantly related to Cannabis Use Disorder (CUD; r=0.19, p=0.01), but only remained significant for men when stratified by gender (r=0.37, p=0.001). Total cannabis use days and days of co-use of cannabis and alcohol were both positively, significantly related to CUD as well (rs=0.33 and 0.24, ps<0.001). Only total cannabis use days, and not co-use days or number of alcohol drinks, were significantly related to increased affective disturbance (Beck Depression Inventory, Beck Anxiety Inventory, and Perceived Stress Scale; rs=0.24, 0.17, 0.15, ps=0.001, 0.030, 0.046 respectively), and this association remained significant for men only once stratified (rs=0.28, 0.34, ps=0.02, 0.005). Levels of pro- and anti-inflammatory cytokines were not significantly related to any symptom measures or substance use patterns.

Conclusions: In the current study, problematic alcohol use was related to problematic cannabis use, but only for men when the sample was stratified by gender. Cannabis use days, but surprisingly not co-use days or number of drinks, were related to multiple measures of affective disturbance, and again only remained significant for men once stratified. Notably, men also had significantly higher levels of affective disturbance, cannabis and alcohol use, and circulating cytokines. This study extends the previous literature to a sample of medically motivated cannabis users that is more balanced in gender, and suggests important links between alcohol and cannabis use and the experience of affective disturbance, and how those experiences may differ by gender. Although cross-sectional, this data suggests use patterns among anxious women may be less problematic than among men, who in this sample are using at higher rates and have stronger associations between use and affective disturbance. Future work should further investigate how alcohol and cannabis use patterns among men using for anxiety may confer more risk for CUD and AUD.

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CHARACTERIZING WITHDRAWAL FROM VAPORIZED CANNABIS FLOWER IN ADULT RATS

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Cannabis use has been associated with physical dependence and withdrawal in humans. Previous studies have tried to model withdrawal in animals using repeated injections of pure synthetic Δ^9-tetrahydrocannabinol (THC), while little work has focused on the withdrawal effect of inhaled cannabis flowers. Considering the vast pharmacokinetic difference between the injection of THC and the inhalation of cannabis flower smoke or vapour, this study aimed to assess whether CB1 antagonist SR141716 (rimonabant) can induce precipitated withdrawal in vaporized cannabis flower exposed rats. In this study, two groups of rats were used (a control group n=8 and a high-THC cannabis group n=8) Animals received either high-THC cannabis vapour (Truro Wedding mint, 33.0% THC) (1.5 g) or air three times a day every 8 hours for 7 days. On the last day, rimonabant was administered intraperitoneally (3mg/kg) and 30 minutes later animals were recorded for 10 minutes to assess somatic signs of withdrawal (eye blinks, paw tremors, body shakes, ptosis, grooming, and scratches). The study also assessed body temperature, locomotor activity, tail flick latency as well as sucrose intake. Results showed rimonabant induced an increase in the total number of somatic signs (eye blinking, head and body shake as well as tremors) in rats that were repeatedly exposed to high-THC cannabis vapour compared to the control rats. Further, cannabis vapour-exposed rats also showed lower sucrose intake compared to their baseline consumption showing potential anhedonia-like effects. Taken together, this study showed that rats repeatedly exposed to vaporized high-THC cannabis flower exhibit precipitated withdrawal.

CORRELATES OF CANNABIS USE DISORDER AND CANNABIS USE AMONG ADOLESCENTS WITH BIPOLAR DISORDER AND MAJOR DEPRESSIVE DISORDER IN THE NATIONAL COMORBIDITY SURVEY-ADOLESCENT SUPPLEMENT (NCS-A)

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Background: Despite substantial evidence regarding prevalence and correlates of cannabis use disorder (CUD) and cannabis use (CU) among adults with bipolar disorder (BD) and major depressive disorder (MDD), little is known about this topic among adolescents.

Methods: The 2001-2004 National Comorbidity Survey—Adolescent Supplement, an in-person survey of mental disorders, implemented a modified version of the Composite International Diagnostic Interview. Participants included adolescents, 13-18 years of age, with BD-I or BD-II (n=295), MDD (n=1112), or general population controls without mood disorders (n=8716). Analyses examined prevalence and correlates of CUD and CU within the BD and MDD groups.

Results: CUD was most prevalent in BD followed by MDD then controls. CU was most prevalent in MDD followed by BD then controls. Several correlates of CUD/CU were evident in both the BD and MDD groups, including conduct disorder, alcohol use disorder, and treatment for depression. BD-specific correlates of CUD/CU included longer depressive episodes, more past-year mania/hypomania, and lower rates of treatment for mania/hypomania. MDD-specific correlates of CUD/CU included history of hospitalization for depression, suicidal ideation and attempts, physical or sexual abuse/assault, lifetime smoking, and past-year stimulant use. Clinical correlates of CUD and CU were largely similar.

Conclusion: CUD and CU are both associated with multiple adverse clinical characteristics in adolescents with BD and MDD in the general population. Evidence that risks of cannabis extends across the spectrum of use is particularly important for adolescents with BD and MDD in whom the consequences have potential to be more severe.

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THE EFFECTS OF CANNABIDIOL ON SOCIAL HEALTH: A DOUBLE-BLIND, RANDOMIZED PLACEBO CONTROLLED TRIAL

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Introduction: Millions of Americans suffer from social anxiety (Stein et al., 2017). The anxiolytic effects of cannabidiol (CBD), a non-intoxicating molecule of the *Cannabis sativa* L. plant, in the context of social anxiety are well-established (Blessings et al., 2015; Linares et al., 2019; Zuardi et al., 2017). To date, it appears that acute, 300mg doses of CBD attenuate socially-relevant threat responding in healthy samples. However, 600mg outperformed placebo in a sample of participants with social anxiety disorder (Bergamaschi et al., 2011). Further, there is promising but limited data from rigorous studies with regard to the effects of repeated dosing in anxious samples (e.g., Masataka et al., 2019). However, no study has examined the effects of CBD on social health, among a sample elevated in trait worry, a defining, cognitive feature of anxiety.

Methods: The current study examined the effects of 300mg CBD, 50mg CBD, and placebo administered daily for 14 days on social health as a secondary analysis of data drawn from a larger study that was designed to examine the effects of CBD on worry among a sample of high trait worriers. Participants (N = 63; M_{age} = 29.27; SD_{age} = 9.58) were elevated in trait worry, meeting a comprehensive list of eligibility criteria (e.g., no past month CBD or THC use). Participants were randomly assigned to condition (300mg CBD, 50mg CBD, placebo) and reported social health (World Health Organization Quality of Life-Brief [WHO-Social]; World Health Organization, 2004) at baseline (prior to randomization), and day 14.

Results: Mixed effects models revealed there was a significant time by condition interaction for 300mg vs 50mg (β = -1.46, t = -2.30, $p < 0.05$, η^2 = 0.12) and 300mg vs placebo (β = -1.71, t = -2.71, $p < 0.01$, η^2 = 0.12). Simple slope tests revealed significant increases in WHO-Social scores from baseline to week two among the 300mg (β = 2.29, CI[1.39, 3.18]), but not 50mg (β = 0.83, CI[-0.07, 1.72]) condition or placebo (β = -0.57, CI[-0.33, 1.47]). Post hoc pairwise trend comparisons revealed the 300mg condition reported significantly greater increases in WHO-Social scores vs placebo (β = 1.71, t = 2.71, $p < 0.05$, d = 1.36), but no significant difference in trend vs 50mg condition (β = 1.46, t = 2.30, p = 0.063, d = 1.36). The trend of WHO-Social score within the 50mg condition did not significantly differ from placebo (β = 0.25, t = 0.40, p = 0.915, d = 0.45).

Conclusions: These data suggest 300mg of CBD improved social health compared to 50mg of CBD and placebo after two weeks of administering CBD. These findings fit with evidence suggesting that CBD attenuates anxious arousal in the context of social anxiety (Blessings et al., 2015; Linares et al., 2019; Zuardi et al., 2017) and extends the literature by suggesting CBD has the potential to improve social health among a vulnerable, untested sample. Future work should further explore the effects of CBD on social health across various vulnerable samples.

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THE EFFECT OF FREQUENT CANNABIS-USE ON COGNITIVE-MOTOR TASKS

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Introduction: Since the legalization of recreational use of cannabis took effect in Canada, many questions have been brought forward regarding its immediate and sustained effect on daily tasks.

Methods: To investigate the effect of cannabis on various brain functions, we created a battery of cognitively demanding, visual-spatial and visual-motor tasks (Go/No-Go, Serial Visual Search, N-Back, Trail Making, and Task Switching). Here, we discuss preliminary findings of two tasks. The first is a speeded Go/No-Go task (80% go, 20% no-go) that measures the ability to inhibit motor impulses. Task performance is analyzed by comparing the proportion of false alarms on no-go trials as well as reaction times on hits and false alarms in frequent cannabis users (N=144), infrequent users (N=111), and non-users (N=469). The second task is a spatial N-Back task (1-Back, 2-Back, & 3-Back) which assesses visuospatial working memory and working memory capacity. This task is analyzed by comparing the proportion and reaction times of correct and incorrect trials in frequent cannabis users (N=29) and non-users (N=51).

Results: Our findings indicate that frequent users, infrequent users, and non-users performed similarly on these tasks. These results may suggest the absence of a negative effect of cannabis on performance of impulse inhibition.

Conclusions: These results may suggest that frequent cannabis-use is not associated with working memory impairments. While there might be immediate effects of cannabis use, our preliminary results show little to no prolonged effects of cannabis on spatial working memory and impulsivity control.

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NOVEL CANNABIGEROL (CBG) DERIVATIVE DM300 OUTPERFORMS CBG IN PHARMACOKINETICS (PK), EFFICACY AND SAFETY FOR INFLAMMATORY BOWEL DISEASE (IBD)

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Introduction: The naturally produced phytocannabinoid cannabigerol (CBG) reportedly holds wide ranging pharmacologic potential. However, oral bioavailability is a critical hurdle, and safety concerns includes cyclooxygenase enzymes (COX-1 or -2) inhibition which are related to gastrointestinal (GI) ulcers and bleeds. Here we present data on a novel, orally bioavailable derivative of CBG named DM300. In contrast to CBG, DM300 is highly bioavailable and demonstrates robust efficacy in multiple models of inflammatory bowel disease (IBD) while lacking COX inhibition.

Methods: PK study: Rats were dosed by oral gavage and timed plasma samples were taken for LC MS/MS analysis for CBG or DM300. Indomethacin-Induced Crohn's Disease (CD) in Rats (RIBD)**:** Rats received injections of indomethacin, inducing the expected CD model phenotype in the small intestine. Body weight was measured along with small intestine weight, gross score, and histopathology. TNBS (2.4.6.-trinitrobenzenesulfonic acid) model: Mice received intracolonic TNBS to induce the UC model phenotype in the colon. Disease activity index (DAI) was calculated, and colons were measured for weight/length and histopathology scoring. COX Inhibition Assays: Human recombinant Sf9 cells were induced with arachidonic acid (1.2 μM)+ ADHP (25 μM) for 3 or 5 min and Resorufin (oxidized ADHP) was detected by fluorimetry. DM300, CBG and controls were tested at several concentrations for IC_{50} determinations.

Results: The peak plasma in rats dosed orally with 30 mg/kg of DM300 was 5,260 ng/mL and the area under the curve (AUC) was 20,284 h*ng/mL, while the same parameters for CBG were 104.4 ng/mL and 254 h*ng/mL respectively. DM300 dosed orally at 50, 200, or 800 mg/kg resulted in statistically significant dose-responsive beneficial effects in the RIBD model with reductions in small intestine necropsy parameters (weight and score), histopathology parameters (73-99% reductions in inflammation and necrosis at 200 or 800 mg/kg), and cytokine levels (IL-1β, IFNγ, and TNFα). Conversely, CBG treatment in the RIBD model resulted in severe toxicity and unexpected death prior to experiment completion. In addition, DM300 dosed orally at 50 mg/kg in the mouse TNBS model showed reductions in colon weight/length ratio, and significantly improved stool consistency, fecal blood score, body weight loss and disease activity index (DAI). CBG demonstrated no differences in colon weight or length from disease control but did result in improved DAI. In Sf9 cells recombinant for human COX-1 or -2 inhibition was undetectable for DM300. The IC_{50} for CBG was 3.0 μM for COX-2. COX-1 inhibition by CBG was not determined by us but has been demonstrated in the literature.

Conclusion: Based on these data DM300 has less toxicity, greater specificity, and enhanced efficacy in comparison with and CBG, especially for CD.

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SAFETY OF ORALLY ADMINISTERED CANNABINOID-BASED MEDICINES: ADVERSE DRUG REACTIONS IN CLINICAL PRACTICE

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Introduction: Existing evidence points towards a favourable safety profile of cannabinoid-based medicines (CBMs), with common adverse effects likely to be mild, such as somnolence, dizziness and dry mouth. Despite the growing popularity of CBD wellness products, severe adverse effects can occur with CBD as well as THC-based oral products. Furthermore, the safety of CBD is generally assessed without consideration of its common combination with THC and specific dosages of THC and CBD are rarely reported in current literature. In this study, we investigated adverse drug reactions (ADRs), cannabinoid profile and dosage reported by patients taking oral CBMs under direct medical supervision.

Methods: Between July 2020 and October 2021, 1993 adult patients consented to participate in the study and were authorized to receive at least one CBM oral product (i.e. oral extracts) at a network of four medical cannabis clinics. In the Canadian medical cannabis market CBD-dominant oral products include those with a minimum ratio of THC:CBD 1:20. ADRs were systematically collected after the initial visit, including details on ADR terms, severity, causality, outcome, seriousness, and details of the suspected product. Association between two categorical variables was assessed with Chi-squares tests (X^2).

Results: 510 patients (24.3%) reported a total of 858 ADRs associated with oil products. Almost half (44%) of ADRs were reported at the 1-month follow-up visit (FUP) and 31% at the 3-month FUP. The majority of ADRs were mild (70%), almost all were possibly (52%), or probably (38%) related to CBMs (Table 1). There was a significant association (ps <0.03) between headaches and palpitations and the product profile (CBD-dominant versus THC:CBD 1:1 or THC-dominant). The proportion of these ADRs was significantly higher with CBD-dominant products than expected (Table 2). Four serious ADRs (SADRs) were reported as they led to a hospitalization: 1. Pulmonary embolism assessed as unlikely related to a CBD-dominant oil (1mg THC and 42mg CBD), 2. Nausea and diarrhea probably related to a THC-dominant oil (79 mg THC, 1.5 mg CBD), 3. Tachycardia probably related to a THC-dominant oil (26.3mg THC and 0.5mg CBD), and 4. Vomiting possibly related to a CBD-dominant oil (0.5 mg THC, 20 mg CBD) and a THC:CBD balanced inhaled dried flower. Treatment was interrupted temporarily in each case but later restarted with a conservative protocol, except for the THC-dominant oil which was ceased completely. All SADRs were recovered by the time of data extraction. Further analysis of dose-dependent effects and co-variant effects of patient demographics such as age, comorbidities, and previous cannabis experience will be completed and presented.

Conclusions: While CBMs are generally safe, this study shows that a significant number of ADRs, including SADRs, can occur even with a controlled, conservative supervision of treatment initiation and titration. Like any other medication, medical supervision by fully trained health care professionals is required to ensure appropriate follow-up of cannabinoid therapies. Controlled data collection and analysis from dedicated cannabis clinics where a high-volume of patients are seen provides an excellent opportunity to improve understanding of the safety of CBMs to determine clinical guidelines that are representative of current medical cannabis products.

A SYSTEMATIC REVIEW AND META-ANALYSIS OF CLINICAL TRIALS INVESTIGATING ACUTE CANNABIDIOL (CBD) IMPAIRMENT: COMPARISON WITH PLACEBO AND DELTA-9-TETRAHYDROCANNABINOL (THC)

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Introduction: Cannabidiol (CBD) is widely believed to be non-impairing. However, there has not been a synthesis of research evaluating the acute effects of CBD on performance and cognition. A meta-analysis was conducted to synthesize available evidence on the magnitude of impairment associated with acute CBD consumption to inform both public and workplace policy.

Methods: Studies were identified through systematic database searches of AMED, EMBASE, CENTRAL, PsychINFO, CINAHL, Clinicaltrials.gov, Medline, MedRxiv, and Web of Science. Adult clinical trials measuring impairment within 0-8 hours after acute CBD administration were included. Measures of impairment included self-report, researcher observation, or objective neurocognitive or psychomotor assessments. The primary outcome was the peak mean difference in impairment measures between CBD and placebo. Effect estimates were calculated using Hedges' g. Pooled estimates were calculated using robust variance estimation (RVE) meta-regression. Moderator analyses were conducted to assess the effect of measure type (subjective vs objective), testing domain, and dose. In studies that included acute THC dose conditions, a secondary analysis was carried out utilizing THC as a positive control for comparison to CBD.

Results: A total of 11,355 studies were screened, 16 studies met inclusion criteria for quantitative synthesis; 8 included a THC dose condition. Included trials were placebo-controlled crossover studies. Doses ranged from 12.5 mg to 4500 mg CBD. The omnibus RVE meta-analysis indicated a significant and small effect size for impairment following acute CBD consumption compared to placebo (Hedges' g = 0.125, 95% CI 0.026 – 0.223, $p = 0.017$). In comparison, the positive control condition of acute THC administration (doses ranged from X to X) had a significant and moderate effect size (Hedges' g = 0.339, 95% CI 0.0728 – 0.605, $p = 0.02$). A significant moderator effect was observed for measure type. This effect reflected larger mean differences between CBD and placebo when subjective measures were used (Hedges' $g_{Subjective} = 0.295$ versus Hedges' $g_{Objective} = 0.052$). When examining effect sizes by neurocognitive test, a small but significant impairment effect was found for tests assessing attention (Hedges' g = 0.041), episodic and semantic memory (Hedges' g = 0.066), and working memory (Hedges' g = 0.046). A slightly larger effect was found for subjective measures of sedation (Hedges' g = 0.334). CBD dose was not a significant moderator (p = 0.767).

Conclusions: Acute CBD consumption was associated with a small, but statistically significant, impairment in cognition compared to placebo. Interestingly, this effect appears to be primarily driven by subjective ratings of sedation. The observed effect for CBD was smaller compared to THC. In order to inform future policy, further investigations should evaluate if the statistical significance translates into functional significance. Additionally, research should examine if tolerance due to consistent CBD use diminishes the observed effect.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOME ANALYSIS OF MEDICAL CANNABIS THERAPY IN CHRONIC PAIN PATIENTS WITH AND WITHOUT SLEEP IMPAIRMENT

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Introduction: In the UK, chronic pain affects an estimated 35.0–51.3% of patients, of which 67–88% experience disruption to sleep. Evidence suggests poor sleep can exacerbate pain severity and its effects on health-related quality of life. Previous studies have demonstrated the therapeutic potential of cannabis-based medicinal products (CBMPs) in separately managing chronic pain and insomnia. However, there has been limited evaluation comparing the effects of CBMPs in those with chronic pain and co-morbid sleep disruption to those with normal sleep quality. This study therefore aims to assess changes in patient related outcome measures (PROMs) following CBMP treatment in chronic pain patients with and without sleep impairment.

Methods: A cohort study of chronic pain patients from the UK Medical Cannabis Registry was performed. Primary outcomes included changes at 1, 3, 6, and 12 months from baseline using a repeated measures one way analysis of variance test and mean differences between the two arms using an independent t-test in the following PROMs: single-item sleep quality scale (SQS), General Anxiety Disorder-7 (GAD-7), EQ-5D-DL, Brief Pain Inventory (BPI), and McGill Pain Questionnaire-2 (MPQ-2). An alpha value<0.050 was statistically significant.

Results: Chronic pain patients enrolled within the UK Medical Cannabis Registry for >12 months (n=1139) were assigned to impaired sleep (n=517) and unimpaired sleep (n=622) cohorts. The mean baseline SQS score was 1.99 (95%CI: 1.90–2.09) in the sleep impaired cohort compared to 6.06 (95%CI: 5.92–6.19) in the sleep unimpaired group. After 12 months, an increase of 1.37±2.20 ($p<0.001$) and 0.15±1.65 ($p=0.257$) in the mean SQS scores was observed in each group respectively, with the sleep impaired arm showing a greater mean difference ($p<0.001$). Both groups exhibited improvements in the GAD-7, ED-5D-5L index value, BPI, and MPQ-2 scores at all timepoints ($p<0.010$), with the sleep impaired arm showing greater mean improvement in all but the MPQ-2 scores ($p<0.010$). 22.2% (n=253) of patients between both groups reported 2817 adverse events within 12 months of starting CBMP treatment, of which 84.4% (n=2378) were mild or moderate in severity.

Conclusion: Assessment of validated PROMs showed an associated improvement in pain, anxiety, and general health-related quality of life in chronic pain patients, although in those with co-morbid sleep impairment tended to experience larger improvements than those without sleep impairment in most domains. These findings suggest that the multimodal effects of CBMPs may lead to supplementary benefits in chronic pain patients with poor sleep quality, through disruption of the reciprocal effects pain and poor sleep quality have on one another.

CANNABIS CRAVINGS PREDICT COMPENSATORY INCREASES IN CIGARETTE USE IN SCHIZOPHRENIA: FINDINGS FROM CANNABIS ABSTINENCE STUDIES

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Introduction: Relative to non-psychiatric controls, people with schizophrenia have higher rates of cannabis and tobacco use. In cannabis recovery, remedying cannabis cravings by addiction substitution is commonly observed, such that an increase in tobacco may compensate for subjective experiences of reductions in cannabis use. We conducted a secondary analysis of two datasets, including participants with and without schizophrenia who underwent a 28-day cannabis-abstinence paradigm to assess the role of changes in cannabis use and cannabis craving on changes in cigarette use.

Methods: A total of 49 participants (schizophrenia n=29; control n=20; 2% female) with cannabis use disorder were recruited. All participants received weekly behavioral support sessions to assist with cannabis abstinence. Timeline Follow-Back for cannabis grams per day (GPD), cigarettes per day (CPD), the Marijuana Craving Questionnaire (MCQ) were administered weekly. Data were analyzed with multiple linear regression models; exploratory conditional process analysis was used to ascertain mechanistic effects of cannabis craving.

Results: Participants with schizophrenia had significantly greater MCQ compulsivity scores at 7-days of cannabis recovery relative to non-psychiatric controls ($t = 2.72$, $df = 46.8$, $p < .01$). Moreover, we found that increases in compulsivity cannabis cravings predicted an increase in cigarette smoking in schizophrenia compared to non-psychiatric controls ($t = -2.27$, $se = 0.59$ $p = .028$). Conditional process analysis results were non-significant, but trended in predicted directions.

Conclusion: People with co-occurring schizophrenia and CUD may be at greater risk for addiction substitution with nicotine during cannabis recovery due to heightened compulsivity craving intensity. Findings are pertinent to the design and implementation of interventions for this common comorbidity in schizophrenia.

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SELF-REPORTED EFFECTS OF CANNABIS ON SYMPTOM MANAGEMENT AMONG PATIENTS UNDERGOING CANCER TREATMENT: PRELIMINARY RESULTS FROM A SURVEY OF PATIENTS WITHIN A COMPREHENSIVE CANCER CENTER

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Background: Cancer is a qualifying condition for the medical authorization of cannabis use in Florida; however, previous exposure, sociodemographic variables, and stigmas foster variable acceptance among physicians and patients. The Sylvester Comprehensive Cancer Center (SCCC) catchment area serves over 6 million residents of diverse ethnic/racial backgrounds with broad cancer disparities and barriers to healthcare access. The purpose of this study was to examine the self-reported effects of symptom management with cannabis among cancer patients in the SCCC catchment area.

Methods: A redcap-based questionnaire was administered to patients ≥ 18 at SCCC classified as being within 5 years of initial treatment. The questionnaire was created in collaboration with 11 United States National Cancer Institute-designated cancer centers to obtain a harmonized set of questions detailing demographics, cancer site, treatment history, and reasons for cannabis use. A preliminary analysis of responses from October 2021 – March 2023, was conducted using SAS to obtain quantify the effect of cannabis use on symptom management among patients undergoing chemotherapy and immunotherapy.

Results: The patient-sample surveyed (n=156) held a mean age of 52.1 years with 52.0% in active treatment and yielded varying prevalence of cancer type; the most common were breast (26.6%), prostate (11.3%), lymphoma (7.3%) and multiple myeloma (5.6%). 76.6 % of patients undergoing chemotherapy reported moderate to significant pain level reduction with cannabis use. Similarly, 71.4% of patients undergoing immunotherapy reported moderate to significant pain level reductions. Moderate to significant stress level reductions were reported by chemotherapy (75.7%) and immunotherapy (62.5%) recipients. Peripheral neuropathy reductions were reported in 52.5% of chemotherapy and 29.4% of immunotherapy recipients.

Conclusions: Preliminary results indicate that the majority of cancer patients who use cannabis report symptom improvement in pain, stress, and peripheral neuropathy in active chemotherapy and immunotherapy treatments. Future studies should examine the specifics between controlled cannabis use and symptom management through treatment regimens via prospective designs. In the meantime, healthcare provider education programs may consider including cannabis use data to help support patient informed decision making when considering alternative symptom management strategies.

MAXIMIZING THE THERAPEUTIC POTENTIAL OF MEDICAL CANNABIS: INNOVATIVE NANO DRUG DELIVERY SYSTEM

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Introduction: Cannabinoids are highly lipophilic molecules, rendering them to be insoluble in water and prone for poor pharmacokinetics (PK). In order to develop pharmaceutical products comprising cannabinoids, it is inevitably required to implement complex drug delivery systems (DDS) for successful integration into medical dosage forms. Majority of pharmaceutical products rely on either solid state or aqueous liquid environment, both being an obstacle for cannabinoids. Appropriate dosage form would combine both high patience compliance e.g., *per-os* and adequate PK profile e.g., short t_{max}, reasonable bioavailability and clear dose-dependence. In the present study, we aimed at developing novel nanoparticle DDS (nanoDDS; referred to as Unlokt™) for cannabinoids and evaluating its pharmacodynamic (PD) and PK properties.

Methods: Protein-core nanoparticles composed of whey isolate with either THC or CBD were prepared using coacervation method and diafiltration with distilled water. Quantity of cannabinoids was analysed using HPLC and physical properties were measured using DLS.
All *in vivo* trials were approved by IACUC and were performed following ethical guidelines for the use of animals in research. *In vivo* PK was performed in rats following test items administration via oral or sub-lingual routes of administration. At multiple time points blood samples form the retro-orbital sinus were collected and subjected for bioanalysis using LC/MS. PK parameters were calculated using non-compartmental analysis of plasma concentrations over time. PD effects of nanoDDS cannabinoids were evaluated using mouse hot plate analgesia assay.

Results: Nanoparticles loaded with either THC or CBD had major population in the 300-400 nm range and zeta potential of -30 mV. The nanoDDS had loading capacity ranging between 30% and 50% w/w. *In vivo* PK analysis of per-os and sub-lingually administered nanoDDS-THC resulted with t_{max} at 30 min and detectable levels as early as 15 and 5 min, respectively. The bioavailability of nanoDDS-THC via per-os was 21%, which is 300% higher than THC in oil carrier (~7%). Similarly, the oral bioavailability of CBD is 300% higher than unformulated control CBD. In hot plate assay, unformulated THC had a bell-shape dose-response. The unformulated THC reached maximal increase in latency at 25 mg/kg and all other doses had lower effect. Surprisingly, nanoDDS-THC led to an S-shaped dose response curve with EC_{50} of 19 mg/kg. Moreover, at 30 min post administration only the nanoDDS-THC exhibited a significant increase in latency while unformulated THC had no effect in all doses up-to 35 mg/kg.

Conclusions: There are several types of nano-DDS such as nanoemulsions, nanoconjugates and nanoparticles. While nanoemulsions are used in some medical cannabis products, they require additional excipients and nanoconjugates are prohibited under current medical cannabis regulations. Based on our results, protein-core nanoparticles without any surfactants provide a viably delivery system to improve poor PK properties of cannabinoids. Moreover, we demonstrated for the first time the contribution of nanoDDS to classic dose-response correlation between THC and complex biological response, suggesting a possible clinical trasnlation for predictable and consistent dosing of THC.

THE EXTENT OF ILLICIT CANNABIS USE IN THE UK TO SELF-TREAT CHRONIC HEALTH CONDITIONS: A CROSS-SECTIONAL STUDY

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Introduction: In 2019, a large-scale cross-sectional survey conducted by The Centre for Medicinal Cannabis estimated that approximately 1.4 million adults in the UK purchase illicit cannabis in an attempt to self-treat chronic physical and mental health conditions. This survey was conducted one year following the rescheduling of cannabis-based medicinal products (CBMPs) in the United Kingdom, but before the first specialist clinics had started treating patients. It is estimated that approximately 32,000 individuals are now prescribed unlicensed CBMPs. The aim of this study was to therefore assess changes to illicit cannabis consumption following increased access to CBMPs.

Methods: Adults over the age of 18 in the UK were invited to participate in a cross-sectional survey though YouGov® between 22nd and 29th September 2022. The responding sample was weighted to generate a sample representative of the adult population of the UK. A series of questions were asked about respondents' medical diagnoses, illicit cannabis use, cost of purchasing illicit cannabis per month, and basic demographics. Modelling of population size was conducted based on an adult (≥ 18 years) population of 53,369,083 according to 2021 national census data.

Results: There were 10,965 respondents to the questionnaire, of which 5,173 (47.2%) and 5,792 (52.8%) were male and female respectively. 5,670 (51.7%) respondents indicated that they were affected by a chronic health condition. The most common reported conditions were anxiety disorders (n = 1,588; 14%). Of those suffering with health conditions, 364 (6.4%) purchased illicit cannabis to self-treat health conditions. Utilising this proportional response of a weighted population sample, 1,781,673 individuals were modelled to consume illicit cannabis for health conditions across the United Kingdom. Of these, 333 (91.5%) reported the financial costs of their illicit cannabis, with 226 (67.9%) spending less than £200 per month and 107 (32.1%) spending more than £200 per month. A total of 88 (24.2%) were unaware that CBMPs were legally available for eligible patients in the United Kingdom.

Discussion: There was a modelled rise in the number of individuals utilising illicit cannabis to self-treat chronic health conditions in the United Kingdom in 2022 compared to previously reported figures in 2019, despite the advent of specialist clinics in the intervening period. There was an increase in both the number of individuals who reported a health condition, as well as the proportion of those with health conditions self-treating with illicit cannabis. One possible explanation for both the increase could be related to the impact coronavirus disease 2019 (COVID-19) has had on both physical and mental health as well as access to treatment. Public health measures taken during COVID-19, whilst necessary, had a demonstrable impact on mental and physical health and the ability to access healthcare. This is a potential cause the increase seen in patients seeking alternative approaches to treatment. In addition to this, the cause is likely multifactorial, with an increase in acceptability and a reduction in stigma towards cannabis as a medicine.

PHARMACOKINETICS OF THC AND CANNABIS ABUSE LIABILITY IN MEN AS A FUNCTION OF FREQUENCY OF CANNABIS USE

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Introduction: After the controlled administration of smoked cannabis, plasma delta-9-tetrahydrocannabinol (THC) is greater in individuals who use the drug most frequently. This may be related either to more efficient inhalation that increases drug absorption, or to changes in cytochrome P450-mediated conversion of THC to the metabolites 11-Hydroxy-THC (11-OH-THC) and 11-Nor-9-carboxy-THC (THCCOOH). Frequent cannabis use results in the accumulation of THC, 11-OH-THC, and THCCOOH in the blood. However, the relationship between these pharmacokinetic differences and factors that relate to abuse liability are unknown. Thus, the aim of the current study was to examine associations between the concentrations of THC and its metabolites and abuse liability, operationalized by drug self-administration and self-reported drug effects.

Methods: In this double-blind, placebo-controlled, within-subjects study, male volunteers that frequently (n = 11) or occasionally (n = 10) used cannabis participated in 3 laboratory sessions. Smoked cannabis (0%, 4%, and 10% THC) was administered in a randomized order (one dose per session). Frequent use was defined as use greater than or equal to 5 days per week. Blood was drawn prior to drug administration (baseline), and 6 time points after controlled cannabis administration, to assess dose- and time-dependent plasma concentrations of THC and the metabolites 11-OH-THC and THCCOOH. Subjective drug effects related to abuse liability and intoxication were assessed with visual analog scales (VAS), including the smoked cannabis rating form (SC-RF). Cannabis's reinforcing effects were assessed with a self-administration task with participants choosing to smoke 0-3 puffs of the cannabis smoked earlier that session at a cost ($1 per puff). Peak change from baseline was calculated for VAS items and for plasma concentrations, each comparing frequent and occasional use.

Results: Participants reported a mean of 6.5 and 1.9 days of cannabis use per week, reflecting frequent and occasional use, respectively. At baseline, THC, 11-OH-THC, and THCCOOH levels were greater after frequent compared to occasional use (i.e., THC >20 ng/mL after frequent vs 2 ng/mL after occasional; $p = 0.016$). Peak concentrations of THC and metabolites were two-fold higher in frequent versus occasional use (ps < 0.05). Across doses, frequent use resulted in more self-administered cannabis than occasional ($p = 0.011$). Self-administration increased in a dose-dependent manner after frequent use only ($p = 0.019$; linear use and dose interaction). As expected, significantly less intoxication (VAS "High"; $p = 0.008$) and cannabis strength (SC-RF "Strong"; $p = 0.014$) were reported after frequent use relative to occasional. However, good drug effect and cannabis liking in the VAS and SC-RF did not differ as a function of use frequency, whereas negative drug effects were only reported after occasional use in the VAS and SC-RF (i.e., "Bad effect"; $p = 0.007$).

Conclusions: In agreement with prior reports, plasma THC was higher after frequent cannabis use, which may suggest inhalation efficiency. Despite these levels, intoxication, but not good drug effect, was reduced, likely due to tolerance. Abuse liability after frequent use is evidenced by the same reported good drug effect, the lack of reported bad drug effect, and increased rates of self-administration compared to occasional use. The increases in self-administration as a function of THC dose underscores the utility of this behavioral measure for future studies.

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COMBINING CANNABIS AND YOGA: PRACTICES AND MOTIVES

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Introduction: Cannabis has long been used for its psychoactive properties, to enhance health, and contribute to spirituality (Frederick et al., 2021). Yoga is recognized as a mind-body intervention that amalgamates physical, mental, and spiritual facets. The therapeutic use of cannabis and yoga share a common history in ayurvedic medicine. The reclamation of using cannabis for wellness is accompanied by interest in the potential synergistic effects of combining cannabis and yoga. Despite public interest and positive anecdote, to date there has been no systematic study describing the practices, practitioners, and consequences of this combined practice. To address this, the current research is the first to describe cannabis use and yoga practices of combined cannabis and yoga (CY).

Methods: The sample consisted of 401 participants who were recruited from online yoga forums and social media posts of prominent experts in CY. The participants filled out an online self report survey, identifying their demographics, cannabis use, yoga practice, and various measures such as the psychological flourishing questionnaire and mystical experiences questionnaire.

Results: Past six months of cannabis use was reported by 90% of participants (n = 361). Respondents reporting CY (n= 288, 72%) were an average age of 34, predominantly female (n = 244), used cannabis more often ($X^2(1, N = 360) = 17.86$, $p = .001$) and were more likely than non-CY to report using for medical purposes ($X^2(1, N = 343) = 17.50$, $p = .001$). CY respondents practiced yoga more often than non-CY ($X^2(1, N = 339) = 17.98$, $p = .001$) and engaged in a more advanced level of yoga ($X^2(1, N = 388) = 32.08$, $p = .001$). The most endorsed motivation for CY was to enhance connection to their body (n = 229, 81%), enhanced physical relaxation (n = 208; 73%) and enhanced mental relaxation (n = 204; 71%). Reduced anxiety was a reported consequence of CY for 80% (n = 156) of CY respondents and reduced pain was reported by 75% (n = 140).

Conclusions: Among yoga practitioners, the CY group utilized cannabis and practiced yoga more often than the non-CY group. Motivations for CY were desires for mental and physical relaxation and overall wellness, which mapped onto the reported outcomes of relief for mental and physical distress. A limitation was the survey being self reported, social desirability could have impacted the results. Our findings indicate that CY practitioners are characterized by health-related motivations, suggesting a need for future research that systematically compares the long-term outcomes of CY. As well research that compares the outcomes of CY to unstructured cannabis use to help develop best practices for the use of medical cannabis.

MANAGEMENT OF PARKINSON'S DISEASE WITH CANNABIS-BASED MEDICINAL PRODUCTS: A PRELIMINARY ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

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Introduction: Parkinson's Disease is the second most common neurogenerative disorder and has a pervasive effect on health-related quality of life. There is increasing evidence of the importance of the endocannabinoid system in pre-clinical models of disease. However, there is a paucity of clinical evidence on cannabis-based medicinal products (CBMPs) in Parkinson's Disease. This study presents a preliminary analysis of those with Parkinson's Disease enrolled in the UK Medical Cannabis Registry (UKMCR).

Methods: Patients prescribed CBMPs for Parkinson's Disease symptoms for longer than one month were identified from the UKMCR. The primary outcomes were changes from baseline in Parkinson's Disease Questionnaire-39 (PDQ-39), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L scales at one month. $p<0.050$ was defined as statistically significant.

Results: Thirty-four patients were identified from the UKMCR that met the inclusion criteria. Twenty (58.82%) patients were male, and the mean age was 66.76 ± 11.60 years. The median Charlson co-morbidity index score was 5.00 (IQR: 3.00-6.00). There was a significant improvement at one month follow up in the EQ-5D-5L index value, as well as the pain and discomfort, and usual activities domains ($p<0.050$). There was no significant change at one month in the PDQ-39, GAD-7 or SQS measures ($p>0.050$). Twelve adverse events were reported by two (5.88%) participants. The majority of adverse events were mild (n=7; 20.59%) or moderate (n=3; 8.82%). There were no life-threatening adverse events.

Conclusions: This preliminary analysis demonstrates a possible association with improved general health-related quality of life secondary to improvements in the ability to perform usual activities, as well as pain in those with Parkinson's Disease. Moreover, the results suggest that CBMPs are well-tolerated in the first month of treatment. However, this must be interpreted with caution considering the small sample size, length of follow-up and the limitations of observational study design.

UK MEDICAL CANNABIS REGISTRY: AN ANALYSIS OF GENERALIZED ANXIETY DISORDER PATIENTS TREATED WITH CANNABIS-BASED OILS AND DRIED FLOWER

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Introduction: Cannabis-based medicinal products (CBMPs) have emerged as a novel pharmacotherapeutic for the treatment of generalized anxiety disorder, despite a paucity of high-quality clinical data. Due to the heterogeneity of CBMPs, evidence is particularly limited on the optimal formulation of the medication. The UK Medical Cannabis Registry was founded in 2019 to collect real-world evidence on patient outcomes with CBMPs. The aim of this study was to analyse changes in patient-reported outcome measures (PROMs) and the incidence of adverse events in a cohort of patients with generalized anxiety disorder.

Methods: This cohort study included patients with generalized anxiety disorder enrolled on the UK Medical Cannabis Registry for greater than 12 months. Participants were assigned to three cohorts prescribed medium chain triglyceride oils, dried flower, or both formulations of CBMP products. Primary outcomes were changes in reported EQ-5D-5L, Generalised Anxiety Disorder 7-item (GAD-7), Single-item Sleep Quality Scale (SQS), and Patient Global Impression of Change (PGIC) questionnaires at 1, 3, 6 and 12 months compared to baseline. Adverse events were reported with the common terminology criteria for adverse events version 4.0. Statistical significance was defined as $p<0.050$.

Results: 302 patients were included in the final analysis, with 43 (14.4%), 167 (55.30%), and 92 (30.46%) patients prescribed oil-based, dried flower, or both CBMPs respectively. The mean age was 38.22 ± 12.03 years. The majority of participants were male (n=211; 69.6%). Most patients had either consumed cannabis previously (n=70; 23.10%) or were consuming cannabis at baseline (n=193; 63.70%). At each time period, improvements were observed in the EQ-5D-5L index value, GAD-7, SQS, and PGIC in all 3 prescription types ($p<0.050$). Patients treated with a combination of both oils and flower experienced a statistically significant improvement in PGIC compared with patients who were prescribed dried flower at 12 months only ($p<0.050$). There was otherwise no difference in outcomes between cohorts ($p>0.050$). 707 adverse events (224.11%) were recorded by 55 (18.21%) patients. The majority of these were mild (n=343; 113.58%) or moderate (n=285; 94.37%). There were no life-threatening/disabling events.

Conclusion: This study demonstrates a positive association between CBMP treatment and improvements in health-related quality of life over 12 months in patients with generalized anxiety disorder. However, apart from the PGIC there were no significant differences between treatment modalities with respect to reported outcomes. However, this must be interpreted within the limitations of a cohort study design, with randomized controlled trials necessary to evaluate optimal preparations of CBMPs for generalized anxiety disorder.

UK MEDICAL CANNABIS REGISTRY: AN ANALYSIS OF CLINICAL OUTCOMES OF MEDICINAL CANNABIS THERAPY FOR ATTENTION-DEFICIT/HYPERACTIVITY- DISORDER

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Introduction: Attention-deficit/ hyperactivity disorder (ADHD) is a neurodevelopmental disorder resulting in core symptoms of inattentiveness, hyperactivity, and impulsiveness. Despite the advent of stimulant and non-stimulant medications for ADHD, adherence rate to current pharmacological treatments are reported to be relatively low due to associated adverse events. The endocannabinoid system has been identified as a potential therapeutic target through interaction with dopaminergic pathways in the brain. However, there is a paucity of high-quality clinical evidence of cannabis-based medicinal products (CBMPs) for ADHD. This study aims to analyse the changes in general health-related quality of life and incidence of adverse events following initiation of CBMP therapy for ADHD.

Methods: An uncontrolled case series of patients receiving CBMP treatment for a primary indication of ADHD from the UK Medical Cannabis Registry was investigated. Participants enrolled <12 months prior to the date of data extraction (8th January 2023) were excluded. The primary outcomes were changes in the following patient-reported outcome measures (PROMs) at 1, 3, 6, and 12 months from baseline: EQ-5D-5L index value, generalized anxiety disorder-7 (GAD-7) questionnaire and the single-item sleep quality score (SQS). Secondary outcomes were the incidence and severity of adverse events as measured utilising the common terminology criteria for adverse events version 4.0. Statistical significance was defined as $p<0.050$.

Results: 68 (male: 55; 80.9%) patients met the inclusion criteria for this study. The mean age of participants was 35.6 (±10.2) years. Most participants (n=55; 80.9%) were consumers of illicit cannabis at the point of enrolment. The mean cannabidiol dose per 24 hours was 28.2 (±31.1) mg, whilst the mean tetrahydrocannabinol dose was 219.4 (±121.7) mg. The mean GAD-7 and SQS scores at baseline were 10.85 (±5.91) and 4.18 (±2.34). There were statistically significant reductions in these scores at each at 1, 3, 6, and 12 months, respectively ($p<0.010$). Improvements were also identified in the general health-related quality of life EQ-5D-5L index value at 1, 3, and 6 months ($p<0.050$). 61 (89.71%%) adverse events were recorded by 11 (16.2%) participants, of which most were moderate (n=26, 38.2%) or mild (n=19, 29.9%).

Conclusions: There was an observed association between initiation of CBMP treatment and improvements in anxiety, sleep quality and the general health-related quality of life in patients with ADHD. Treatment was well tolerated within the 12-month period. The results of this study must be interpreted with caution as a causative effect cannot be proven with the present study design. These results, however, do provide additional support for future evaluation within randomised controlled trials.

CANNABIS USE IN THE UK: A QUANTITATIVE COMPARISON OF INDIVIDUAL DIFFERENCES IN MEDICAL AND RECREATIONAL CANNABIS USERS

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Introduction: There is a paucity of research, especially in the UK, which investigates individual differences in cannabis users such as age, gender, motivations for use, and mental health. Further, there is no current research in the UK which compares these outcomes between medical cannabis users (MCUs) and recreational cannabis users (RCUs). Therefore, the current study aims to investigate these individual differences and compare them between RCUs and MCUs in the UK.

Methods: Using a cross-sectional survey design, data was collected online using Question-Pro between 14th June and 14th July 2022. Participants were RCUs and MCUs currently living in the UK. RCUs were invited to take part via social media. MCUs were recruited from Sapphire Medical Clinics, which provides treatment with prescribed cannabis-based medicinal products in the UK. The survey assessed participants' demographic data and cannabis use frequency, as well as PTSD symptoms (PCL-5), depression symptoms (CES-D), trait and state anxiety (STAI), and several cannabis use motives (CMMQ) using validated questionnaires. Chi-square and independent sample t-tests were used for comparison of categorical variables and normally distributed continuous variables. Differences in each questionnaire were analysed using analyses of variance (ANOVAs) and t-tests. Statistical significance was considered where $p<0.05$.

Results: 161 participants completed the survey. MCUs were older, consumed cannabis more often and had higher "Sleep" motive on the CMMQ ($p < 0.05$). MCUs had higher prevalence in self-reporting current diagnoses of neurological problems, mood disorders and anxiety disorders. RCUs had significantly higher scores on several motives (e.g., "Enjoyment", "Coping", "Experimentation", "Boredom", "Celebration") and higher state anxiety scores ($p < 0.05$). Most common motives for both groups were "Enjoyment", "Low Risk" and "Sleep". The two groups did not differ on gender, "Low Risk" motive, PTSD symptoms, depression scores, and trait anxiety scores ($p > 0.05$). Furthermore, there was no difference between the two groups on self-reported prevalence of substance use related disorders, and past consumption of alcohol, tobacco, and caffeine ($p >0.05$).

Conclusions: The current study demonstrates a difference in age and motivations for cannabis consumption between RCUs and MCUs, but also shows areas of potential overlap in mental health outcomes, past substance use, and gender. Whilst these findings are UK-specific, they indicate that RCUs experience higher state anxiety, despite no difference in underlying trait anxiety highlighting the need for yet further evaluation of potential anxiogenic/anxiolytic properties of cannabis. These findings hold significant implications for future research, clinical practice, and legislation.

PHYTOCANNABINOIDS IMPROVE BLOOD PRESSURE SENSITIVITY IN FEMALE POST-CONCUSSION SYNDROME PATIENTS: CASE SERIES

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Introduction: Cannabidiol (CBD) is gaining research interest due to its neuroprotective effects that are devoid of intoxication. Research is now available to show that post-concussion syndrome (PCS) can disrupt autonomic function. CBD administration to patients with PCS has been shown to help restore blood pressure (BP) dynamics and improve heart rate variability. The relationship between the changes in heart rate and blood pressure is reflected by the baroreflex sensitivity (BRS). However, BRS has not been assessed in PCS following CBD administration. The purpose of this study was to observe the influence of daily administration of CBD on BRS parameters in three female PCS participants.

Methods: Heart rate was collected using a 3-lead electrocardiogram (ECG) and beat-to-beat BP was collected using finger photoplethysmography. Three participants first completed a 5-min seated rest period, followed by a 5-min seated controlled breathing protocol (0.1 Hz; participants inhaled for 5-seconds, followed by 5-second exhalation) performed for 5-minutes. The BP and ECG data was inspected visually to remove any artifact or excessive noise. Using Ensemble-R software (Elucimed Ltd., Auckland, NZ), systematic and monotonic three-beat increases or decreases in systolic BP and R-R intervals during controlled breathing were used as the threshold, with a regression curve fitted to the BP and ECG R-R signals. The slope of the regression curve was calculated to attain the upward sequences (BRS-up), downward sequences (BRS-down), and the average of the entire controlled-breathing protocol (BRS-pooled). CBD was self-administered twice per day by the participants under the guidance of their medical doctor, with dosage ranging from 25mg to 400 mg per day. Venous blood samples were collected to analyse plasma CBD (peak concentrations). Participants were assessed every 16 ± 5 days for up to 58 days.

Results: Participant 1 took a maximum dose of 400 mg per day for 29 days, resulting in an increase in BRS-down from 6.5 to 6.8ms/mmHg, BRS-up from 7.0 to 15.0 ms/mmHg, and BRS-pooled from 6.7 to 10.7 ms/mmHg. Participant 2 took a maximum dose of 200 mg for 24 days (peak 26.36ng/mL), resulting in an increase in BRS-down from 11.2 to 12.5 ms/mmHg, BRS-up from 15.9 to 20.2 ms/mmHg, and BRS-pooled from 13.2 to 15.4 ms/mmHg. Participant 3 took a maximum dose of 50 mg for 38 days (peak 12.24ng/mL) with an increase in BRS-down ms/mmHg from 5.5 to 7.2 ms/mmHg, BRS-up from 8.6 to 14.3 ms/mmHg, and BRS-pooled from 6.8 to 10.6 ms/mmHg.

Conclusions: Chronic, daily CBD administration in females with PCS appears to improve BRS parameters (4.4% to 53.3%). This appears to be with doses as low as 50 mg/day and as high as 400 mg/day. Though speculative as only three cases are presented, these findings are indicative of improved cardiac autonomic function in patients with PCS following CBD administration. More double-blinded, randomized controlled trials with a controlled dosing regimen are required.

UK MEDICAL CANNABIS REGISTRY: A COHORT STUDY OF PATIENTS PRESCRIBED ADVEN® PRODUCTS

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Introduction: Whilst there is increasing evidence of the effects of cannabis-based medicinal products (CBMPs) on health-related quality of life, it is typically of low quality. A major limitation of the current literature is the heterogeneity of studied CBMPs. Whilst examining effects across multiple products provides insight to outcomes in clinical practice, it limits internal validity. The present study aims to analyse changes in health-related quality of life in patients prescribed a homogenous group of CBMPs throughout therapy.

Methods: A cohort study was conducted utilising data from the UK Medical Cannabis Registry. Inclusion criteria were those prescribed medium chain triglyceride oils, dried flower or both formulations (Adven® 20, 50, and EMT, Curaleaf International, Guernsey, UK) and were enrolled on the UK Medical Cannabis Registry ≥ 12 months. Primary outcomes were changes in patient-reported outcomes (Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L) at 1, 3, 6, and 12 months from baseline. The secondary outcome was an adverse events analysis. Statistical significance was defined as $p<0.050$.

Results: 1,378 patients prescribed Adven® CBMPs were included in the final analysis. 641 (46.5%), 235 (17.1%), 502 (36.4%) patients were treated with oils, dried flowers, or a combination of the two, respectively. 733 (53.2%) participants were female, whilst 645 (46.8%) were male. The mean age was 46.3 ± 7.2 years. The most common conditions were chronic non-cancer pain (n=395; 28.7%), fibromyalgia (n=162; 11.8%), neuropathic pain (n=136; 9.9%), and generalised anxiety disorder (n=120; 8.7%). Statistically significant improvements were found in all the PROMs among all the routes of administration, including Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS) and EQ-5D-5L at 1, 3, 6, and 12 months from baseline ($p<0.010$). Those prescribed dried flower only or both oils and dried flower experienced greater improvements in GAD-7, SQS, and EQ-5D-5L index values at 12 months ($p<0.050$). There was no significant difference in outcomes between those prescribed dried flower only or dried flower with oils. 3663 (263.6%) adverse events were recorded by 297 (21.6%) patients. The most common adverse events were fatigue (n=271; 19.7%), somnolence (n=250; 18.1%) dry mouth (n=246; 17.9%) lethargy (n=221; 16.0%), and headache (n=205; 14.9%).

Conclusion: There was an associated improvement in self-reported anxiety, sleep quality and health-related quality of life in patients treated with the CBMPs studied in this analysis. Those prescribed treatment formulations including dried flower were most likely to show a clinical improvement. However, these results must be interpreted with caution given the limitations of study design.

CANNABIS USE PATTERNS IN PATIENTS SEEKING TREATMENT IN A UK SAMPLE OF MEDICAL CANNABIS PATIENTS

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Introduction: Cannabis based medicinal products (CBMPs) have been available in the UK under specialist medical providers since 2018. It has been shown that up to 70% of patients prescribed CBMPs have previously consumed cannabis. However, there is limited data to understand the motives of prior cannabis consumption and how this may be altered after being prescribed CBMPs. This in turn contributes to stigma attached to the use of CBMPs as a risk intensive treatment option. The aim of this study was to further analyse the relationship between patients receiving CBMPs and their past and present use of cannabis.

Methods: Patients prescribed CBMPs within the past 3 months by Sapphire Medical Clinics were invited via email to participate in this cross-sectional survey study. The questionnaire was designed by a multidisciplinary group of academics to capture demographic information and medical conditions, as well as assess prior and present illicit cannabis consumption. The survey was distributed via Qualtrics (Seattle, Washington, United States) and analysed using descriptive statistics.

Results: From 6849 patients invited to participate, 1100 (16.1%) respondents completed the questionnaire. 1086 (98.7%) reported having a medical diagnosis from a medical professional, and 1078 (99.3%) reported currently administering CBMPs as prescribed by the clinic. Responses indicated that several patients also consumed illicitly purchased cannabis to self-treat their condition (n=235; 21.6%)). The majority of participants (n=770; 70.0%) reported using cannabis for recreational purposes prior to being prescribed CBMPs. However, 609 (79.1%) of these patients reported no longer using cannabis for recreational purposes. Only one (0.1%) participant started consuming cannabis recreationally after being prescribed CBMPs. Some patients (n=291; 26.5%) also reported having medical conditions for which they hadn't sought a diagnosis from a healthcare professional. Several individuals reported consuming cannabis for undiagnosed conditions (n=241; 82.8%). Most of these received a prescription for CBMPs (n=234; 80.4%), whilst 24.4% (n=71) consumed illicit cannabis for this reason.

Discussion: Use patterns of patients seeking treatment with CBMPs compared to unregulated cannabis are complex. It is difficult to determine if responses to questions regarding recreational use can be separated from the potential that it is in fact "self-medication". Self-reporting of cannabis for recreational use appears to reduce once access to CBMPs occurs. Moreover, it is rare for individuals to start consuming cannabis recreationally after initiation of treatment with CBMPs. From the patients surveyed the majority are using legitimate CBMPs under the supervision of a medical professional. In terms of reducing risk attached to using unregulated cannabis to treat medical conditions, medically supervised use of CBMPs is instrumental in reducing the potential for harm.

RELATING BLOOD THC LEVELS TO IMPAIRMENT OF COGNITIVE AND PSYCHOMOTOR FUNCTIONING

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Introduction: Within the cannabis literature, there is growing evidence that behavioural impairment may not be linearly correlated with levels of THC in blood. However, cognitive and behavioural impairment appears to be greater at higher than lower levels of THC. A recently published study of the effects of smoked cannabis on simulated driving 30 mins after exposure found no correlation between THC levels in blood and the degree of lateral control (i.e., weaving) demonstrated by participants. However, when participants were divided into those whose blood THC was above versus below 5 ng/mL, the upper per se threshold set out in the Criminal Code of Canada, participants above the threshold demonstrated greater impairment of lateral control. Using a similar approach, the current study examined the effects of smoked cannabis on cognitive and psychomotor skills 60 mins after exposure.

Methods: Twenty-eight healthy cannabis users aged 19-29 years with recent history of binge drinking smoked cannabis (12.5% THC) ad libitum alone or with alcohol (target breath alcohol content [BrAC] 80 mg/dL). Blood THC levels were measured 30 mins after smoking (45 mins after drinking). Approximately 60 mins after smoking (75 mins after drinking), participants completed a verbal free recall (VFR) test, Digit Symbol Substitution Test (DSST), Continuous Performance Test (CPT), Useful Field of View Test, and grooved pegboard (GPB) task. Spearman correlations between blood THC levels and all outcome measures were assessed. Outcome measures after cannabis and alcohol+cannabis were compared to the same measures after placebo when blood THC levels were above or below the 5 ng/mL threshold (Wilcoxon signed rank tests). Outcome measures after cannabis and alcohol+cannabis among participants above the 5 ng/mL threshold were compared to those of participants below the 5 ng/mL threshold (Mann-Whitney U tests).

Results: In both the cannabis (above threshold: n=12, below: n=14) and alcohol+cannabis (above threshold: n=18, below: n=8) conditions, immediate recall measures from the VFR task were negatively correlated with blood THC levels. Under cannabis only, the percent retained positively correlated with blood THC levels. As well, time to complete the GPB with the non-dominant hand was positively correlated with blood THC levels. When comparing cognitive functioning post-drug in the cannabis condition to placebo, participants with blood THC above 5 ng/mL demonstrated poorer immediate recall on the VFR task. Participants with blood THC below 5 ng/mL demonstrated no impairment on any outcome. In the alcohol+cannabis condition, participants with blood THC above 5 ng/mL demonstrated impairment on the VFR task and CPT, poorer performance and longer completion times on the DSST, and longer completion times with both the dominant and non-dominant hands on the GPB task and on the divided and selective attention tasks of the UFOV. Participants with blood THC below 5 ng/mL demonstrated impairment on the VFR task, poorer performance on the DSST, and longer completion times with the dominant hand on the GPB task. When directly comparing cognitive functioning of participants with blood THC above versus below 5 ng/mL, significant results were found in the cannabis condition for the VFR and GPB tasks: participants above threshold recalled significantly fewer words but had a higher percent retained. Participants above threshold took longer to complete the GPB with their non-dominant hand. In the alcohol+cannabis condition, participants above threshold recalled fewer words on the VFR task.

Conclusion: The VFR task was most sensitive to cannabis impairment, in both the cannabis and alcohol+cannabis conditions, using both correlational analysis and a split based on the 5 ng/mL threshold. Analyses using the 5 ng/mL threshold were better able to detect the THC-related cognitive impairment, which was more evident in the alcohol+cannabis condition, sometimes even among those with blood THC levels below 5 ng/mL.

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

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Introduction: As cannabis-based medicinal products (CBMPs) are prescribed with increasing frequency, there is a need for the adoption of pharmacovigilance approaches to study the long-term effects of CBMPs on individuals. Whilst randomised controlled trials are always necessary within the context of biomedical research, observational research provides supplementary benefits through real-world, longitudinal evaluation of prescribing outcomes in larger populations. The UK Medical Cannabis Registry, the largest patient registry in the UK, was devised to help collect this necessary data. The aim of the present study is to analyse changes in patient reported outcome measures (PROMs) and adverse events in patients prescribed CBMPs.

Methods: This case series was conducted utilising the UK Medical Cannabis Registry. Inclusion criteria were those enrolled for a minimum of 12 months and had completed all necessary PROMs at baseline. Primary outcomes were changes in PROMs (generalised anxiety disorder-7 (GAD-7), single-item sleep quality scale (SQS), and EQ-5D-5L) at 1, 3, 6, and 12 months after starting CBMP therapy. Adverse events were reported in accordance with CTCAE v.4.0. Statistical significance was defined as $p<0.050$.

Results: 2,637 patients within the UK Medical Cannabis Registry met the inclusion criteria. The mean age was 43.49 ± 14.03. 1,508 (57.19%) and 1,128 (42.78%) participants were male and female. The most common primary indications for CBMP therapy were chronic non-cancer pain (n=688; 26.09%), generalised anxiety disorder (n=302; 11.45%), fibromyalgia (n=286; 10.84%), neuropathic pain (n=233; 8.84%), and post-traumatic stress disorder (n=152; 5.76%). 1,912 (72.51%) participants had previously consumed cannabis prior to enrolment. Improvements in GAD-7, SQS, and EQ-5D-5L index value scores were seen at 1, 3, 6, and 12 months compared ($p<0.001$). Clinically significant improvements in sleep quality and generalised anxiety were observed in 18.96% (n=500) and 20.06% (n=529) of individuals at 12 months. Individuals prescribed dried flower in isolation or in combination with oil-based CBMPs were more likely to have an improvement in EQ-5D-5L index value, SQS, and GAD-7 scales compared to those prescribed oil-based CBMPs alone ($p<0.001$). 6,390 adverse events (mild: 2707, 102.65%; moderate: 2750, 104.29%; severe: 925, 35.07%; life-threatening/disabling: 8, 0.30%) were reported by 562 (21.21%) participants.

Conclusion: This study suggests that CBMPs are associated with positive changes to health-related quality of life and are well tolerated up until 12 months follow up. One in 5 individuals reported clinically significant changes in generalised anxiety and sleep quality, despite generalised anxiety and insomnia being the primary indication for treatment in only 11.45% and 2.20% of cases indicating potential adjunctive effects beyond the initial treatment indication.

AWARENESS OF MEDICAL CANNABIS REGULATIONS AMONG UK POLICE OFFICERS – A CROSS-SECTIONAL STUDY

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Introduction: Cannabis-based medicinal products (CBMPs) were rescheduled in the UK in November 2018. Since this time there has continued to be a rise in the number of patients prescribed unlicensed CBMPs, with approximately 32,000 patients receiving treatment by the end of 2022 for conditions such as chronic pain, generalised anxiety disorder and post-traumatic stress disorder. Despite this, it has been shown that approximately half of the general population are unaware of this change in scheduling, with patients prescribed CBMPs continuing to perceive themselves as being subject to stigma. Concerns around interactions with the criminal justice system and police officers may contribute to perceived stigmatisation. The aim of this study was to therefore assess awareness of current legislation governing CBMPs among police officers in the UK and whether they had received training on the subject.

Methods: A cross-sectional survey study was conducted between 24th October – 1st November 2022. The survey was designed by a multi-disciplinary team of academic clinicians and a cognitive neuroscientist with expertise in qualitative research. The questionnaire was distributed to serving police officers in the UK by Opinium Research, weighted to derive a representative sample (London, UK). Data was analysed utilising descriptive statistics.

Results: In total, 200 police officers completed the survey. 109 (54.5%) of respondents were male and 91 (45.5%) were female. 96 (48.0%) were aged between both 18-34 and 35-54, whilst 8 (4.0%) were aged over 55. Two-fifths (n=80; 40.0%) of participants had previously encountered someone who claimed to be in receipt of CBMPs. 143 (71.5%) respondents knew that CBMPs were legal on prescription in the UK, whilst 42 (21%) and 15 (7.5%) participants thought that they were not legal or were not sure of their legal status. 47 (23.5%) participants had received no formal training on this topic, whilst 85 (42.5%) had received training but believed it to be inadequate. 177 (88.5%) of police officers subsequently believed they would benefit from more training on CBMPs and how to identify legal medical cannabis patients.

Conclusion: Despite CBMPs being legally available on prescription for patients in the UK since 2018, a significant proportion of police officers are still unaware of this. Beyond this, police officers identified that they would benefit from further education on this topic. This may contribute to the stigma perceived by current medical cannabis patients. Implementation of national and local education focused at providing information on the legalities of CBMPs, what conditions they are prescribed for and which formulations they may be found in are necessary to help improve knowledge among police officers.

NON-DESTRUCTIVE AND RAPID MONITORING OF CANNABINOID DEGRADATION IN HEMP INFLORESCENCE DURING STORAGE: KINETIC MODELING USING A TIME-BASED APPROACH

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Introduction: The current reference method for cannabinoid quantification is gas or liquid chromatography, which is costly, time-consuming, and has a large environmental impact. Fourier Transform Near Infrared (FT-NIR) is a rapid and nondestructive spectroscopic analytical technique. NIR instrumentation costs considerably less than chromatography instrumentation, making it an attractive option for producers of hemp to implement and is substantially easier for untrained operators. More transparency with hemp compliance testing has been of increased importance due to testing issues and mislabeling. A critical source of variation in product from the initial analysis could be the degradation of cannabinoids during distribution and storage. The objective of this study was to design a method of analysis that can be implemented in real-time, leading to a more accurate cannabis analysis.

Methods: Dry baths with lids were set to different temperatures (-20°C, 2°C, 25°C, 35°C, 45°C, 55°C). Around 1.5 grams of homogenized inflorescence were weighed into tubes and placed in the appropriate temperature condition. Two samples were removed from each of the temperature conditions at determined time points and stored at -40°C until analysis so that no further degradation would occur. Inflorescence samples were analyzed by reference ultrahigh performance liquid chromatography- triple quadrupole mass spectrometry (UPLC-MS/MS) to obtain cannabinoid concentrations (CBD, CBG, Δ9-THC, CBN, and acidic forms). Whole and ground hemp samples were sampled with a handheld FT-NIR Scanner (16 nm resolution, 1350-2500 nm range) with a 10-second exposure time. Multivariate chemometric techniques (SIMCA and PLSR) allowed for the classification and correlation of spectral data with reference UPLC-MS/MS data. For kinetics modeling, data were fit to the Weibull distribution and the Arrhenius equation to determine the shelf-life rates of degradation.

Results: PLSR models show the quantification of major cannabinoids from the inflorescence with low detection limits (0.021% w/w Δ9-THC) and high quantification limits (20.7% CBD acid). PLSR models show reproducible and sensitive results (Rcv>0.95). SIMCA models show good class separation and classification of the various temperature and time points. The kinetics equations show the degradation of cannabinoids follows first-order kinetics. These kinetic models can be used to determine the appropriate shelf life of hemp inflorescence.

Conclusions: Cannabinoid degradation and quantification are important traits for maintaining the quality control of hemp products. This study provides a rapid and nondestructive alternative testing method to UPLC. FT-NIR with chemometrics provides a simple solution for chemically complex matrices, as well as determine the optimal storage conditions of hemp inflorescence. Producers and growers of hemp would benefit greatly from an easy-to-use analytical tool like FT-NIR.

MONITORING THE EFFECT OF MEDICAL CANNABIS THERAPY ON THE MAGNETO-ELECTRIC FIELD IN HUMANS ALLOWS SAFE DOSING AND MINIMIZES PATIENTS' SIDE-EFFECTS

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Humans as all other organisms are surrounded by a magneto-electric field (MEF), whose characteristics are affected by the organism's health (1). This field can be measured by a GDV camera by measuring the coronal discharge of fingers and has been used to screen for various diseases as diverse as anxiety (1) colon cancer(2) and diabetes (3). The following study reports on 289 consecutive patients treated by medical cannabis therapy (MCT) for at least two years. The indication was chronic low back pain and all patients were monitored by patient reported outcome measures (PROMs) and MEF change. The PROMs used were Oswestry Disability Index (ODI), SF-12 version 2 and the Brief Pain Inventory (BPI).

Methods: All patients were examined by a single physician (D.R.), minimal requirements for consideration of medical cannabis therapy were persistent chronic low back pain for at least one year treated by at least three pharmaceuticals including a narcotic, NSAIDs and CNS acting pain modifying agents (most commonly duloxetine) as well as physiotherapy.

The inclusion criteria included: age over 18 years, failure of at least one year analgetic therapy, advanced imaging studies indicating an organic symptom origin, ability to consent to MCT.

Exclusion criteria included: psychosis, uncontrolled epilepsy, army conscripts or prisoners, pregnancy or intended pregnancy.

MCT was begun as 60 mg THC containing infloresences. Dosage schedule was once a night a night for one month and then TIC. Dosage increase by 10 grams per month, was considered after three months. Patients' were monitored at 3 months, 6 months, one year and two years. Patient visits included physician evaluation, ODI, BPI and SF-12 and MEF assessment including two parameters: stress and energy.

Results: 93% of patients continued the MCT during the two years follow-up period. BPI decreased from 9±1.5 intensity and 8.7±1.8 pain intervention scale, at baseline to 5.5±2.3 and 4±3.2 at 3 months and 1.3±0.9 and 1.8±2 at two years. MCT dose increased from 20 grams at baseline to 35±10 grams at one year and 43±8 at two years. ODI decreased from 85±12 to 24±22 at two years and SF12 PCS changed from 34±8 at baseline to 52±12 at two years. MEF changes during MCT included energy decrease by 4±3% and anxiety decrease by 0.5±0.2 units. Patients whose repeated scans indicated anxiety increase (n=34) were treated by administration of additional CBD rich chemovar (10 grams per month). 28/34 patients had decreased anxiety at the next visit. No patients required license cancellation.

Conclusions: Long term MCT therapy is safe in LBP patients, PROMs indicate high patient satisfaction. MEF allows monitoring of MCT effects and mitigation of side effects preserving patients' vitality and decreasing anxiety. It is recommended that a combined MEF-PROMs based index is used as a dosage adjustment algorithm.

EFFECT OF MEDICAL CANNABIS THERAPY ON AUTONOMIC NERVOUS SYSTEM BALANCE AND ACTIVATION

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Medical cannabis therapy is used extensively in Israel with approximately 2% of the population holding a license for medical cannabis. The most common method of application in Israel is by smoking and the most common grade is THC20%:CBD4%. The following investigation was undertaken in order to assess the effect of medical cannabis therapy on autonomic nervous system (ANS) balance and activation. The assessment was performed using a CLA INSIGHT instrumentation. The system evaluates three parameters: heart rate variability, skin conductivity and skin temperature. An AI analysis comparing to several tens of thousands of prior measurements allows determination of two essential characteristics of the autonomic nervous system: 1. Degree of activation and 2. Sympathetic to parasympathetic balance.

Methods: 121 patients seeking treatment by medical cannabis at one of the authors clinic (DR) were assessed prior to therapy initiation and at least 3 months following therapy initiation. The degree of ANS activation and balance was measured as well as brief pain inventory questionnaire and Oswestry disability index.

Results: BPI improvement average 6 grades after at least 3 months of therapy ($p<0.001$), ODI changed by 3 months by an average of 32% ($p<0.01$). The change in ANS activity averged 22 points (100 max), and balance by 34 percent ($p<0.01$). The correlation between BPI and ANS balance was high ($R>0.9$), the correlation between BPI and ANS activity was lower ($R<0.7$) though significant. The same was true for ODI changes.

Conclusion: It appears that medical cannabis therapy using smoking T20:C4 strains appears to relieve in a consistent manner pain and disability related to chronic low back pain. The mechanism of action appears to involve rebalancing of the autonomous nervous system activity and in particular vagus nerve activation leading to parasympathetic activation. It is important to measure ANS activity and add interventions leading to ANS activation as it has been previously related to lack of spontaneous tissue repair. The optimal therapy for chronic low back pain might involve a multimodality mind-body approach and ANS assessment can serve as an objective measure of treatment success.

CLINICAL OUTCOME ANALYSIS OF PATIENTS WITH MULTIPLE SCLEROSIS – ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

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Introduction: Disease-modifying therapies are the cornerstone for treatment of multiple sclerosis (MS). Yet, there is a need to develop novel therapeutics for the symptomatic sequalae of the disease. Cannabis-based medicinal products (CBMPs) have been suggested as a potential therapy for the MS-associated pain, spasticity, and co-morbid mental health disorders. Whilst randomised controlled trials of nabiximols has demonstrated clinically significant effects, a paucity of clinical evidence limits further therapeutic applications of other CBMPs for patients with MS. The aim of this study was to therefore assess changes in MS-specific and general health-related quality of life outcomes alongside adverse event incidence in patients from the UK Medical Cannabis Registry (UKMCR) prescribed unlicensed CBMPs for MS.

Methods: A case-series of patients prescribed CBMPs for MS symptoms from the UKMCR was analysed. Health-related quality of life was assessed at 1-, 3- and 6-months using changes from baseline in patient-reported outcome measures [MS Quality of Life-54 (MSQoL-54), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L]. The safety profile of CBMP treatment in this population was assessed through reporting the frequency and severity of adverse events. $p<0.050$ was defined as statistically significant.

Results: 141 patients met the inclusion criteria for the study. 41 (29.70%), 22 (15.60%) and 78 (55.32%) patients were respectively treated with oils, dried flower, or both. There was an improvement in the following subscales of the MSQoL-54 at 6 months: change in health scale, cognitive function, mental health composition, physical health, role limitations due to physical limitation and due to emotional problems, as well as social and sexual function ($p<0.050$). Significant improvements were also observed in GAD-7, SQS, and EQ-5D-5L index value ($p<0.050$). There were 146 (103.50%) adverse events reported by 21 patients (14.89%) - the majority of which were rated as mild (n=47; 33.30%) or moderate (n=72; 51.10%) in severity. The most frequently reported adverse events were fatigue (n=14; 9.90%) and lethargy (n=10; 7.10%).

Conclusions: This preliminary analysis presented an association between CBMP treatment and improved general health-related quality of life measures for MS patients. The majority of patients tolerated CBMP treatment within the first 6 months. However, due to the notable limitations of this study, definitive conclusions cannot be drawn, and results must be analysed with caution.

MEDICAL CANNABIS FOR NEUROLOGIC DISORDERS: A RETROSPECTIVE CHART REVIEW

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Introduction: Real world medical cannabis data in the treatment of neurologic conditions lacks details regarding cannabis varieties, indications for treatment, patient outcomes, and adverse effects. The Neurology Centre of Toronto by Numinus (NCT) is an outpatient, publicly funded, community-based neurology clinic with a robust medical cannabis program using standardized patient-and condition-specific treatment protocols. Real-world use of medical cannabis for the treatment of neurological disorders in both children and adults can be sampled from this historical data at NCT. Resultant ecological data will inform research and clinical practice.

Methods: This study was a retrospective patient chart review. All patients that underwent medical cannabis treatment between 2018-2022 (inclusive) are included. Demographic data, medical history, cannabinoid-specific dosing, and condition-specific outcomes were manually extracted from each patient's medical records. After extraction, the outcomes were analyzed and compared grossly against all patients and within specific subgroups. These subgroup divisions included age, sex, diagnoses, target condition, and follow-up time.

Results: The patient cohort consisted of adults and children with neurological diagnoses. Medical cannabis therapy was had treatment-specific targets. Preliminary analyses yielded N=190 patients. Most patients were using medical cannabis to target epilepsy (55%), headache (14%), or concussion symptoms (12%). In epilepsy patients with at least a 90-day follow-up (EPF; n=62), 34%, 50%, and 16% reported an improvement, no change, and worsening in seizure frequency, respectively. Fifteen percent, 82%, and 3% reported an improvement, no change, and worsening of quality of life, respectively. The mean age was 15.4 years [3.4, 56.1] Sixty-six percent of the EPF group were treated with CBD and THC; the rest were treated with CBD alone. At the time of the conference, the sample size of this study is expected to triple. The results to be presented will show gross sample and condition-specific results in conditions of epilepsy, headache, and concussion. For statistical comparisons, nonparametric tests, t-tests, or Chi-square tests will be used, depending on distribution normality and variable type.

Conclusion: This historical, retrospective review will report on the largest adult and pediatric cohort of medical cannabis users in neurology from a single clinic. It will provide evidence of real-world applications of a structured medical cannabis program in neurology, and insight into medical cannabis dosages, treatment protocols, adverse effects, and outcomes.

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THE EFFECTIVENESS OF WATER-SOLUBLE CANNABINOID MEDICINES TO TREAT REFRACTORY-EPILEPSY ASSOCIATED WITH NEURODEVELOPMENTAL AND BEHAVIORAL DISORDERS: A CASE REPORT

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Introduction: Lenox-Gastaut Syndrome is a severe form of epilepsy that begins in early childhood. Autism Spectrum Disorders are neurodevelopmental disorders characterized by impaired interaction and social communication. Oppositional Defiant Disorder is classified as a conduct disorder, usually manifesting itself in young children. The future of cannabis use in nanomedicine offers promising solutions for clinical practice. Water-soluble nano-cannabinoid medicines are presented as an alternative to overcome the challenges of sustained cannabinoid delivery (low aqueous solubility, low cell penetration and first-pass metabolism). In this article, the objective is to report the clinical case of a pediatric patient where the use of water-soluble nano-based cannabis medicine offered benefits in controlling the symptoms of the conditions described above.

Methods: We report a pediatric case where the results were evaluated through medical records and the mother's report on the patient's evolution. Scientific research was carried out to support the therapeutic decision and the discussion of this case.

Results: Here we report an 11-year-old, male, 42 kg, resident of an indigenous village, with Lenox-Gasteau syndrome, ASD and ODD, having 15 to 20 seizures per day and using several antiepileptic drugs. In April 2021, we started introducing five products as follows: Raw full spectrum oil raw <0.3% THC (8.4mg CBDA/ml) 5ml 3x/day (126mg CBDA/day); full spectrum oil <0.3% THC (21mg CBD/ml) 5ml 3x/day (315mg CBD/day); concentrated full spectrum oil <0.3% THC (240mg CBD/ml) 0.1ml 2x/day (48mg CBD/day); concentrated full spectrum oil <0.3% THC (170mg CBD/ml) 0.1ml 3x/day (51mg CBD/day); THC oil intranasal spray (5mg/spray) 3x/day (15mg THC/day). Total daily CBD dose was 540mg (12.8mg/kg/day) and daily THC dose was 15mg (0.3mg/kg/day). Seizures dropped to 10-12 per day. Despite a partial improvement in the condition, the patient and his family still had a very poor quality of life. In November 2022, it was decided to change the therapeutic plan discontinuing all the oil-based cannabis products, except the nasal spray, and starting with three water-soluble nano-cannabinoid formulations to increase bioavailability and attain greater clinical results: Full spectrum THC/CBD 1:1 (10mg/ml:10mg/ml) 0.1ml 4x/day (4mg CBD/day, 4mg THC/day); Full spectrum <0.3% THC (20mg CBD/ml) 2ml 4x/day (160mg CBD/day); THC oil intranasal spray (5mg/spray) 1x/day (5mg THC/day). Total daily CBD dose is 164mg (4mg/kg/day), total daily THC dose is 9mg (0.2mg/kg/day). We also tried using a water-soluble intranasal spray, but it triggered patient's rhinitis, then we decided to keep the oil-based spray. After 3 months of treatment, there was a drastic seizures reduction of more than 80%. Currently, the patient no longer has seizures during the day, leaving only a few spasms and occasional nocturnal seizures. The results were achieved with about 1/3 of the previous dose of CBD and lower THC dose. There was also improvement in symptoms related to ASD and ODD, such as interaction with family members, improvement in speech, attention and less aggressiveness.

Conclusions: The results reported here are very promising and indicate that innovative and advanced water-soluble nano-based delivery system can be an alternative to increase bioavailability and efficacy of cannabinoids at lower doses. Benefits of the cannabinoid medicines controlling seizure, ASD and ODD symptoms were observed. There were no concerns regarding toxicity and scarce side effects. The family reported significant improvement in quality of life.

FEAR OF COVID-19 DIAGNOSIS AND INCREASED CANNABIS USE AMONG ADULTS LIVING WITH A CHRONIC HEALTH CONDITION: RESULTS FROM THE COVID-19 CANNABIS HEALTH STUDY

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Introduction: Limited literature exists on the impact of the unprecedented SARS-CoV-2 (COVID-19) virus on cannabis consumers living with a chronic disease. According to global reporting, millions of adults consume cannabis to manage chronic health conditions; it is crucial to explore how the COVID-19 pandemic impacted the cannabis use patterns of adults with a chronic disease. The purpose of this study was to examine how the fear of COVID-19 diagnoses impacted cannabis use during the pandemic.

Methods: The COVID-19 Cannabis Health study is a cross-sectional online survey aimed to examine how the COVID-19 pandemic impacted adult cannabis users by collecting self-report data on cannabis use patterns and health conditions throughout the COVID-19 pandemic. A subsample of (N=1,466) cannabis consumers living with a chronic disease was generated for analysis. Prevalence estimates and chi-square analyses were conducted using SAS.

Results: Majority (81.59%) of the sample was non-Hispanic white, 52.15% female, 11.94% lived outside of the US, and the mean age was 47.13 years (±15.05). 59.31%of the sample feared a COVID-19 diagnosis; 35.12% of adults who feared COVID-19 diagnosis consumed cannabis for both recreational and medicinal reasons compared to 25.96% of adults that did not fear COVID-19 diagnosis. Of the 17.02% of the subsample that reported a change in their cannabis route, 42.21% of adults fearing diagnosis consumed cannabis via pipe/bowl before the pandemic and reported a switch to consuming via edibles (35.71%) since the pandemic; while 31.11% of those with no fear of diagnosis consumed via pipe/bowl prior to the pandemic and 28.09% consumed via pipe/bowl since pandemic. A significant difference was found in the relationship between cannabis dose and fearing COVID-19 diagnosis (p=0.0312):61.67% of adults who feared COVID-19 increased their cannabis dosage compared to 38.33% of adults with no fear of diagnosis increased their use.

Conclusion: About 62% of adults who feared COVID-19 increased their cannabis dosage; therefore, it is crucial to examine the short and long-term impact of this increase on physical and mental health. Generalizability of current results to non-White populations is challenging so future research is encouraged to recruit diverse samples.

THE IMPACT OF IMPROVING THE MENTAL STATE ON THE PHYSICAL CONDITION, UTILIZING CANNABIS TREATMENT

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There is a belief that improvement in one's mental state can improve one's physical condition. Therefore, this research aims to examine the relationship between psychological and physical health by utilizing cannabis as a treatment for both in a naturalistic setting among patients.

The data used in this research is collected in real-time from the Strainprint® Journal and Outcomes Tracker Mobile App (the "App") from authorized medical cannabis patients across North America. Each time a patient treats with cannabis they track an electronic patient-reported outcome ("a session" or a "ePRO"). Each ePRO identifies: (i) the symptom(s) being treated (whether physical symptoms only or physical and mental at the same time - anxiety or depression), along with both pre- and post- medication severity ratings - on a scale from 1-10 - resulting in an efficacy score (the higher the efficacy score, the more significant the reduction in the severity of the symptom); (ii) the estimated dose; (iii) the route of administration; and (iv) the legal product used (including batch-level ingredient details from COA).

Patients in this study were broken out into the following four groups, based on the type of condition they are treating: (i) *Neuromuscular*: Physical Only (n=105,398 sessions, n=2,515 patients), Physical and Mental (n=23,051 sessions, n=972 patients); (ii) *Neurological*: Physical Only (n=85,947 sessions, 4,777 patients) Physical and Mental (n=8,986 sessions, n=957 patients); (iii) *Pain and Inflammation*: Physical Only (n=311,306 sessions, n=6,395 patients), Physical and Mental (n=16,186 sessions, n=1,793 patients); or (iv) Gastrointestinal: Physical Only (n=105,216 sessions, n=4,423 patients), Physical and Mental (n=10,244 sessions, n=1,036 patients). Efficacy scores were compared between sessions that treated only physical symptoms to those that simultaneously treated physical and mental symptoms. The severity of one's anxiety also broke out sessions or depression (high, medium, low) as determined by the severity on the 10-point scale before they used cannabis.

Results: The average efficacy score of cannabis for physical symptoms, grouped by:

Neuromuscular: Efficacy is 46% when treating anxiety and/or depression in the same session as physical symptoms and 43% treating only a physical symptom. Efficacy was highest (49%) for those treating severe anxiety with physical symptoms.

Neurological: Efficacy is 44% when treating anxiety and/or depression in the same session as physical symptoms and 45% treating only a physical symptom. Efficacy was highest (51%) for those treating severe anxiety with physical symptoms.

Pain and Inflammation: Efficacy is 40% when treating anxiety and/or depression in the same session as physical symptoms and 41% treating only a physical symptom. Efficacy was highest (46%) for those treating severe anxiety with physical symptoms.

Gastrointestinal: Efficacy is 50% when treating anxiety and/or depression in the same session as a physical symptom and 48% treating only a physical symptom. The reported efficacy was highest (54%) for those treating severe anxiety with physical symptoms.

Overall, cannabis was most effective for physical symptoms when patients were also treating severe anxiety. This suggests the importance of working with patients to understand their mental health while treating their physical health.

PHARMACOKINETICS AND ABSOLUTE BIOAVAILABILITY OF TWO NANOEMULSION FORMULATIONS OF Δ^8-TETRAHYDROCANNABINOL IN RATS

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Introduction: The consumption of Δ8-tetrahydrocannabinol (Δ8-THC) has increased steadily over the last several years. Like with Δ9-THC, the pharmacokinetic properties of oral intake are not ideal. Absorption in oil formulations is typically slow and variable, which often leads to inaccurate dosing or unintended overconsumption when additional doses are taken. Nanoemulsions are kinetically stable systems with particle sizes less than 250 nm that are formed when two immiscible liquids are exposed to high shear forces (e.g. from ultrasonic cavitation) in the presence of emulsifying agents (e.g. NanoStabilizers®). Nanoemulsions may be an improved delivery vehicle for molecules such as THC. The objective of this study was to characterize the pharmacokinetics (PK), including absolute bioavailability, of two nanoemulsion formulations of Δ8-THC in rats.

Methods: Adult male Sprague-Dawley rats (N=6 per group) were dosed with one of three formulations containing Δ8-THC. A liquid nanoemulsion and reconstituted powder nanoemulsion were administered by oral gavage at a dose of 10 mg/kg. The third formulation was prepared in a mixture of propylene glycol/ethanol/water (4.5:4.5:1 v/v) and administered at 0.6 mg/kg via intravenous injection. Venous blood samples were collected at 8 timepoints and plasma was isolated for quantification of Δ8-THC and two metabolites, 11-hydroxy-Δ8-THC (11-OH-Δ8-THC) and 11-carboxy-Δ8-THC (COOH-Δ8-THC), using an LC-MS/MS method. Non-compartmental PK parameters were calculated using Phoenix WinNonlin software.

Results: The median Δ8-THC Tmax of the liquid nanoemulsion was 0.667 h [range 0.667-1.5 h] and the median Tmax of the reconstituted powder nanoemulsion was 1 h [range 0.667-1.5 h]. These values compare favorably with a study in rats where the Tmax of THC in sesame oil was 4 h [range 2-4 h] (Izgelov et al., 2020). The Δ8-THC Cmax of the two nanoemulsions were comparable, with median values of 123 and 149 ng/mL, respectively. The 11-OH-Δ8-THC Cmax of the two nanoemulsions were also similar, with median values of 39 and 32 ng/mL, respectively. The absolute bioavailability of both formulations was approximately 10%.

Conclusions: The liquid nanoemulsion and the reconstituted powder nanoemulsions of Δ8-THC showed comparable PK properties. Both nanoemulsions demonstrated favorable PK profiles when compared to historical studies of oil formulations.

MACHINE LEARNING DIRECTED DESIGN OF AN ADVANCED ORAL FORMULATION OF CANNABIDIOL

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Introduction: The design and development of advanced oral formulations of drug remains challenging compared to conventional pharmaceutical products such as tablets and capsules. To circumvent the monotonous trial and error experiments that are commonly associated with pharmaceutical formulation development, our group has been working on data-driven strategies that harness advances in machine learning (ML) and experimental automation. In this work, we demonstrate how combining automated experimentation and ML can guide the design of promising lipid-based nanoparticles (LNPs) encapsulating the natural product cannabidiol (CBD).

Methods: Miniaturized batches of LNPs with varying compositions were prepared using an automated nanoprecipitation workflow on a liquid handing robot. ML analysis was used to explore the relationships between composition, properties, and performance for CBD-loaded LNPs. The pharmacokinetics and biodistribution of select LNP formulations were evaluated following oral administration to female Sprague Dawley rats (20 mg/kg).

Results: Tree-based ML models were found to best fit the collected experimental data. This data-driven approach to formulation development resulted in the identification of an optimal CBD-loaded LNP formulation within two days. This CBD-loaded LNP exhibited similar pharmacokinetics to a formulation equivalent in composition to the commercial product Epidiolex.

Conclusions: These results demonstrate that ML and automation can facilitate the expedited design of LNP formulations for enhanced oral delivery of hydrophobic natural products.

WITHDRAWN

THE EFFECT OF PRENATAL THC AND CBD EXPOSURE ON NEUROANATOMY AND BEHAVIOUR IN THE ADULT RODENT

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Introduction: Existing human and rodent studies have found some convergent and some contradictory findings on the impact of prenatal cannabinoid exposure on offspring. In children and adolescents, reported behavioural effects include a variety of cognitive changes (reviewed in *McLemore and Richardson*, 2016). Using a mouse model developed to reflect pre- and peri-gestational THC and CBD exposure, we examined the effects on neuroanatomy and a range of behaviours in young adult offspring.

Methods: Beginning 7 days prior to mating and continuing throughout pregnancy until gestational day 17.5, pregnant mice were subcutaneously injected once daily with either 5mg/kg THC, 60mg/kg CBD, or vehicle (1:4:15 95% EtOH:Tween80:saline). At 9 weeks of age, male and female offspring brains were fixed and imaged using MRI. Beginning at the same timepoint, a separate cohort of mice underwent a battery of behavioural tests which included accelerod (motor coordination), elevated plus maze (anxiety), open field (locomotion and anxiety), and novel object recognition (cognition and memory).

Results: No differences in brain structure volumes were detected when comparing drug-exposed adult offspring brains to vehicle controls in neither males nor females. However, we found sex-specific behavioural differences in the elevated plus maze and novel object recognition which point to alterations in anxiety and novelty-seeking behaviour. In the elevated plus maze male, but not female, CBD- and THC-exposed animals spent significantly more time in the open arms compared to vehicle controls, indicating a lower stress or anxiety response (ANOVA with Tukey's multiple comparisons test, $p<0.05$). Consistent with increased time spent in open arms, CBD-exposed males exhibited more overall and unprotected head dipping behaviour (ANOVA with Tukey's multiple comparisons test, $p<0.01$). In the novel object recognition test, female CBD-exposed mice displayed an altered object interaction time course favouring exploration of the novel object (ANCOVA, $p<0.05$).

Conclusion: Gestational exposure to cannabinoids affects behaviour in adult rodents without additional intervention. Specifically, we saw a reduction in anxiety-like behaviour in THC and CBD-exposed male mice, and an increase in novelty-seeking behaviour in CBD-exposed females. Interestingly, these behavioural changes occurred without alterations in the volume of brain regions, none of which were significantly different across groups when measured using MRI.

ADOLESCENT EXPOSURE TO LOW-DOSE THC DISRUPTS ADIPOSE ORGAN HOMEOSTASIS IN ADULTHOOD

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Introduction: One of cannabis' most iconic effects is the stimulation of hedonic high-calorie eating – the 'munchies' – yet habitual cannabis users are on average leaner than non-users. We asked whether this unexpected phenotype might result from lasting changes in energy balance established during adolescence, when habitual use of the drug often begins. In our previous study, we found that daily low-dose administration of cannabis' intoxicating constituent, delta-9-tetrahydrocannabinol (THC), to adolescent mice causes an adult metabolic phenotype characterized by reduced fat mass, increased lean mass and utilization of fat as fuel, partial resistance to diet-induced obesity and dyslipidemia. The purpose of the present study was to elucidate the persistent effects of adolescent THC exposure on adipose organ homeostasis which might underlie the paradoxical impact of THC on systemic metabolism.

Methods: Mice were given THC (5 mg/kg) by intraperitoneal injection (i.p.) from postnatal day (PND) 30 to PND 43. After reaching adulthood (PND 70), acute thermoregulatory responses were assessed under low ambient temperature, which simultaneously stimulates thermogenesis in BAT and lipolysis in WAT. White and brown adipose tissues were subjected to untargeted transcriptomic, proteomic, and metabolomic analyses, as well as to immunohistochemical (IHC) investigations.

Results: We found that mice exposed to THC in adolescence display enhanced thermogenesis and impaired cold- and beta-adrenergic receptor-stimulated lipolysis. This phenotype is associated with multiple molecular anomalies in both brown and white adipose tissues, which include a striking ectopic expression of proteins normally found in muscle, such as titin and troponin. IHC studies revealed substantial titin accumulation in the cytosol of brown adipocytes. Moreover, heightened anabolic processing was observed in brown adipose tissue of THC-exposed mice.

Conclusion: The results indicate that adolescent exposure to THC promotes an enduring 'pseudo-lean' state that superficially resembles healthy leanness but might in fact be rooted, at least in part, in adipose organ dysfunction.

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INVESTIGATING THE EFFECTS OF VAPORIZED CANNABIS ON ADOLESCENT NEURODEVELOPMENT AND BEHAVIOUR IN RATS

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Introduction: Since legalization of cannabis in Canada, there has been a push for research that backs public policy on safe cannabis consumption, including the impacts of cannabis use during adolescence. Roughly one third of youth and young adults consume cannabis, yet little research is available on the associated risks. Cannabis exerts its effects through acting on the endocannabinoid (eCB) system, which is known to be involved in neurodevelopment and behaviour (planning actions, emotional reactivity, and navigation). At present, clinical research in adolescence has found a relationship between cannabis-induced neuroanatomical changes and cognitive-emotional changes that could predispose one to mental health conditions. However, much of this research has been difficult to replicate as clinical adolescent research is retrospective and cannot control for the amount or timing of cannabis exposure. Pre-clinical models allow for control over dosage and timing of exposure. However, past animal research 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 more translational pre-clinical models. This project aims to investigate the effects of varied patterns of vaporized cannabis inhalation on adolescent 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 conditions: 1) 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. MRI scans were taken within five days pre- and five days post-vapour exposure, to quantify individual differences in neurodevelopment of regions of interest. Following the vapour exposure period, rats were also subjected to behavioral tests, including fear conditioning (FC) and for fear learning and working memory performance; as well as anxiety-like behaviours using a light-dark box (LDB) analysis.

Results: Compared to non-users, HF cannabis-exposed rats showed significantly reduced cortical white matter and a significant decrease in volume of the cortex and nucleus accumbens, regions that are involved in learning working memory, and reward salience. Preliminary analysis found that rats in the HF exposure group had poorer performance on learning tasks compared to the non-user group. Analyses on the weekly and daily usage groups are ongoing. For the FC paradigm, there was an interaction between sex, group (HF vs non-user), and conditioned stimulus/unconditioned stimulus (CS/US; tone/footshock) administration during conditioning. The HF exposure group exhibited less freezing behaviour than the non-user group across a number of CS/US presentations, in both males and females. Thereby rats exposed to cannabis took longer to associate a tone with a footshock, suggesting impaired learning. There were no significant differences in memory extinction or retrieval across any conditions. This observed learning deficit could correlate with our finding that HF-cannabis exposure decreased cortical volume in the cortex. Lastly, in the LDB paradigm, HF cannabis-exposed rats spent significantly less time in the dark side of the box than the non-user rats. This suggests that the cannabis-exposed rats either show a decrease in anxiety-like behaviours or have impaired risk-assessment function.

Conclusions: This research has shown that chronic, HF cannabis exposure impacts rat neurodevelopment by significantly decreasing cortical volume. This structural change could be correlated with the observed functional changes; both impairment in fear learning and in risk-assessment.

PRENATAL EXPOSURE TO THC AND CBD PRODUCES SEX-SPECIFIC EFFECTS ON ADOLESCENT NEUROPSYCHIATRIC OUTCOMES

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Introduction: Cannabis is one of the most widely used substances during pregnancy and is often perceived as safe. However, prenatal cannabinoid exposure (PCE) is associated with several neuropsychiatric outcomes, such as cognitive impairments and increased vulnerability to mood disorders. Investigation into the underlying mechanisms of these effects is needed to better understand the potential risks. The impact of distinct cannabis constituents [Δ^9-tetrahydrocannabinol (THC) vs. cannabidiol (CBD)] and sex-specific outcomes remain unclear. This project utilized a rat model of PCE to assess long-term neuropsychiatric liability in both male and female offspring. THC and CBD were assessed separately and in combination.

Methods: Pregnant Wistar rats were given daily injections (i.p.) of THC (3 mg/kg), CBD (30 mg/kg), a combination (THC+CBD), or vehicle from gestational days 7-22. Offspring underwent a battery of behavioural tests during adolescence (postnatal day 35-45) to assess changes in anxiety-like behaviours, object and social memory, and sensory gating. *In vivo* electrophysiology and RT-PCR were then used to determine neuronal and gene expression changes in the prefrontal cortex (PFC) and ventral hippocampus (vHipp).

Results: Adolescent CBD and THC+CBD exposed female offspring exhibited anxiety-like behaviours, while THC+CBD male offspring spent more time in the open arms of the elevated plus maze. All PCE male offspring displayed reduced temporal order object recognition, but only CBD female offspring were impaired on this task. Social recognition was impaired in all PCE groups regardless of sex. Prenatal CBD and THC+CBD reduced sensory gating in only male offspring. Sex and treatment-specific effects on neuronal activity in the PFC and vHipp were also observed. Prenatal THC tended to increase delta, theta and alpha oscillatory patterns in males, while THC+CBD reduced the power of these frequencies in both males and females. Prenatal CBD reduced delta power in male vHipp and reduced theta and alpha oscillatory patterns in female vHipp. Preliminary results also suggest that prenatal exposure to CBD reduced the firing rate of PFC pyramidal neurons in both male and female offspring. PCR analysis revealed upregulated CB1 receptor gene expression in the PFC of CBD and THC+CBD exposed males and females and in the vHipp of THC+CBD males. Glut/GABAergic-related gene expression was altered in the PFC and vHIPP of all PCE male offspring groups. CBD and THC+CBD exposed females exhibited changes in serotonin 5-HT_{1A}R (a target of CBD) and energy metabolism-related gene expression.

Conclusions: PCE differentially affected male and female offspring. Female offspring may be more susceptible to the effects of prenatal CBD, while males seem more impacted by THC. Changes observed in the PFC and vHipp may underly the long-term effects of PCE.

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ADOLESCENT VAPORIZED CANNABIS USE ELICITS LEARNING AND BRAIN CONNECTIVITY CHANGES IN ADULTHOOD

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Introduction: In recent years, cannabis-derived vaping products have become popular among adolescents. As cannabis usage becomes more mainstream, more research is needed to investigate the long-term effects of vaporized cannabis use on brain development and function, particularly looking at use starting in adolescence. Thus, this study aimed to investigate how vapor exposure to 3 types of cannabis in adolescent rats impacts brain development through behavioural tests and magnetic resonance imaging (MRI).

Methods: Sprague Dawley rats were divided into four groups (7/group for males and 8/group for females) and exposed to either high-cannabidiol (high-CBD) cannabis flower, high-D-9-tetrahydrocannabinol (high-THC) cannabis flower, balanced CBD + THC cannabis flower, or air at post-natal days (PND) 28-42 using a volcano vaporizer. Blood samples were collected post-exposure on the final day of cannabis administration (day 14) to check plasma cannabinoid levels. On days 0 and 14 of exposure, rats underwent a cannabinoid tetrad test (e.g., locomotor activity, tail-flick latency, rectal temperature, and catalepsy). In adulthood (PND68 onward), all rats underwent 12 days of Pavlovian autoshaping, followed by active avoidance, prepulse inhibition and finally diffusion and functional MRI analysis using a 9.4T MRI.

Results: Analysis indicated significant plasma cannabis levels were found in both sexes. For the cannabinoid tetrad test, all-male groups showed significant increase in body temperature following cannabis exposure when compared to baseline, whereas tail flick latency only significantly increased for the THC-exposed group. For females, all treatment groups showed a significant increase in tail-flick latency following exposure when compared to baseline. Analyzing Pavlovian autoshaping using Pavlovian Conditioned Approach (PCA) showed male rats exposed to high-THC and high-CBD cannabis had less lever-directed behaviour after the 3rd session compared to controls and balanced cannabis-exposed groups. Interestingly, all female rats were found to show high lever-directed behaviour (sign-trackers) with no significant differences between the exposure groups. Male active avoidance showed significant differences in avoidance learning lower avoidance between the treatment groups with all three cannabis exposed groups showing impaired avoidance learning. The female active avoidance data showed significant differences between the THC and CBD-exposed groups only. Both males and females showed no treatment group differences in PPI. Regarding MRI data in male rats, we found a single network with altered functional connectivity amongst the four groups and two networks with altered structural connectivity amongst the four groups.

Conclusion: Adolescent cannabis vapor exposure to both CBD and THC-containing cannabis, can lead to lasting impacts on behavioural, cognition, and brain structure and functionality in adulthood with clear sex differences that need to be further investigated.

LONG-TERM BEHAVIOURAL EFFECTS OF VAPORIZED THC AND NICOTINE CO-EXPOSURE IN ADOLESCENCE

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Introduction: Adolescents are vaping nicotine and cannabis at high rates in North America (Smith, D.M. et al. J Cannabis Res 3, 39 (2021)). However, there is limited research on the long-term effects of their co-use. In adolescence, the brain undergoes a critical period of development where it is particularly vulnerable to the effects of exogenous substances including nicotine and Δ9-tetrahydrocannabinol (THC), the main psychoactive cannabinoid in cannabis (Thorpe, H.H.A. et al. Pharmacol Ther, 206 (2020)). The long-term objective of this research is to identify long-lasting behavioural effects of adolescent concurrent THC and nicotine use in males and females.

Methods: Four groups of Sprague Dawley rats (n=8/sex/treatment, post-natal day PND28) received once-daily passive administration in adolescence for 14 days of either vaporized vehicle (control), nicotine, THC, or combined THC and nicotine. Blood was collected on the last day of exposures to confirm THC and nicotine blood concentrations. The cannabinoid tetrad test measured locomotion, hypothermia, tail-flick analgesia, and catalepsy, to assess behavioural outcomes of adolescent THC exposure. In adulthood (PND 85), rats underwent behavioural tasks including locomotor reactivity to a novel environment (LRNE), and autoshaping/sign-tracking. Autoshaping involves the delivery of a food reward after the presentation of the conditioned stimulus lever (CS+), regardless of whether the rat interacts with the lever. Pavlovian Conditioned Approach (PCA) scores were used to evaluate the performance of rats as sign-trackers or goal-trackers depending on their preference of interacting with the CS+ lever or the food cup.

Results: In the tetrad test, the combination group displayed significantly increased tail-flick latency in females only. While there was no significant difference between groups in LRNE, both female THC and combination groups spent significantly more time in the center zone in the LRNE test. During autoshaping, sign-tracking behaviour was significantly higher in the male combination group compared to controls. There was no significant difference between groups in sign-tracking in the female rats.

Conclusions: In male rats, concurrent use of vaporized nicotine and THC in adolescence results in significantly higher sign-tracking behaviour during adulthood which indicates a long-lasting impact on reward learning. In female rats, no significant differences in sign-tracking were observed, but a decrease in pain sensitivity and anxiety levels in an open field was found in combination groups. Thus, chronic adolescent administration of nicotine and THC produced sex-dependent differences in short- and long-term behavioural effects.

IN-VITRO EXPOSURE OF HUMAN OOCYTES TO CANNABIS ALTERS CHROMOSOMAL ORGANIZATION AND SEGREGATION – A SINGLE CELL NEXT GENERATION SEQUENCING STUDY

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Introduction: Cannabis legalization is increasing worldwide and was legalized in Canada in 2018.It is the third most used substance by women of childbearing age and THC has been shown to reach the follicular niche and affect supportive granulosa cells in humans. Thus, knowledge of its effects on female reproduction is of utmost importance.

Methods: GV oocytes were collected from 25 consenting patients (135 oocytes) undergoing IVF-ICSI at the CReATe Fertility Centre. GV oocytes were split into three groups: control (n=44), THC1 (n=46) (physiological concentration of Δ9-THC, based on previous human studies) and THC2 (n=45) (supraphysiological concentration, based on animal studies), and cultured for 24h in Sage OneStep+Menopur (7.5IU/mL). To reduce inter-sample variability, we selected patients with similar demographics and stimulation parameters that also had at least 1 MII oocyte for each treatment group. cDNA from 39 oocytes (10 patients) was synthesized from single-oocytes using SmartSeq v4. Libraries were constructed (NexteraXT) and sequenced (NovaSeq S1 2x150bp). Bioinformatics (Partek Flow) and pathway analysis were conducted to identify key pathways altered by THC treatment. Differentially expressed genes (DEG) were defined as -2>FC>2, p-value<0.05.

Results*:* Preliminary results showed increased maturation rate following exposure of human GV oocytes to cannabis (31.8% for Ctrl (n=44), 34.8% for THC1 (n=46) and 46.7% for THC2 (n=45)), however this analysis was underpowered and still ongoing. When assessing the oocyte transcriptome; principal component analysis (PCA) clustered based on oocyte maturation level (PC1 30.0%), but not based on cannabis treatment. Of oocytes that matured to metaphase-II, there were 189 differentially expressed genes (DEG) in THC1 vs Ctrl; 66 genes were upregulated, and pathway analysis revealed an enrichment in electron transport chain, ATP metabolic process, and response to toxic substances. The 123 downregulated genes were associated with regulation of chromosome segregation and duplication, vitamin D signalling, and cell development. Furthermore, 6 genes previously associated with oocyte maturation were differentially expressed (*ACTG1*, *ANK2*, *PWWP2A*, *LIN7C*, *TM4SF1*, and *KLHL28*). In oocytes exposed to even higher (supraphysiologic) concentrations of cannabis, upregulated genes (n=6) were associated with transcription initiation and activation, while the downregulated genes (n=36) were associated with nuclear DNA replication and DNA methylation.

Conclusion: This is the first study investigating the impact of cannabis on human oocytes. We demonstrated a significant change in ooplasm mRNA, potentially altering meiotic spindle formation/organization and cytoplasmic maturation. With cannabis legalization increasing worldwide, further investigation into its consequences is critical for clinical consultation and legalization guidelines.

A SCOPING REVIEW ON CANNABIS EQUITY INITIATIVES IN JURISDICTIONS WITH REGULATED CANNABIS MARKETS

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Introduction: Cannabis equity initiatives – either to support the inclusion of groups disproportionately harmed under cannabis prohibition in the employment and economic opportunities in the legal cannabis industry, or to grant amnesty for cannabis offences – have been implemented in a number of jurisdictions where non-medical cannabis markets have been legally regulated. Our work aims to explore the best practices and available evidence on these initiatives.

Methods: A scoping review was conducted using a structured approach. English-language peer-reviewed records were found through keyword searches of electronic databases including Scopus, Medline (via Ovid), Embase (Ovid), PsycINFO (Ovid), CINAHL, Web of Science's Science Citation Index, Social Sciences Citation Index, Emerging Sources Citation Index, Sociological Abstracts (ProQuest), EconLit, Criminal Justice Abstracts, and Business Source Premier (EbscoHost). Studies were included if they contained original data on the impacts of cannabis equity initiatives in jurisdictions with regulated non-medical cannabis markets or if they were a systematic review of such studies.

Results: We screened 2068 titles and abstracts and 106 full texts, with 1 study meeting inclusion criteria. This paper found gender and racial inequity in senior positions in the cannabis industry in Massachusetts, United States, where participation was low for women of colour and Latinos of all genders. A post-hoc grey literature review was undertaken, with 3 reports selected for inclusion. These reports all discussed the potentially exploitative experiences of participants of equity initiatives in relationships with non-equity applicant incubator partners and investors, and recommended that there be more oversight of these relationships by licensing bodies.

Conclusions: There is a dearth of information on the effectiveness of cannabis equity initiatives. Current cannabis equity initiatives may not be addressing inequity through inclusion of people of colour and women in senior positions within the legal cannabis industry. Significant research is needed to identify the effectiveness of current cannabis equity initiatives to improve policy where it exists and inform policy in jurisdictions where it has not yet been implemented.

ASSESSMENT OF CLAIMED VS FOUND LEVELS OF Δ^9-THC AND CBD IN SIXTEEN NON-FLOWER CANNABIS PRODUCTS AVAILABLE IN THE COMMERCIAL MARKET PLACE

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Introduction: The enactment of marijuana laws in many states coupled with the Hemp Improvement Act (The 2018 Farm Bill) has resulted in the proliferation of several cannabis derived products. The quality of these products is suspect at best. We have previously reported on the analysis of several cannabis flowers products acquired from dispensaries in three major cannabis producing states (Colorado, California, and Oregon). Through the same mechanism by which we received those flowers samples we were able to receive samples from a variety of non-flower cannabis products for this investigation. The goal is to determine how accurate the labeling of these products is for Δ^9-THC and/or CBD. Previous reports have shown large discrepancies between the claimed and found concentrations for some products. This study encompasses several products that have not been previously examined.

Methods: Samples from 16 different products were acquired through legal channels from dispensaries in California, Colorado, and Oregon. All samples (a total of 126 samples) were analyzed by a validated GC/FID method for seven major cannabinoids. Since analysis was carried out on underivatized extracts the values obtained represent the total cannabinoids (acid and neutral). The seven major cannabinoids analyzed are Δ^9-THC, CBD, Δ^8-THC, CBC, CBG, THCV and CBN.

Results: The products analyzed are Beverages (3), Chocolate containing products (11), Cookies (4), Fruit Chews (5), Gummies (15), Pills/Tablets/Capsules (5), Suckers (1), Badder (2), Distillates/ Distillate Cartridges/Distillate Vapes (12), Live Resin/Resin Cartridges (16), Oils and Oil Cartridges (16), Sauce (live sauce and sugar sauce): (5), Shatter (10), Suppositories (1), Tinctures (11) and Waxes (9) for a total of 126 products. Within each product the number of samples with >20% deviation between the labeled amount and the found values were identified.

Conclusions: It is concluded that the number of samples with labelled amounts of cannabinoids (CBD and THC) outside the ±20 of the analytical value is excessive and calls for tightening quality control measures on cannabis derived products.

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INVESTIGATION OF CANNABINOID CONTENT IN CANNABIS BEVERAGE PRODUCTS: VARIABILITY AND ACCURACY

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Introduction: The dynamic nature of the rollout of legal cannabis edible products in Canada in Fall 2019 has led to widespread availability of many new products arriving on the market, with beverages increasing in popularity. Cannabis edible product have a regulated limit of 10mg of the cannabinoid tetrahydrocannabinol (THC) per serving, however concentration of THC can vary with product volume in the case of beverages. There is no regulated limit in the case of CBD, so concentrations of CBD in these beverages can vary to a greater degree. Consumers may be uncertain how to select products safely and reliably, especially consumers with medical conditions or those taking prescription drugs, as the risk for cannabis-drug interactions is present. If labelled cannabinoid content is not representative of actual cannabinoids present, risk of overdose or unexpected outcomes increases. To date, there has been no evaluation of cannabinoid content variability in cannabis beverage products. This research was undertaken with the goal of informing safe use and quality control of non-solid cannabis edible products. This study aimed to characterize major cannabinoid content (THC and CBD) in beverages available through the Ontario Cannabis Store (OCS). Variability in cannabinoid content was determined within and between products and product categories, and relative to labelled values.

Methods: Samples of beverages were taken and diluted in methanol for better quantification of cannabinoids. Two different sample preparation methods were tested and compared. Direct sampling and a secondary method of freeze drying and reconstitution of samples in methanol was performed. High Performance Liquid Chromatography (HPLC) was used to identify and quantify cannabinoid presence in beverages. Comparisons between beverages of the same lot, different lots, and beverage categories were performed.

Results: Among the tested beverages, total THC and CBD values ranged from 3.5-10mg and 10-40mg, respectively. Cannabinoid concentrations ranged from 10-45µg/mL (THC) and 28-400µg/mL (CBD). Intra-product variability ranged from <1% to over >14%. Both THC and CBD content showed results that were higher and lower than labelled content, with discrepancies from labelled values ranging from <-80% to >200%. Cannabinoid content (THC or CBD) of fewer than half of the beverages was within 15% of their labelled values: the limit for variability of THC products. No consistent differences in variability and label accuracy were detected between product types (ex: juice, tea, sparkling beverage).

Conclusion: This work suggests that cannabinoid discrepancies between beverages and labels may exist in products easily accessible to consumers through the OCS. These kinds of discrepancies can have implications in terms of reliability of labelling and consumer use. Values lower than labelled can result in undesired effects while values higher than labelled could result in unexpected outcomes such as overdose or illness. The risk for cannabis-drug interactions at the metabolic level also increases if cannabinoids as found in higher concentrations in a product. This work can be used to better inform cannabis production and consumption. This study can provide a foundation of knowledge to better inform health care practitioners and consumers alike on cannabis products.

MULTINATIONAL MEDICAL CANNABIS USE PATTERNS FOR MENTAL AND PHYSICAL HEALTH CONDITIONS AND PRODUCT CHARACTERISTICS

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Introduction: Medical use of cannabis is allowed in many countries. In the U.S., states set up their own systems due to federal prohibition. Canada, which permits medical and recreational cannabis use, manages medical cannabis federally, but provinces regulate retail sales. Australia, which permits medical use, has a highly regulated medical model.

Methods: In this paper, we describe the conditions for which medical cannabis is used across these three countries. We use self-reported data specifying reasons for medical use, product choice, and product composition from the 2021 survey wave of the International Cannabis Policy Study. Simple differences in weighted proportions reported across countries are assessed using t-tests. Regression models are then used to assess differences controlling for demographics.

Results: We find that the same reasons are generally given for medical use despite jurisdictional policy variance. Medical patients in all three countries report use primarily for pain (AUS: 44.3%; CAN: 53.4%; US: 54.3%), anxiety (53.0%; 53.5%; 60.9%), depression (46.1%; 42.1%; 46.1%), sleep problems (37.6%; 48.4%; 42.4%) and post-traumatic stress disorder (28.8%; 17.5%; 24.5%). Herb products followed by edibles were the top two products used across all countries, although half of all medical patients report using both, despite limited evidence for these modes of administration for medical use.

Conclusions: Despite jurisdictional differences in medical policies, evidence from self-reports suggest similar reasons for using medical cannabis and product choice. Further investigation is necessary to understand if this is due to access via illicit markets or confounding reporting with recreational use.

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BRIEF COMPOSITE CANNABIS ASSESSMENT TOOL: A NEW CANNABIS USE MEASURE

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Introduction: The changing landscape of legal cannabis use in Canada highlights the need for more nuanced assessment of cannabis users that go beyond purely problematic aspects of use. The Brief Composite Cannabis Assessment Tool (BCCAT) was developed with the purpose of providing a brief measure that characterizes consequences of cannabis use, while also differentiating between problematic, therapeutic, and recreational cannabis use.

Methods: A preliminary set of 25 candidate items was were administered via online survey to 384 undergraduate students, and principal axis analysis was conducted on their responses to identify the factor structure and inform the selection of items. We then administered the reduced 12-item measure to a second sample of 437 adult cannabis users, and a large sample of 2032 medical cannabis patients. Factor structure of the items was examined in both samples using principal components analysis. Reliability and validity was evaluated by examining internal consistency of the BCCAT subscales, by examining correlations between subscale scores and responses on gold-standard measures of cannabis motives and consequences, and by examining pairwise comparisons of subscale scores from medical and nonmedical cannabis users.

Results: Factor analysis of responses to the 25 candidate items suggested a 3-factor structure for Therapeutic Use, Problematic Use, and Recreational Use. When administered to medical and non-medical cannabis users, the BCCAT subscales demonstrated excellent internal consistency (all $\alpha > .800$). Validity was established by presence of convergence and discrimination of cannabis use characteristics assessed by each subscale. For example, higher scores on Therapeutic Use were associated with using cannabis for common medical concerns such as sleep disturbance ($r = .737$, $p<.001$), and weak associations with non-therapeutic use motives such as social conformity and experimentation ($r = -.008$, $p>.05$). Medical users scores on Therapeutic Use were greater than for Recreational Use ($t=30.878$, $p<.001$) and Problematic Use ($t=99.20$, $p<.001$), and were also greater than Therapeutic Use scores in nonmedical users ($t=53.130$, $p<001$)

Conclusion: The BCCAT addresses concerns with previous cannabis use assessments as it measures therapeutic, non-problematic, and problematic cannabis use patterns, and has been validated in medical, community, and university samples. The BCCAT is suitable for a diverse range of medical and research settings, and can provide distinct profile scores for each cannabis user.

ADVERSE EVENTS OF MEDICAL CANNABIS REPORTED TO A SAFETY CALL CENTER

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Introduction: In Minnesota, patients with specific qualifying medical conditions are certified by a licensed healthcare provider to use medical cannabis (MC) products in accordance with state law. The corresponding legislation includes a provision mandating accessible post-market surveillance for all patients (Minnesota House of Representatives, 2018). Mandated collection of adverse events (AEs) data provides the framework for analysis of the AEs associated with the use of MC. Our objective was to describe the AEs reported to a mandatory call center.

Methods: This is a retrospective study that analyses all calls reporting AEs to a central designated center (Safety Call, LLC) over a 6-year period (2015-2021). Calls reporting information not recognized as AEs (i.e., overdose, expired product dispensed, efficacy issue, device breakage) were excluded from the analysis. Information collected during the calls included: exposure characteristics (symptom(s), severity, treatment applied), caller identity (exposed individual, family member, healthcare provider), date and time of the call, demographics (gender, age) and information on medical cannabis product(s) exposed (i.e., name, formulation). Data were categorized based on patients' gender (male, female, unknown), age groups (<18, 18-64, ≥65 years), qualifying condition(s), severity of the symptom(s) (i.e., asymptomatic, minor, moderate, major), and the composition of the product (CBD only, CBD dominant, CBD:THC balanced, THC only, and THC dominant). Reported symptoms were further classified and reported by the organ system based on the international statistical classification of diseases and related health problems ICD-10. R software (V.4.0.3) was used for data analysis.

Results: The dataset included 237 calls from 225 unique individuals reporting 692 AE symptoms. Most patients were female (59.1%). Of the calls received, 49.3% were from individuals aged 18-64 years and 40.0% were from 65+ individuals. Median (range) age of callers was 60 years (4 to 95 years). Intractable pain was the main qualifying condition associated with the use of MC (40.1%). Most of the reported AEs were minor (71.7%), followed by moderate (26.6%), and major symptoms (0.8%). The 2 calls reporting major symptoms included cough, dyspnea/shortness of breath, respiratory, irritation, worst anxiety, chills/rigors, dizziness/vertigo, drowsiness/lethargy, emesis/vomiting, feeling high, fever/hyperthermia, insomnia/sleep disorder, lung infection, nausea. AEs were mostly associated with THC dominant products (39.8%), followed by those that were CBD-THC balanced (23.3%). Capsules were the formulation associated with most symptoms (36.8%). Symptoms classified as "Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified" (ICD-10 R) were the most reported (72.3%), specifically "Symptoms and signs involving cognition, perception, emotional state and behavior" (ICD-10 R40-46) (32.4 %), "Symptoms and signs involving the digestive system and abdomen" (ICD-10 R10-19) (20.4 %), and "General symptoms and signs" (ICD-10 R50-69) (19.0%).

Conclusions: This study provides a comprehensive analysis of AEs associated with MC use in a statewide system. Notably, our findings suggest that THC use is associated with a higher incidence of AEs compared to CBD only or CBD and THC use, underscoring the importance of more stringent monitoring of its use. Furthermore, there is a need for further research on subpopulations, including older MC users, to gain deeper insights into the potential risks and preventative strategies for AEs in these groups.

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PRELIMINARY DATA CO MPARING THE EFFECT OF ACUTE VAPORIZED CANNABIS TO ORAL OXYCODONE AND PLACEBO ON STANDARDIZED FIELD SOBRIETY TEST PERFORMANCE

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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. Thus, the primary aim of this double-blind, within-participants crossover trial is to determine the efficacy of acute cannabis exposure to oxycodone for pain analgesia. This trial not only includes chronic spine pain patients (spontaneous pain relief results presented in separate abstract), but concurrently investigates the analgesic efficacy of cannabis on experimental pain in a group of healthy individuals.

Methods: After informed consent and screening, participants attended 3 separate 4-hour study visits, with 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. Participants received one of the following drugs across the 3 visits: active vaporized cannabis (placebo capsule), active oxycodone (placebo cannabis), and placebo/placebo. Enrollment continues in this study; therefore, final data analyses have not been completed. Descriptive statistics were performed on a subset of data collected from participants who completed all three study visits. The data remain blinded and are referred to here as drugs A, B, and C (3 drug treatments: Cannabis, Oxycodone, and Placebo). Data are presented as means.

Results: To date, a total of 38 participants have completed the study. Participants who received Drug A displayed a reduction in their ability to perform the one leg stand component of the test, whereas those who received Drug C exhibited a marked increase in the occurrence and severity of Horizontal Gaze Nystagmus. Administration of Drug B did not impair participants' ability to pass any portion of the field sobriety test. Furthermore, the administration of Drug A, Drug B, or Drug C did not affect the participants' ability to pass the walk and turn portion of the field sobriety test.

Conclusions: These initial data appear to show differences in the effects of the administered drugs on participants' performance during the field sobriety test. While Drug A was associated with a reduced ability to perform the one leg stand component of the test, Drug C led to a marked increase in Horizontal Gaze Nystagmus occurrence and severity. Interestingly, Drug B did not have a significant impact on participants' performance during the test. These results suggest that oxycodone and vaporized cannabis can have varying effects on field sobriety test performance and further research is needed to explore the implications of these findings for forensic and clinical contexts. The study is expected to be completed by the beginning of 2024.

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THE ADVERSE HEALTH EFFECTS OF MICROBIAL CONTAMINANTS OF CANNABIS: A SCOPING REVIEW

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Introduction: Whilst many potentially pathogenic organisms have been identified from the surface of dried *Cannabis sativa* L. flower, the prevalence of adverse events secondary to ingestion or inhalation of cannabis contaminated by microorganisms is not currently know. Moreover, whilst the effects of regulations on cannabis quality and the underlying health of consumers is presumed to affect the risk associated with consuming cannabis, this has not been fully examined. Therefore, this review primarily aimed to explore the adverse health effects of microbial contaminants of cannabis. Secondary aims were to investigate whether factors, such as underlying health conditions and/or source of cannabis may affect health outcomes.

Methods: A systematic review was conducted of EMBASE, MEDLINE, and PubMed databases up to 1st February 2023 in line with PRISMA-ScR guidance. The literature search and data extraction were performed by two independent co-authors with discrepancies resolved by a senior author. Inclusion criteria extended to any study methodology which reported adverse health effects due to microbials where cannabis consumption was reported in the same individuals. Studies were excluded if they did not constitute original research.

Results: In total 700 studies were screened, from which 28 met inclusion criteria, reporting outcomes in 119 patients. The most reported contaminants responsible for adverse health effects were *Aspergillus spp.* (study n=19), resulting in invasive aspergillosis (n=8), disseminated aspergillosis (n=4), or hypersensitivity pneumonitis (n=4). Other organisms responsible for adverse outcomes included Blastomyces (n=2), Pseudomonas aeruginosa (n=2), Cryptococcus neoformans (n=2), Nocardia spp. (n=2), Fusarium (n=1), Rhizopus spp. (n=1), and Geotrichum candidum (n=1). Cannabis was confirmed as being the definitive cause of the negative health effects through microbial testing in 8 (28.6%) studies. Cannabis was illicitly sourced or purchased from recreational dispensaries in the United States in 17 (60.7%) and 3 (10.7%) studies, respectively. Source of cannabis was unreported in 8 (28.6%) of studies, whilst no studies reported adverse effects from microbial contaminants in legal medical cannabis patients. Underlying immunosuppression and chronic lung disease were reported in 14 (50.0%) and 3 (10.7%) studies.

Conclusions: Whilst it is well-known that cannabis is commonly contaminated by pathogenic microorganisms, this study is the first to attempt to summarize the evidence of the potential adverse effects associated with these. The available evidence is of low quality and limited to poorly defined case series or case reports. However, the data does support a potential association between cannabis contaminated with microbials and adverse effects on health. Importantly these effects are not limited to those with underlying immunosuppression or chronic lung disease. Whilst further high quality research is necessary, the paucity of reports in legal medical cannabis patients suggests that higher standards in these settings may negate this potential risk.

TETRAHYDROCANNABINOL METABOLITE ASSOCIATIONS WITH DECISION-MAKING PHASES ON A CANNABIS PURCHASE TASK

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Introduction: With increasing legislation moving toward legalizing recreational cannabis, examining the biological and behavioural determinants of cannabis misuse is crucial. Applying a behavioural neuroeconomic approach can elucidate mechanisms underlying maladaptive decision-making for cannabis consumption. The Marijuana Purchase Task (MPT) captures cannabis valuation and can identify problematic cannabis use. To date, no studies examine the neural correlates of the task, or THC metabolite relationships with decisions on the MPT. This presentation will discuss THC metabolite associations with decision-making on the MPT.

Methods: Twenty participants reporting recreational dry flower cannabis use at least twice/week from the community underwent functional magnetic resonance imaging (fMRI). The MPT asks individuals to choose how many grams to consume at a range of prices ($0-$100/gram), which produces a demand curve. The curve includes: (1) steady consumption at low costs (inelastic/approach trials); (2) consumption choices sensitive to price increases (elastic/ambivalent trials); and (3) no consumption (suppressed/avoid trials). Participants completed a urine screen on which was analyzed for THC metabolites using mass spectrometry urinalysis.

Results: Individuals demonstrate the three distinct phases of the curve on the task (Figure 1), with the greatest mean consumption for inelastic trials, and no consumption during suppressed trials (Figures 2 and 3). THC glucuronide (THC-Glu) shows significant negative correlations with activity in the anterior cingulate cortex (ACC; r = -.51), caudate (r = -.70) , and left (r = -.67) and right (r = -.50) putamen during approach trials. There are no significant relationships between THC glucuronide and activity during ambivalent trials. During avoid trials, THC glucuronide shows significant negative correlations with activity in the right ACC (r = -.56), left (r = -.52) and right (r = -.59) caudate, and right putamen (r = -.52). Higher THC-Glu is significantly, positively correlated with Intensity (r=.57), and Omax (r=.82) and inversely correlated to Elasticity (r=-.51; Figure 4) on the MPT (all ps<0.05).

Conclusions: THC-Glu is associated with neural and behavioural economic demand on the MPT. Findings suggest individuals with greater THC-Glu are less sensitive to increasing prices and are more likely to consume more cannabis at higher prices. These findings have implications for legislation surrounding cannabis availability/pricing, and interventions.

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FREQUENT LOW-DOSE THC IN ADOLESCENCE INDUCES PERSISTENT AND SEXUALLY DIMORPHIC EFFECTS ON BRAIN TRANSCRIPTOME IN RESPONSE TO THE VIRAL MIMIC POLY (I:C)

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Introduction: The endocannabinoid (ECB) system is critically involved in neural network formation and modulating synaptic plasticity. Epidemiological and preclinical research studies have found that chronic exposure to delta-9-tetrahydrocannabinol (THC) during adolescence causes persistent neurobiological changes that may affect cognition. The immune system, where the ECB system also plays a modulatory role, and central nervous system are deeply interconnected, and perturbations in either can have detrimental effects on cognition. The present study aimed to explore how adolescent THC exposure might impact the immune response to viral challenge in the brain using the double-stranded RNA mimic, polyinosinic:polycytidylic acid (Poly (I:C)), which activates certain Toll-like receptors and leads to the production of type-1 interferons. Understanding the impact of adolescence exposure to cannabis on the long-term consequences of viral infections in the central nervous system (CNS) is important, especially in the post-coronavirus disease (COVID)-19 era.

Methods: Mice were given THC, 5 mg/kg, by intraperitoneal injection (i.p.), from postnatal day (PND) 30 to PND 43 and left untreated until young adulthood. At PND 70, mice received i.p. injections of Poly (I:C) (12 mg/kg) or control saline. Four hours after the injection, brains were collected for gene expression analysis using bulk RNA sequencing (RNAseq). Comparative transcriptome analyses were carried out in the basal or Poly (I:C)-stimulated condition between adolescent THC-exposed (ado-THC) versus adolescent vehicle-exposed (ado-vehicle) groups. Both male and female mice were tested and compared for possible sexual dimorphisms in the response to adolescent THC exposure and/or Poly (I:C).

Results: In both male and female mice, Poly (I:C) administration upregulated many immune-related genes enriched in gene ontology (GO) categories including defense response to virus, immune system process, and negative regulation of viral genome replication. Adolescent THC exposure slightly increased this effect in males, while producing an overall decrease in females. In addition, Poly (I:C) elevated the transcription of genes related to translation and ribosomal subunit-encoding genes in males but reduced them in females. Adolescent THC exposure dramatically reversed these effects in both sexes. Poly (I:C) down-regulated genes were enriched in GO categories including nervous system development and synaptic transmission in control male mice, but this change was sex-specific and abrogated in adolescent THC-exposed mice.

Conclusion: We found that administration of the viral mimic Poly (I:C) caused significant transcriptional alterations in mouse brain, which were sexually dimorphic and modified by adolescent THC exposure. The results suggest that adolescent cannabis use may persistently impact the brain's response to immune challenges and affect synaptic function differentially in males and females.

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CHANGES IN CANNABIS USE PATTERNS IN PSYCHIATRIC POPULATIONS PRE- AND POST- LEGALIZATION OF RECREATIONAL CANNABIS USE IN CANADA: A REPEATED CROSS-SECTIONAL SURVEY

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Introduction: To examine the changes in daily and average 30-day cannabis use amongst individuals reporting past 12-month experiences of specific mental health disorders and among those without past 12-month experiences of any mental health disorder before and after Canadian legalization of recreational cannabis use.

Methods: Data came from Canadian respondents in Wave 1 (August – October 2018), Wave 2 (September – October 2019), and Wave 3 (September – November 2020) of the International Cannabis Policy Study. Participants (N = 13,256) self-reported past 12-month anxiety, depression, post-traumatic stress disorder, schizophrenia, bipolar disorder, or an alcohol/substance use disorder. Changes in daily and average 30-day cannabis use from Wave 1 to Wave 3 were assessed using self-report survey questions. Changes in cannabis use were also examined among non-psychiatric persons.

Results: After adjustment for covariates, the odds of using cannabis daily increased only in individuals with schizophrenia between Wave 1 and Waves 3 (*aOR* = 5.60, *95% CI*: 1.96 – 16.00). Similarly, significant increases in the average frequency of average 30-day cannabis use between Wave 1 (*M* = 12.80, *SD* = 1.65) and Wave 3 (*M*=20.30, *SD* = 1.03) were only evident in individuals with schizophrenia [$F(1,2)=4.58$, $p<.05$). No significant changes in daily and average past 30-day cannabis use were observed in non-psychiatric populations and individuals reporting past 12-month anxiety, depression, post-traumatic stress disorder, bipolar disorder, or an alcohol/substance use disorder.

Conclusions: Our findings suggest that the recent legalization of recreational cannabis use in Canada in 2018 significantly increased regular cannabis use rates only amongst individuals with self-reported schizophrenia symptoms. These findings highlight the need for public health programs targeting cannabis use prevention among these vulnerable psychiatric populations.

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TRENDS IN CANNABIS USE, BLOOD PRESSURE AND HYPERTENSION IN MIDDLE-AGED ADULTS: FINDINGS FROM NHANES, 2009-2018

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Background: Epidemiologic studies investigating the relationship between Cannabis Sativa L. (i.e., cannabis or marijuana) and blood pressure and hypertension have yielded conflicting findings.

Methods: Cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) were analyzed for five 2-year cycles between 2009 to 2018 representing 9,783/ (unweighted)/92,033,212 (weighted) respondents who answered questions about cannabis use, use of antihypertensive medication, and had their blood pressure measured by trained examiners. Multivariable logistic regression models accounting for sampling weights and adjusting for socio-demographic characteristics, alcohol/cigarette use, and other cardiovascular risk factors were used to assess associations between cannabis use and both blood pressure and prevalent hypertension.

Results: A history of monthly cannabis use was reported by 25% (n=2,228) of respondents. Significant upward trends were seen over time in: current use of cannabis (p for trend < 0.01); current use by those with a history of monthly use (p for trend = 0.02); and mean SBP and DBP (p for trend = < 0.01 for both), but not in the prevalence of hypertension (p for trend = 0.23). Slightly less than half of all respondents (i.e., 47.6%) had hypertension. In adjusted models, compared to never use, there were no significant associations between cannabis use and blood pressure: mean differences in SBP: 1.35 mmHg (95% CI: -0.77 to 3.47) and DBP: 0.07 mmHg (95% CI: -1.44 to 1.58), or prevalent hypertension: OR=0.88 (95% CI: 0.62-1.24). Analyses of duration, recency (i.e., past month) and frequency (i.e., days past month, times per month, and joints or pipes per day) of use yielded similar findings.

Conclusions: Cannabis use was not associated with either blood pressure or prevalent hypertension in a nationally representative sample of middle-aged US adults. These results support prior findings on the lack of associations between cannabis use and blood pressure.

INVESTIGATING PHYTOCANNABINOID ACTIVITIES AT HUMAN 5-HT$_{1A}$ RECEPTORS USING THE MEMBRANE POTENTIAL ASSAY

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Introduction: 5-HT$_{1A}$ is a G-protein coupled receptor widely distributed in the central nervous system, which mediates inhibitory serotonin (5-HT) neurotransmission and has been implicated in various clinical conditions such as anxiety and depression. The biological activities of some phytocannabinoids are widely attributed to the modulation of 5-HT$_{1A}$ receptors. For example, 5-HT$_{1A}$ is implicated in cannabidiol's interference with Δ^9-tetrahydrocannabinol (THC)-induced conditioned gaping, anxiolytic, anti-emetic, and antidepressant effects. The target is also reported to mediate the antipsychotic and anti-emetic effects of THCV and CBDA, respectively. Besides major phytocannabinoids, the pharmacology of several minor phytocannabinoids at the 5-HT$_{1A}$ receptor is unknown. Therefore, we aimed to systematically investigate the *in-vitro* activities of phytocannabinoids at human 5-HT$_{1A}$ receptors.

Methods: We examined the actions of cannabinol (CBN), cannabinolic acid (CBNA) cannabigerol (CBG), cannabigerolic acid (CBGA) cannabichromene (CBC), cannabichromevarin (CBCV), cannabichromevarinic acid (CBCVA), cannabicyclol (CBL), cannabidivarinic (CBDV) cannabidivarinic acid (CBDVA), cannabidiolic acid (CBDA), and THC, in AtT20 cells stably transfected with human 5-HT$_{1A}$ receptors. We used FLIPR membrane potential assay to measure cellular hyperpolarisation in Flexstation 3 plate reader.

Results: 5-HT, a non-selective endogenous agonist of 5-HT$_{1A}$ receptor activated AtT20-5-HT$_{1A}$ receptors with a pEC$_{50}$ value of 8.1 ± 0.14 (7.5 nM) and 8-OH-DPAT, a selective 5-HT$_{1A}$ agonist also caused a cellular hyperpolarisation with a pEC$_{50}$ of 8.3 ± 0.1 (4.5 nM). The activities of both 5-HT and 8-OH-DPAT were blocked by 95% and 94%, respectively, with WAY100635 (100 nM), a 5-HT$_{1A}$ receptor antagonist. We next screened a 10 µM concentration of the above phytocannabinoids and found that all neutral phytocannabinoids caused modest changes in cellular hyperpolarisation which was not significantly different from the vehicle control response. CBGA and CBDA caused substantial cellular hyperpolarisation. However, these changes were also observed in wild-type cells suggesting the effect was non-specific to the 5-HT$_{1A}$ receptor.

Conclusion: Our data shows that the tested phytocannabinoids do not activate 5-HT$_{1A}$ receptors using the membrane potential assay. Future studies should investigate their agonist, antagonist and allosteric activities using orthogonal assays.

CLINICALLY RELEVANT DRUGS MODULATE THE EFFECT OF AMB-FUBINACA IN MALE AND FEMALE MICE

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Introduction: Synthetic cannabinoids (SCs) are a structurally diverse and fast evolving class of "designer drugs". In New Zealand, a specific SC, AMB-FUBINACA (AMB-FUB), has been implicated in 58 deaths since 2017 (Morrow et al., EClinicalMedicine. 25 (2020) 100460). Furthermore, 35% of seized AMB-FUB samples in New Zealand also contain a component of "party pills", para-fluorophenylpiperazine (pFPP) (Johnson et al., Forensic Sci Int. 307 (2020) 110107). The first aim of this research was to investigate the mechanisms underpinning AMB-FUB toxicity and the impact of pFPP on AMB-FUB activity *in vivo*. A CB_1 receptor antagonist/inverse agonist, SR141716, or Rimonabant, was also utilised in the present study as a potential antidote to AMB-FUB toxicity.

Methods: Male and female C57BL/6 mice (10-12 weeks old) were administered a single dose of AMB-FUB (3 or 6 mg/kg), pFPP (10 mg/kg) or 7% polysorbate-80 in 0.9% NaCl vehicle intraperitoneally. Mice were co-exposed to AMB-FUB (3 mg/kg, i.p.) and pFPP (10 mg/kg, i.p.) to investigate the impact of this drug combination. To study the potential rescue of AMB-FUB toxicity, Rimonabant was administered (3 mg/kg, i.p.) 15-minutes following a 6 mg/kg AMB-FUB dose. Rectal temperature measurements were taken 15 minutes prior to drug administration, as a baseline, and then every 15 minutes until 3 hours post-dose. Convulsions caused by drug administration were recorded. Data were analysed by a two-way, repeated measures ANOVA followed by Tukey's post-hoc tests ($p<0.05$ considered significant).

Results: When compared to pFPP treatment alone, which did not induce hypothermia in mice, the combination of pFPP and AMB-FUB led to significant exacerbation of AMB-FUB-induced hypothermia. The combination did not affect convulsion frequency in male or female mice when compared to 3 mg/kg AMB-FUB alone. Rescue from 6 mg/kg AMB-FUB using Rimonabant (3 mg/kg) was successful in attenuating hypothermia from 60-90 minutes post-AMB-FUB in male mice only. In both sexes, Rimonabant post-treatment caused a significant reduction in convulsion number. Specifically, male and female mice administered Rimonabant both had an average of 5 convulsions post Rimonabant, compared to 24 and 27 convulsions, respectively, in the 6 mg/kg AMB-FUB alone treatment groups within the same time period.

Conclusions: As a New Zealand-specific case of poly-pharmacy, the combination of AMB-FUB and pFPP appears to exacerbate hypothermic response, but not convulsions, in mice. Conversely, AMB-FUB-induced hypothermia and catalepsy can be partly reversed using Rimonabant. The results from this study provide further insight into mechanisms underpinning AMB-FUB adverse outcomes and can help inform potential overdose treatment strategies.

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MUTANT MICE EXPRESSSING AN INTERNALIZATION-RESISTANT FORM OF CB_1R DISPLAY ENHANCED TOLERANCE TO CANNABINOIDS

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Introduction: Although cannabinoids such as delta-9-tetrahydrocannabinol (Δ^9-THC) exhibit clinical efficacy in pain, tolerance to the antinociceptive effects develops with repeated treatment. The focus of our work is to investigate the mechanisms responsible for the acute response and tolerance to different cannabinoid agonists. We previously found that tolerance to cannabinoids is reduced in S426A/S430A mutant mice expressing a desensitization-resistant form of cannabinoid receptor 1 (CB_1R) that disrupts the classic mechanism of G protein-coupled receptor kinase (GRK)/βarrestin2-mediated CB_1R desensitization. The objective of our current work is to assess the role of CB_1R internalization and trafficking on cannabinoid tolerance using a novel six point mutant mouse strain expressing an internalization-resistant form of CB_1R that was recently produced in our laboratory.

Methods: Knock-in mice were produced that express serine/threonine to alanine point mutations for six putative G protein-coupled receptor kinase phosphorylation sites in the distal C-terminus of CB_1R that are required for the efficient internalization in transfected cells. The acute response to CP55,940, Δ^9-THC, and WIN 55,212-2 were assessed by performing cumulative dose response curves. Antinociception was measured using the tail-flick and hotplate tests while cannabinoid-induced hypothermia was assessed by measuring core body temperature. Tolerance to the antinociceptive and hypothermic effects of once daily injections of 0.6 mg/kg CP55,940, 30 mg/kg Δ^9-THC, and 10 mg/kg WIN 55,212-2 were determined. The duration of effect for bolus injections of 0.6 mg/kg CP55,940, 30 mg/kg Δ^9-THC, and 10 mg/kg WIN 55,212-2 were also measured.

Results: We find that the maximal effects of CP55,940 on hypothermia and tail-flick antinociception is reduced in six point mutant mice relative to wild-type littermate controls. The potency of WIN 55,212-2-induced tail-flick antinociception is also reduced in six point mutants. We also find a shorter duration for the acute antinociceptive effects of CP55,940 and the hypothermic effects of CP55,940, Δ^9-THC, and WIN 55,212-2 in six point mutant mice. Six point mutant mice also display enhanced tolerance to the antinociceptive and hypothermic effects of 0.6 mg/kg CP55,940 relative to wild-type littermate controls.

Conclusions: This work establishes six point mutant mice as a novel model to study the role of CB_1R internalization, trafficking, and resensitization *in vivo*. Preliminary data shows that cannabinoid tolerance is increased in six point mutant suggesting that the normal processes of internalization, trafficking, and resensitization of CB_1R might play an important role in counteracting the development of cannabinoid tolerance.

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THE INTERNALIZATION OF CANNABINOID RECEPTOR 1 IS SELECTIVELY INHIBITED BY SGIP1

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Introduction: SH3-containing GRB2-like protein 3-interacting protein 1 (SGIP1) is an interacting partner of cannabinoid receptor 1 (CB1R). SGIP1 hinders CB1R internalization, increases the interaction of CB1R with β-Arrestin 2 and G Protein-Coupled Receptor Kinase 3, and decreases ERK1/2 signaling (Hajkova et al., Neuropharmacology 107 (2016) 201–214; Gazdarica et al., J. Neurochem 160 (2022) 625– 642). SGIP1 knock-out mice exhibit an anxiolytic-like phenotype, altered nociception, increased sensitivity to Δ9-THC and morphine, and delayed onset of tolerance, while their cognitive and motor skills are intact (Dvorakova et al., M. Br J Pharmacol. 178 (2021) 1588– 1604). In the present study, we tested whether SGIP1 affects the internalization of other neuroreceptors, namely μ-opioid receptor (MOR), Δ-opioid receptor (DOR), dopamine receptor 1 and 2 (DR1 and DR2, respectively). Furthermore, we studied the role of carboxyl-termini in CB1R-SGIP1 interaction using CB1R and MOR chimeric receptors.

Methods: Chimeric CB1R and MOR receptors were created by swapping sequences that follow intracellular helix 8. The receptor internalization was measured by the Homogenous Time-Resolved FRET (HTRF) in HEK293 cells transiently expressing SNAP-tagged receptor with either an empty vector or SGIP1. Upon receptor stimulation with an agonist, the HTRF signal between Lumi4-Tb (terbium cryptate)-labeled receptor (donor) and fluorescein in the media (acceptor) was measured using Mithras LB 940 microplate reader for 60 minutes at 37°C.

Results: SGIP1 hindered the internalization of CB1R, but it did not affect the internalization of MOR, DOR, DR1, or DR2. However, the presence of SGIP1 decreased the internalization of both MOR/CB1R and CB1R/MOR chimeric receptors.

Conclusions: While SGIP1 impairs the internalization of CB1R, it does not alter the internalization of MOR, DOR, DR1, or DR2. Coexpression of SGIP1 with chimeric receptors MOR/CB1R and CB1R/MOR decreased the internalization of the activated receptors. Our findings imply that, while the C-tail of CB1R is sufficient to facilitate interaction with SGIP1, other regions of CB1R can also be involved in this interaction.

AXIALLY CHIRAL CANNABINOIDS DISPLAY ENHANCED CB_2 SELECTIVITY

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Introduction: Cannabinol (CBN) is a largely unutilized lead compound for drug development targeting the endocannabinoid system but has potential as a scaffold due to its biaryl framework. The addition of a C10 substituent to CBN results in a CBN analog that is axially chiral (*ax*CBN) and three-dimensional in its ground state, in contrast to the relatively planar ground state of CBN. We evaluated the cannabinoid receptor pharmacology of two axially chiral CBN analogs (i.e., *ax*CBN-3, *ax*CBN-4), and one racemic axially chiral CBD analog (i.e., *rac*-*ax*CBD-2).

Methods and Results: In [^{3}H]CP55,940 competition binding, *ax*CBN-3 had sub-nanomolar affinity at hCB_1 (K_i = 0.661 nM and hCB_2 (K_i = 0.933)—360-fold and 134-fold higher than CBN at hCB_1 (K_i = 240 nM) and hCB_2 (K_i = 136 nM). Additionally, unlike CBN, *ax*CBN-3 also demonstrated affinities for two distinct hCB_1 binding sites, binding a second, higher affinity, site likely indicative of the active conformation. *ax*CBN-3 and *rac*-*ax*CBD-2 demonstrated selectivity for hCB_2 over hCB_1 by 4.8-fold and 6.2-fold, respectively, in contrast to 1.7-fold and 2.3-fold for CBN and CBD, respectively. In [^{35}S]GTPγS binding and the TRUPATH Gαi1/β3γ9 activation assay, *ax*CBN-3 acted as a high potency and efficacy agonist, with higher potency at hCB_2 (TRUPATH pEC_{50} = 8.46; [^{35}S]GTPγS pEC_{50} = 8.97) than hCB_1 (TRUPATH pEC_{50} = 7.58; [^{35}S]GTPγS pEC_{50} = 8.07). *ax*CBN-4 also acted as an hCB_1 and hCB_2 agonist, though hCB_1 agonism was only apparent in [^{35}S]GTPγS binding. *rac*-*ax*CBD-2 produced weak partial hCB_1 agonism in [^{35}S]GTPγS binding (pEC_{50} = 6.05; E_{max} = 37.7%), but was inactive in hCB_1 TRUPATH Gαi1/β3γ9 activation, in contrast to its moderate potency and efficacy in hCB_2 activation assays ([^{35}S]GTPγS pEC_{50} = 6.38, E_{max} = 80.5%; TRUPATH pEC_{50} = 6.75). Induced-fit docking (Glide-XP) was used to predict binding interactions of *ax*CBN-3 and *rac*-*ax*-CBD-2 within hCB_1 and hCB_2. *ax*CBN-3 was predicted to have affinity in hCB_1 (XPgscore -13.285 kcal/mol) and hCB_2 (XPgscore -13.711 kcal/mol), while *rac*-*ax*-CBD-2's affinity in hCB_1 (XPgscore -12.488 kcal/mol) was predicted to be lower than in hCB_2 (XPgscore -13.117 kcal/mol), possibly due to multiple predicted steric clashes within hCB_1 and the presence of beneficial binding interactions in hCB_2.

Conclusions: The present findings demonstrate that axially chiral cannabinoids can possess affinity and functional activity at cannabinoid receptors, with desirable pharmacological properties, such as high affinity and hCB_2 selectivity. Future work should address the downstream signaling, *in vivo* effects, and therapeutic potential of the compounds evaluated here, which will guide the design and optimization of future axially chiral cannabinoids.

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CANNABINOID RECEPTOR INTERACTING PROTEIN 1a (CRIP1a) BINDS THE Gαi1 MYRISTOYLATED N-TERMINUS WITH LIPID AND SEQUENCE SPECIFICITY

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Introduction: The human endocannabinoid system (ECS) includes the neuromodulatory protein CB_1 cannabinoid receptor (CB_1R), a G protein coupled receptor (GPCR) abundant at neuron axon terminals which predominantly transmits signals via inhibitory heterotrimeric G proteins (Gi/o). Cannabinoid receptor interacting protein 1a (CRIP1a) regulates CB_1R-mediated activation of the Gi/o alpha subunits (Gαi/o) via an unknown mechanism. Upon solving the CRIP1a crystal structure, we discovered structural homology to a family of lipidated-cargo carriers. Notably, Gαi/o subunits are irreversibly lipidated by myristoyl moieties. Presently, we investigate the role for CRIP1a in ECS regulation by exploring its potential to bind Gαi/o subunits as lipidated cargo.

Methods: Full-length recombinant human CRIP1a and human Gαi1 (Gαi/o subtype) proteins were purified from *E. coli*. Gαi1 myristoylation was achieved *in vivo* via co-expression with the yeast N-myristoyltransferase (Nmt1). Gαi1 activation/inactivation state was achieved *in vitro* using GTPγS or GDPβS, respectively. Peptides mimicking lipidated ECS proteins were synthesized and linked to fluorescein isothiocyanate (FITC). The binding affinities between the FITC-peptides and CRIP1a or Gαi1 were determined using fluorescence polarization. The binding between CRIP1a and Gαi1 was qualitatively assessed using immobilized metal chelate affinity His6-tag pull-downs.

Results: Purified, un-lipidated, recombinant human Gαi1 is capable of binding its own N-terminal myristoylated peptide mimic. Purified recombinant human CRIP1a is also capable of binding this peptide mimic, although with much higher affinity. For both proteins, peptide binding affinity is dependent upon primary sequence, peptide length, and lipidation status. Removal of the myristoyl moiety completely abrogates peptide binding to both Gαi1 and CRIP1a. Binding between CRIP1a and full-length myristoylated Gαi1 occurs *in vitro* independent of monomeric activation state.

Conclusions: Our results demonstrate that Gαi1 binds to its own myristoylated N-terminus, which could explain the cytosolic solubility of Gαi/o monomers despite their hydrophobic N-termini. Our results further demonstrate that CRIP1a binds to Gαi1 via this myristoylated N-terminus. These data offer an explanation for the role of CRIP1a in ECS signaling, whereby CRIP1a serves as a neuronal lipidated-cargo carrier specific to Gαi/o subunits. As a myristoyl-binding Gαi/o carrier, CRIP1a could extract Gαi/o from the membrane and/or shuttle Gαi/o across various sites, thereby altering Gαi/o accessibility for CB_1R-mediated activation and signaling.

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NEGATIVE ALLOSTERIC MODULATION OF CB_1 CANNABINOID RECEPTOR SIGNALING SUPPRESSES OPIOID-MEDIATED TOLERANCE AND WITHDRAWAL WITHOUT BLOCKING OPIOID ANTINOCICEPTION

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Introduction: CB_1 negative allosteric modulators (NAMs) represent a pharmacological approach to circumvent unwanted side-effects of CB_1 antagonists. We reported that GAT358, a CB_1 NAM, suppressed opioid-mediated reward as well CB_1-mediated catalepsy and hypothermia while sparing cannabinoid antinociception. Whether GAT358 alters beneficial antinociceptive effects of opioids is unknown. Impact of GAT358 in models of persistent pain have not been explored.

Methods: We characterized effects of GAT358 on opioid-induced tolerance and physical dependence in morphine-dependent mice. We used the formalin test to evaluate effects of GAT358 in the presence and absence of morphine on nociceptive behaviors and neuronal activation in the lumbar spinal cord using Fos immunohistochemistry.

Results: In morphine-dependent mice, GAT358 attenuated morphine tolerance in the hotplate test without blocking acute morphine antinociception. GAT358 also reduced opioid-induced slowing of colonic motility. GAT358 also mitigated the somatic signs of naloxone-precipitated opioid withdrawal following chronic morphine dosing. Furthermore, GAT358 produced antinociception in the presence and absence of morphine in the formalin test. GAT358 preferentially suppressed the phase 2 of formalin-evoked nociceptive behaviors and did not impede the antinociceptive effects of morphine in formalin-treated rats. GAT358 also reduced the number of formalin-evoked Fos protein-like immunoreactive cells in lumbar spinal cord laminae associated with nociceptive processing in the same animals quantified for pain behavior.

Conclusions: The CB_1-NAM GAT358 suppresses opioid-mediated unwanted side-effects including tolerance and withdrawal without blocking opioid antinociception. GAT358 also exhibited antinociceptive effects in the presence and absence of morphine.

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CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A) CO-LOCALIZES WITH Gαi

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Introduction: The CB_1 cannabinoid receptor (CB_1R) is a CNS G-protein coupled receptor (GPCR) that modulates neuroprogenitor development, neural commitment and migration, and neurotransmitter release by endocannabinoid-stimulated dissociation of Gαi and Gβγ dimers to interact with their effectors. Cannabinoid receptor interacting protein 1a (CRIP1a) is an abundant 20 kDa protein in the CNS, and is a beta-barrel protein similar in structure to homologous proteins that serve as intracellular carriers of lipidated proteins from one membranous organelle to another. In the present study, we explored the ability of CRIP1a to interact or co-localize with Gαi, and localize with cellular organelles in intact cells.

Methods: Murine N18TG2 neuroblastoma and human SH-SY5Y neuroblastoma cells were cultured on glass coverslips coated with poly-D-lysine. Cells were fixed and permeabilized before fluorescent staining with antibodies to cell organelles of interest. Proximity ligation assay (PLA) was then performed between CRIP1a and Gαi1 or Gαi3. Cells were imaged using a Zeiss 710 confocal microscope, and the PLA puncta were counted and quantified using Zen software. CB1 agonists were used to activate the CB_1R-G heterotrimer in nuclear-free homogenates of cultured N18TG2 neuronal cells. Immunoprecipitations (IPs) from homogenates or cytosolic and membrane fractions were performed with antibodies to peptide epitopes of CRIP1a. IPs were analyzed by western blot analysis for CRIP1a, and Gαi1/3. Band densities were quantitated using LI-COR Empiria software for statistical analyses.

Results: CRIP1a co-localizes with Gαi1 and Gαi3 in mouse and human neuroblastoma cells. Within 30-90 seconds of N18TG2 cells exposure to 100 nM CP55940, it was found that CRIP1a and Gαi co-localized near the plasma membrane (stained with Na/K-ATPase or extracellular-directed CB_1R antibodies). Both CRIP1a and Gαi were detected in a nuclear-free, and detergent-free homogenate of N18TG2 after CRIP1a IP using an antibody directed to the CRIP1a surface away from the proposed site of Gαi interaction.

Conclusions: Collectively these data suggest CRIP1a acts as a cargo-carrying protein that carries Gαi1/3, specifically co-localized to the cellular plasma membrane. This co-localization of CRIP1a and Gαi1/3 is CB_1R-agonist dependent. We postulate that CRIP1a might function to sequester Gαi when Gβγ is modulating calcium channels, or carry Gαi to different cellular locations or organelles.

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CB1-R ALLOSTERIC MODULATOR DRUG DISCOVERY AND CHARACTERIZATION

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Introduction: Antagonism of CB1-R has been shown to have therapeutic potential in obesity, substance use disorders as well as certain psychiatric disorders[1]. However, orthosteric antagonists of CB1-R have been associated with serious adverse psychiatric effects[1]. Allosteric modulation could represent a promising strategy to indirectly reduce CB1-R signalling without the negative side effects associated with ligands targeting the orthosteric site. As such, our lab is interested in the development of negative allosteric modulators (NAMs) of CB1-R, specifically for the treatment of psychosis-related behaviours. In 2020, we reported the discovery and characterization of ABM300[2], a novel compound related to the prototype NAM (ORG27569[3]). This compound behaved as a NAM both *in vitro* and *in vivo* in two genetic models where it ameliorated behaviours of psychosis. Here we demonstrate that ABM300 also has similar effects in two pharmacological models of psychosis. Moreover, we present data on novel NAMs, that have high potency *in vitro* with improved drug metabolic stability and pharmacokinetic properties.

Methods: Using the PathHunter® β-Arrestin assay, compounds are screened *in vitro* against an EC_{20} of the CB1-R agonist, CP55940. Candidate drugs then undergo pharmacokinetic studies to reveal *in vitro* metabolic stability (human and rodent), permeability and solubility. Finally, the behavioural effects of promising compounds are characterized in the Dopamine Transporter Knockout (DATKO) genetic model as well as the MK-801 and amphetamine (AMPH) pharmacological models of psychosis-like behaviours.

Results: In the MK-801 and AMPH models we recapitulated the ability of ABM300 (10mg/kg) to ameliorate the hyperlocomotive phenotype, as seen previously in two genetic models of psychosis (DATKO and GluN1KD). Additionally, we found that ORG27569, which has an IC_{50} of 34nM *in vitro*, ameliorates psychosis-like phenotypes in both the MK-801 and the AMPH behavioural models. We have also identified ABD1085, a novel NAM, as a potential drug candidate. It demonstrates high potency *in vitro* (IC_{50} 14nM) and has a favorable pharmacokinetic profile. Despite this, ABD1085 did not behave as a NAM in either the DATKO genetic model or the MK-801 pharmacological model at 10mg/kg. Furthermore, two additional novel NAMs, ABM338 (IC_{50} 20nM) and ABM407 (IC_{50} 28nM), have been identified as hit compounds showing good potency in our *in vitro* screening and will be tested in our behavioural models.

Conclusion: These results confirm previous data obtained in genetic models, demonstrating that ABM300 is effective in ameliorating psychosis-like phenotypes in two pharmacological models. This effect is also observed with ORG27569 in the pharmacological models. Finally, we have developed novel molecules that are potent NAMs *in vitro*, still undergoing *in vivo* characterisation.

[1] Mielnik, C.A., *et al.* (2021) *Prog Neuropsychopharmacol Biol Psychiatry.* **2**; 106:110163.
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DEGLYCOSYLATION OF CORTICAL CANNABINOID TYPE-1 RECEPTOR REDUCES ITS FUNCTION

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Introduction: A significant advancement has been made in delineating the molecular mechanisms of cannabinoid type 1 (CB1) receptor-mediated signaling in normal physiology and disease conditions. However, the characterization of CB1 receptors and the functional significance of their post-translational modifications are still understudied due to lack of availability of specific reagents.

Methods: In this study, we characterized different forms of glycosylated and non-glycosylated CB1 receptors in the frontal cortex of adult mice by western blot, confocal immunofluorescence and agonist-stimulated G-protein coupling methods.

Results: We identified seven forms of CB1 receptors in the synaptic membrane fraction of frontal cortex of mice with apparent molecular weights of 31, 48, 50, 51 and 55 kDa, by using two specific antibodies and brain tissue devoid of CB1 receptors. Both 48 and 55 kDa forms are highly expressed compared to the others. In addition, incubation of samples at increased temperature resulted in aggregation and formation of many other CB1 receptor forms at higher molecular weights, thereby depleting the receptor abundance at 31, 48, 50 51 and 55 kDa.

Confocal immunofluorescence analysis revealed the presence of the highly glycosylated CB1 receptors (55 kDa) mainly in the plasma membrane of cell body which are partially co-localized with the antibody that recognizes receptors at lower molecular weights (31, 48, 50 and 51 kDa). These specific immunolabeling were absent in the frontal cortex of CB1 receptor deficient mice. The enzyme peptide:N-glycosidase F (PNGase F) markedly reduced the CB1 receptor immunoreactivity at 48 and 55 kDa and resulted in the detection of CB1 receptors at 31, 34, 37, 46 and 48 kDa. This deglycosylation led to the reduction of the CB1 receptor-mediated G-protein activation in the synaptic membrane of the frontal cortex.

Conclusions: Our findings suggest that CB1 receptors exist in highly glycosylated forms leading to molecular weights at around 48-55 kDa and that the non-glycosylated form is present at much lower molecular weight (~31 kDa) than previously recognized. The deglycosylation diminishes the ligand-induced G-protein activation of the CB1 receptors in the frontal cortex. Thus, the dysregulation of glycosylation levels of CB1 receptors may play an important role in the etiology of diseases.

POSTIVE ALLOSTERIC MODULATION OF THE CB1 RECEPTOR REDUCES SPONTANEOUS WITHDRAWAL SIGNS IN NICOTINE-DEPENDENT MICE

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Introduction: The endocannabinoid system, which consists of CB1 and CB2 receptors, endogenous cannabinoids, and their respective biosynthetic and degradative enzymes has been implicated in nicotine addiction. Furthermore, genetic deletion or pharmacological inhibition of the CB1 receptor eliminates the rewarding properties of nicotine, indicating that the CB1 receptor plays a necessary role in nicotine reward. In contrast, CB1 receptor agonists decrease withdrawal signs in nicotine-dependent rodents. However, the well described cannabimimetic side-effects associated with CB1 receptor agonists dampen enthusiasm for their use as a strategy to ameliorate nicotine withdrawal. Alternatively, CB1 allosteric modulators offer clinical promise for targeting the CB1 receptor with minimal side effects. These ligands bind allosteric (i.e., secondary) sites resulting in conformational changes of the receptor that can increase (positive allosteric modulator; PAM) or decrease (negative allosteric modulator; NAM) the potency and efficacy of orthosteric ligands. Pre-clinical screens have identified ZCZ011 as a lead test compound in this class of drugs. Of note, ZCZ011 reduces withdrawal signs in opioid-dependent and THC-dependent mice. However, the effectiveness of CB1 allosteric modulators to ameliorate nicotine withdrawal symptoms remains to be explored. In the present study we tested whether the CB1 PAM would ameliorate somatic withdrawal in nicotine-dependent mice.

Methods: This preliminary study employed a spontaneous nicotine withdrawal mouse model. Subjects were implanted with nicotine or saline minipumps, which were removed on day 14. Nineteen hours after minipump removal, mice received an intraperitoneal injection of either vehicle or ZCZ011 (40 mg/kg) and observed for somatic withdrawal signs (head/body shakes, paw tremors, jumping, backing) for 30 min as described previously (Malin et al., 1994; Jackson et al., 2008). Locomotor activity was assessed using Any-maze software.

Results: The mean total number of withdrawal signs (+/- S.E.M.) in the chronic nicotine group was 21.3 (+/- 2.5) signs, which significantly differed ($p < 0.01$) from the number of signs observed in the control mice (i.e., 10 (+/- 1.4) signs. In addition, ZCZ011 significantly ($p < 0.001$) reduced somatic withdrawal signs of nicotine-dependent mice (i.e., 6.5 (+/- 2) signs) compared with nicotine-dependent mice that received vehicle. Interestingly, ZCZ011 also reduced locomotor behavior, but this hypomotility was not attenuated in CB1 (-/-) mice.

Conclusions: These data provide proof principle that the CB1 PAM ZCZ011 attenuates spontaneous withdrawal signs in nicotine-dependent mice. Moreover, CB1 (-/-) mice provide a useful tool to discern between CB1 receptor-dependent and independent effects. Accordingly, the hypomotility observed in the present study was CB1 receptor independent. Ongoing studies are employing CB1 (-/-) mice to ascertain whether the actions of ZCZ011 in reducing nicotine withdrawal are CB1 receptor dependent.

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EVALUATING SIGNALLING BIAS FOR SYNTHETIC CANNABINOID RECEPTOR AGONISTS AT THE CANNABINOID CB_2 RECEPTOR

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Introduction: Synthetic cannabinoid receptor agonists (SCRAs) remain one of the largest classes of new psychoactive substances, with novel compounds continually emerging in the recreational market worldwide. The compounds are increasingly associated with severe toxicity and even death, in contrast to the phytocannabinoid Δ^9-tetrahydrocannabinol (THC). However, no clear molecular mechanism of toxicity has yet been established, with pharmacological data on newly detected SCRAs often limited due to the rapid structural evolution of these drugs. The majority of studies thus far have focused on the pharmacology of SCRAs at the cannabinoid CB_1 receptor (CB_1), despite many compounds also possessing equivalent or even greater affinities for the cannabinoid CB_2 receptor (CB_2). In order to build a more comprehensive pharmacological profile of this class of cannabinoids, we characterised the *in vitro* activity of a structurally diverse panel of SCRAs at CB_2.

Methods: The functional activities of 12 novel SCRAs were assessed in key receptor signalling and regulatory pathways in CB_2-expressing HEK293 cells, including G protein activation by bioluminescence resonance energy transfer (BRET), phosphorylation of ERK1/2 (AlphaLISA) and β-arrestin translocation (BRET). The activity profiles of the ligands were further evaluated using operational analysis to identify potential ligand bias.

Results: All SCRAs behaved as agonists in the CB_2 signalling pathways examined, albeit with varying potencies and efficacies. The activity profiles of the compounds were relatively balanced compared to the reference ligand, CP55940. However, the SCRAs exhibited activity profiles distinct to THC, most notably in the translocation of β-arrestin.

Conclusions: These findings demonstrate CB_2 is able to accommodate a chemically diverse array of ligands to generate canonical agonist activity. Further study is required to elucidate the physiological effects of the signalling pathways regulated by CB_2 in order to identify potential links to the toxicity of SCRAs.

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POSITIVE ALLOSTERIC MODULATION OF CB1 CANNABINOID SIGNALING REVERSES PATHOLOGICAL PAIN IN A PROBE-DEPENDENT MANNER WITHOUT PRODUCING TOLERANCE OR UNWANTED SIDE EFFECTS

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Introduction: Cannabinoid CB1 receptor (CB1) activation and mobilization of the endocannabinoid 2-arachidonoylglycerol (2-AG) has been implicated in suppression of acute and chronic pain. However, inhibitors of 2-AG deactivation that target the hydrolytic enzyme monoacylglycerol lipase (MAGL) can produce tolerance and physical dependence, effects which are undesirable in a therapeutic agent. We asked whether a CB1 positive allosteric modulators (PAMs) that exhibited probe dependence for 2-AG *in vitro* could suppress neuropathic pain without producing unwanted CNS side effects or tolerance.

Methods: AM12717, a potent CB1 positive allosteric modulator (PAM), AM12716, the less active enantiomer, and AM11517, a racemic mixture of both analogs, were assessed for their respective abilities to attenuate chemotherapy-induced peripheral neuropathy (CIPN) induced by treatment with paclitaxel. We also asked whether efficacy of each agent was sustained following repeated dosing and whether observed pharmacological effects were mediated by CB1R mechanisms. Cannabimimetic side effects of all three compounds were assessed alongside a reference CB1 agonist CP55,940. We assessed the ability of each compound to shift the dose response of CP554,940 in the cannabinoid tetrad. GTPγS binding assays were used to assess each compound's ability to alter 2-AG-stimulated CB1 receptor efficacy in mouse striatum.

Results: AM12717 and AM11517 showed sustained efficacy in reversing paclitaxel-induced mechanical allodynia with no signs of tolerance in a 14-day chronic dosing paradigm. AM12716 failed to produce sustained efficacy and CB1R agonist WIN55,212-2 produced tolerance and hypoactivity. Thus, AM12717 and AM11517 lacked cardinal signs of direct CB1 activation. Anti-allodynic efficacy of both AM12717 and AM11517 was blocked by the CB1R antagonist AM251 but not the CB2 antagonist AM630. No cannabimimetic effects of AM11517, AM12716, or AM12717 were observed in the tetrad and none of the agents shifted the dose response of CP55,940 in the tetrad. By contrast, GTPγS binding assays confirmed that each respective compound enhanced 2-AG stimulated CB1 receptor efficacy in the striatum, with AM12717 showing the most pronounced enhancement in this assay.
Conclusions: Enhancing the signaling of 2-AG through a probe-dependent CB1 positive allosteric modulator is sufficient to produce a beneficial therapeutic and pharmacological profile. These studies support our hypothesis that CB1 positive allosteric modulators suppress neuropathic pain without producing tolerance or cardinal signs of CB1 activation.

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TARGETING CANNABINOID RECEPTOR 1 (CB_1R) WITH NOVEL FOUR-ARM BITOPIC ANTAGONISTS

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Introduction: Synthetic compounds that interact with the cannabinoid receptors or modulate endocannabinoid signaling hold great therapeutic potential in the treatment of several disorders such as metabolic disorders, neurological diseases, and psychiatric disorders. Despite the useful effects of such compounds interacting with the ECS, currently, a very low number of cannabinoid-based medications are available in the clinic. To obtain novel pharmacological tools and to investigate allosteric/dualsteric strategy, a series of novel four-arm diarylpyrazoline compounds were designed, synthesized, and evaluated *in vitro* and *in vivo* assays.

Methods: The novel diarylpyrazoline-based ligands were initially evaluated as racemic ligands in *in vitro* radioligand displacement experiments on mice brain homogenate and CB_1R or CB_2R expressed cell membranes. Component enantiomers having high CB_1R affinity were further studied in detail for their allosteric potential and/or bitopic nature in [^{35}S]-GTPγS binding assays. The pharmacokinetics and pharmacodynamics properties of the ligands were investigated.

Results: In *vitro* radioligand binding experiments revealed that the tested compounds have high affinity and selectivity to CB_1R in the subnanomolar and low nanomolar range (0.15 nM-8 nM). Furthermore, in functional assays using [^{35}S]-GTPγS binding, tested compounds retain high potency for CB_1R antagonism with 1.3- 9.5 nM IC_{50}. Interestingly, despite fully displacing orthosteric radiolabelled CB_1R ligands (^{3}H-CP55940,^{3}H-Rimonabant) in binding experiments, four compounds behaved as non-competitive CB_1R antagonism in GTPγS binding with Schild plot analysis. In pharmacokinetics experiments, four non-competitive antagonists provided good systemic exposures with Cmax at 200-300 nM using 3 mg/kg intraperitoneal injections. Three compounds (MRI2479, MRI2588 and MRI2265 provided comparable oral bioavailability with Cmax at 100-300 nM.

Conclusions: We generated potent and selective bitopic CB_1R antagonists with favorable pharmacokinetic properties. Studies are in progress to explore the pharmacology of these novel bitopic CB_1R antagonists. Outcomes of our research will improve understanding of the pharmacological and molecular mechanisms of allosteric and/or bitopic modulation of CB_1R.

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PHYTOCANNABINOIDS 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 possible medicinal use of cannabis, particularly 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" The goal of this study is to determine if the combination of these two constituents of the cannabis plant Beta-Caryophyllene (BCP) and cannabidiol (CBD), works synergistically to mitigate chronic pain.

Methods: Male C57BL6/J mice (20-25 grams) were purchased from Jackson Laboratories (Bar Harbor, Maine, USA). We used a chronic inflammatory pain model (Complete Freund's Adjuvant, CFA) and two pain-like behavioral tests. We determined the analgesic effects of CBD and BCP individually and in combination and monitored for adverse effects.

Results: CBD and BCP administered intraperitoneally (i.p.) at doses of 0.1-100 mg/kg produced a dose-dependent reduction in mechanical allodynia and thermal hypersensitivity. The combination of the two compounds was tested at this fixed-dose ratio (based on the ED50 of each compound). The combination produced dose-response suppression of mechanical allodynia and cold allodynia. Isobolographic analysis demonstrated that this combination is synergistic. We also tested this combination for CB1-associated side effects. There were no significant effects in body temperature, locomotion, or motor function compared to the vehicle.

Conclusions: The present data show that in a chronic pain state, BCP and CBD synergistically produce an analgesic effect with safety profiles, suggesting that this combination can serve as alternative analgesic therapy for chronic pain and support the entourage effect of cannabinoids.

This study is supported by NIH NS116489.

THE EFFECTS OF THE FATTY ACID BINDING PROTEIN 5 (FABP5) INHIBITOR ART26.12 IN PACLITAXEL-INDUCED NEUROPATHY IN RATS

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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 and selective FABP5 inhibitor ART26.12 is under development at Artelo Biosciences under a licence agreement with Stony Brook University, with successful data in oxaliplatin-induced peripheral neuropathy presented to the ICRS in 2022. The aim of the present study was to examine the potential of ART26.12 against a second chemotherapy agent, paclitaxel (Taxol®), in male and female rats.

ART26.12 (25 or 50 mg/kg BID PO) treatment was initiated for 22 days to adult male and female Sprague Dawley rats (from day -2 to day 20). On Day 0, Day 2, Day 4, and Day 6, paclitaxel (2 mg/kg IP) was administered. On Days 15, 16, 19 and 20, mechanical allodynia and thermal hyperalgesia were assessed 2 hours after ART26.12 dosing. Though not approved for chemotherapy-induced peripheral neuropathy, Duloxetine (30 mg/kg PO) was administered as a positive control.

In paclitaxel-treated male rats, ART26.12 (25 or 50 mg/kg BID) prevented the mechanical allodynia on day 15, 16, and 19 in a manner similar to duloxetine (Figure 1). The ART26.12-treated groups did not have a reduced withdrawal threshold (allodynia) compared to baselines values over the entire study. ART26.12 only prevented the cold allodynia on days 15 and 16 at the 25 mg/kg dose. Body weight reduction was seen over the course of the study in the duloxetine treatment group, but not with ART26.12.

In paclitaxel-treated female rats, ART26.12 (25 or 50 mg/kg BID) prevented the mechanical allodynia on day 15, 16, and 19. In female rats, there was a dose-dependency not observed in male rats. Duloxetine was less effective as an analgesic in female rats than male rats, with ART26.12 (50 mg/kg BID) showing greater efficacy than duloxetine on Day 15 to Day 20. ART26.12 only prevented the cold allodynia on days 15 and 16 at both doses.

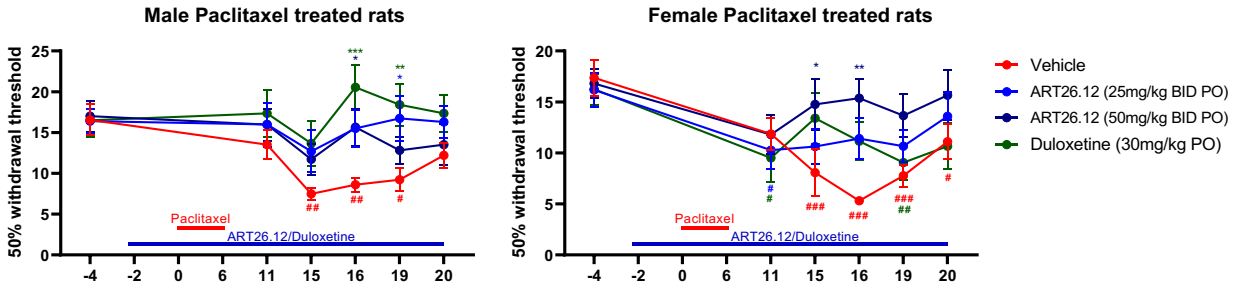

Figure 1 Von Frey thresholds for vehicle and test compound treated groups. *p<0.05, **p<0.01 indicate significant reversal of mechanical allodynia by treatment when compared to the respective group's vehicle values. #p<0.05, ##p<0.01, ###p<0.001 indicate significant decrease in von Frey threshold when compared to the respective group's baseline values. Values are presented as mean ± s.e.m.

In summary, we have shown that ART26.12 reduces mechanical and cold allodynia associated with a second chemotherapeutic agent, paclitaxel, as previously observed in the oxaliplatin model of peripheral neuropathy. These data suggest a common mechanism of action of ART26.12 capable of preventing chemotherapy-induced allodynia from both taxane and platinum based agents, and support further development of ART26.12 as a novel therapeutic agent as a potential treatment for neuropathic pain.

THE EFFECTS OF THE FABP5 INHIBITOR ART26.12 IN A RAT MODEL OF DIABETIC NEUROPATHY

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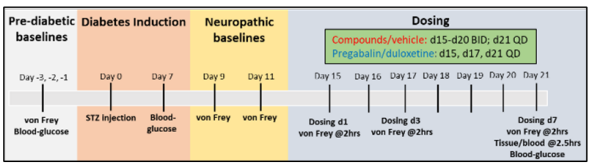
Inhibitors of fatty acid binding protein 5 (FABP5) are effective in multiple models of pain, inhibited by antagonists of CB_1, TRPV1 and PPARα. The potent and selective FABP5 inhibitor ART26.12 is under development with Artelo Biosciences under a licence agreement with Stony Brook University, with successful data in oxaliplatin-induced peripheral neuropathy presented to the society in 2022. The aim of the present study was to examine the potential of ART26.12 in another peripheral neuropathy; the streptozotocin (STZ)-induced model of painful diabetic neuropathy.

ART26.12 showed selectivity against a broad panel of enzymes and receptors with no off-target effects of concern. ART26.12 has no *in vitro* toxicological effects, and has a NOAEL (No-Observable-Adverse-Effect-Level) of 1000 mg/kg/day for 14 days in rodents, and is tolerated at a dose of 1000 mg/kg/day for 14 days in dogs. ART26.12 displayed dose-dependent plasma exposure following oral administration of solution or suspension formulations.

Male Wistar rats were treated with STZ which selectively ablates insulin-producing β cells in the pancreas (55mg/kg IP) on day 0. By day 9-11 (neuropathic baseline, NeuP), animals had developed neuropathy as assessed by measurement of withdrawal threshold using calibrated von-Frey monofilaments applied to the plantar surface of the hindpaw, and diabetes (measured via blood glucose levels, ~30 mmol/L). Animals were treated orally with ART26.12 (25 or 100 mg/kg, BID) from day 15 for seven days, with von Frey measurements on day 15, 17 and 21, approximately 2 hr after dosing. Duloxetine was given on test days as an example of standard care.

On day 15 (D15), after the first dose of ART26.12, withdrawal thresholds were significantly higher than neuropathic baseline levels with the higher dose of 100 mg/kg, suggesting reduced mechanical allodynia (See Figure 1). On the third (D17) and seventh days (D21) of dosing, both 25 and 100 mg/kg ART26.12, and duloxetine, significantly increased withdrawal thresholds to similar levels. Blood glucose levels were not different between groups on day 21. Additionally, animals treated with ART26.12 lost less weight than Duloxetine-treated ones.



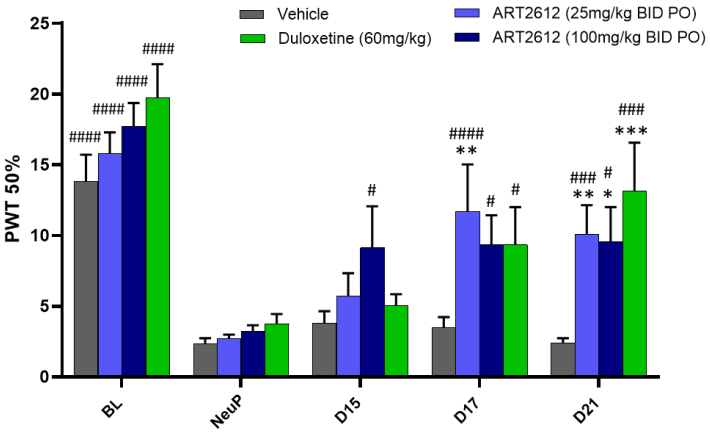


Figure 1: Mechanical allodynia measured using von Frey filaments. Data were analysed with a Two-Way Repeated Measures ANOVA followed by Holm-Šídák's post hoc analysis. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 indicate statistically significant difference when compared to the vehicle group at the same timepoint (between-group comparison). #p<0.05, ##p<0.01, ###p<0.001, and ####p<0.0001 indicate statistically significant difference when compared to the NP timepoint in the same group (within-group comparison). Data is expressed as mean ± S.E.M.

In conclusion, we have shown that ART26.12 reduces mechanical allodynia in a rat model of diabetic neuropathy. DMPK and toxicological studies continue to show a desirable drug profile for ART26.12. These data support the further development of ART26.12 as a novel therapeutic agent in peripheral neuropathies.

GPR55 ANTAGONIST KLS-13019 REVERSES AND PREVENTS CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY (CIPN) IN RATS

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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 and morphine discrimination. Peripheral neuropathy was induced in 16 rats with once daily 1mg/kg paclitaxel injections for 4 days. For acute reversal, rats were then administered 0, 3, 10, or 30 mg/kg KLS-13019 on days 7 and 14 and allodynia was assessed by a Von Frey test. In a chronic dosing paradigm, rats received once daily injections of 0, 1, or 10 mg/kg KLS-13019 for four days post-paclitaxel treatment and allodynia was assessed over the course of the following 42 days. For prevention, rats were co-administered 10 mg/kg KLS-13019 during paclitaxel treatment and allodynia was assessed over the course of the following 42 days. Additionally, in an effort to characterize the *in vivo* pharmacology of KLS-13019 compared to opioid-class drugs, a cohort of rats was trained to discriminate between morphine and saline via alternative and mutually exclusive lever presses as the "correct response" to treatment.

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. With chronic dosing, allodynia was reversed with both doses tested for the entire duration of the CIPN phenotype. In the reversal treatment paradigm, allodynia did not develop in the rats who received KLS-13019 during paclitaxel treatment. After drug discrimination training, rats dosed with KLS-13109 did not respond as though they had received morphine.

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.

ACETAMINOPHEN PRODUCES ANTINOCICEPTION THROUGH A DIACYLGLYCEROL LIPASE-DEPENDENT MECHANISM IN RODENT MODELS OF INFLAMMATORY AND POST-SURGICAL PAIN

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Introduction: Acetaminophen has been commonly used as an over-the-counter pain and fever reliever. Acetaminophen was first synthesized more than a century ago, but its mechanism remains unclear. Interestingly, acetaminophen has been reported to produce antinociceptive effects through a CB1-dependent mechanism, presumably through generation of an active metabolite, AM404. Here we asked whether acetaminophen suppressed allodynia through an endocannabinoid mechanism that requires both diacylglycerol lipase (DAGL) and CB1 using mechanistically distinct models of persistent pain.

Methods: We assessed the role of diacylglycerol lipase and CB1 in the antinociceptive effects of acetaminophen using mouse models of inflammatory and post-surgical pain.

Results: Acetaminophen, injected intraperitoneally (30-300 mg/kg i.p.), attenuated mechanical allodynia induced by intraplantar injection of CFA without altering CFA-induced edema. Pre-treatment with the diacylglycerol lipase (DAGL) inhibitor RHC80267 or either of two distinct CB_1 receptor antagonists (AM251 and rimonabant) blocked acetaminophen-induced anti-allodynic efficacy in CFA-treated mice. Acetaminophen, administered orally, also attenuated incisional injury-induced mechanical allodynia; anti-allodynic efficacy of acetaminophen was, similarly, blocked by the DAGL inhibitor RHC80267. Acetaminophen (300 mg/kg i.p.) also produced antinociception in the hotplate test; this effect was attenuated by RHC80267, administered at a dose that did not itself produce antinociception. Acetaminophen (300 mg/kg i.p.) produced hypolocomotion and hypothermia and did not produce tail-flick antinociception whereas lower doses were ineffective in these assays.

Conclusions: Our results suggest that antinociceptive effects of acetaminophen in animal models of persistent pain requires both DAGL alpha and CB1 activation. These findings support a potential mechanism of acetaminophen-induced analgesic action involving the enzyme DAGL.

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ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH OSTEOARTHRITIS: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

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Introduction: Osteoarthritis is the most common form of arthritis affecting synovial joints. Pain is cardinal symptom of osteoarthritis, limiting mobility and usual activities. Osteoarthritis is typically a progressive disorder in most individuals, however there are limited appropriate pharmacological therapies for long-term management of associated chronic pain. Cannabis-based medicinal products (CBMPs) have an increasing pre-clinical and clinical evidence base for treating chronic pain. However, this is data is heterogenous and there is a paucity of data specifically in individuals with osteoarthritis. The primary aim of this study was to assess changes in pain-specific and general health-related quality of life in patients with osteoarthritis following initiation of CBMPs.

Methods: Data was extracted from the UK Medical Cannabis Registry for individuals receiving treatment with CBMPs for chronic pain secondary to osteoarthritis who were enrolled a minimum of 12 months prior to data extraction (8th January 2023). Primary outcomes were changes in Brief Pain Inventory (BPI) pain interference and severity subscales, as well as the McGill Pain Questionnaire (MPQ2) at 1, 3, 6, and 12 months from baseline. Secondary outcomes were changes in the following validated patient reported outcome measures: the EQ-5D-5L, Generalised Anxiety Disorder-7 (GAD7) questionnaire, and Single-Item Sleep Quality Scale (SQS). The common terminology criteria for adverse events version 4.0 was used for adverse event analysis. $p<0.050$ was determined as statistically significant.

Results: 77 patients met the inclusion criteria for this case series. The mean age of the cohort was 60.04 (±14.27) years, whilst 40 (51.9%) patients were female. The majority of patients (n=48; 62.3%) reported consuming illicitly-sourced cannabis at baseline. Improvements were detected in the MPQ2 and BPI pain interference and severity subscales at each time period up to and including 12 months ($p<0.050$). Improvements were seen in the SQS and EQ-5D-5L index scores at 1 ,3, and 6 months only ($p<0.050$). There was a reduction in GAD-7 scores at 1 and 3 months only ($p<0.050$). There were 17 (22.1%) patients who reported 218 (283.1%) adverse events, which were predominantly mild or moderate (n=180; 233.8%).

Conclusion: Following initiation of symptomatic treatment for osteoarthritis-associated chronic pain participants reported improvement in pain severity and interference at up to 12 months, with supplementary benefits in other measures at shorter follow ups. CBMPs were largely well-tolerated by most individuals highlighting the benefits of patient registries as a pharmacovigilance measure. Whilst these results are promising, further evaluation against placebo control is required in randomised control trials are necessary. Moreover, additional analysis is required to assess the disease modifying effects of cannabinoids for osteoarthritis.

TOWARD A COMPREHENSIVE UNDERSTANDING OF DAGL-BETA ROLE IN OSTEOARTHRITIS-RELATED PAIN

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Introduction: Osteoarthritis (OA) is a chronic joint disease, accompanied with local inflammation, in which cartilage degenerates as a result of its mechanical and biochemical disturbances. The endocannabinoid system (ECS), a biological system linking pain transmission and immunological system possesses multiple novel targets for the treatment of pain. In particular, diacylglycerol lipase beta (DAGLβ), an enzyme responsible for the biosynthesis of the endocannabinoid 2-AG, is highly expressed on macrophages and has been implicated in inflammation and pain. Thus, we used complementary genetic and pharmacological approaches in the sodium monoiodoacetate (MIA) mouse model of OA to test whether DAGLβ represents a potential target to treat OA pain. For comparison, we assessed the monoacylglycerol lipase inhibitor MJN110 and pan CB1/CB2 receptor agonist CP55,940.

Methods: An intra-articular injection of MIA (0.56mg/10uL NaCl) was given to induce OA in rear right knee joint of male and female C57BL6/J (C57) mice as well as in DAGLβ wild type (WT) and DAGLβ knockout (KO) mice. The von Frey test was used to assess mechanical nociception on days 2, 10, 14, 21, 28, 31, 34, 37, 39 post-OA induction. On days 31-39 C57 mice were given an i.p. injection of vehicle (1:1:18 ethanol:emulphor:NaCl), DAGLβ inhibitor (KT109; 40 mg/kg), MJN110 (5 mg/kg) or CP55,940 (0.3 mg/kg) with 72 h between each test day to minimize carry over effects. The von Frey test was performed directly before drug treatment, 2 h after KT109 or MJN110 treatment and 30 min after CP55,940 treatment.

Results: DAGLβ-WT and DAGLβ-KO mice developed similar rates and magnitudes of MIA-induced allodynia. Moreover, KT109 did not reverse OA-induced hyperalgesia in mice, indicating that neither deletion nor inhibition of DAGLβ reduces MIA-induced nociception. Moreover, the MAGL inhibitor MJN110 did not affect MIA-induced nociception as well. In contrast, the CB1/CB2 receptor agonist CP55,940 reversed MIA-induced allodynia in male mice, but not female mice. Finally, females showed increased vulnerability to MIA treatment and developed allodynia even in the contralateral paw (which was not observed in male mice).

Conclusions: Here we found that genetic deletion or pharmacological inhibition of DAGLβ did not ameliorate MIA-induced allodynia. These findings do not exclude the role of ECS in OA treatment and a potential role of other ECS elements for the modulation of pain, as CB1 and CB2 receptor agonists effectively reduce nociception in laboratory animal models of OA pain. In addition, female mice displayed considerably more sensitivity than male mice to the allodynic and toxic effects of MIA, which is consistent with the observation that human women are more likely than males to experience OA. In sum, the results indicate that DAGLβ may not be a viable target to treat OA pain, despite its proven role in inflammatory pain models.

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β-CARYOPHYLLENE INDUCES PRURITUS IN MICE

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Introduction: Pruritus is the sensation that invokes a desire to scratch. Treatments for clinical presentations of pruritus are usually resistant to pharmaceutical intervention, such as antihistamines. The endocannabinoid system is a potential target for pruritus treatments. For example, WIN 55,212-2 (WIN) reduces scratching via CB_2. β-caryophyllene (BCP) is a naturally available sesquiterpene found in *cannabis sativa* previously shown to be a CB_2 and PPAR-γ agonist, which have both been shown to reduce scratching upon activation. The potential antipruritic activity of BCP was investigated using an established 5-HT model of pruritus. We hypothesized that BCP would reduce scratching via CB_2 or PPAR-γ receptors.

Methods: Adult male and female C57BL/6J mice were administered 5-HT or Compound 48/80 to induce scratching. WIN 55,212-2 (0.1-0.3-1-3mg/kg, i.p.) or BCP (12.5-25-50-100-200mg/kg, s.c.) was administered to reduce scratching. In a separate group of mice, rimonabant (3 mg/kg, i.p.) or SR144528 (3mg/kg, i.p.) were administered to probe receptor mechanism of WIN. After injection with the pruritic agent, mice were immediately placed in sound-attenuating chambers, video recorded for 30 min, and hind paw scratching was quantified by a blinded observer. Pruritic activity of BCP alone (25-50-100mg/kg, s.c.) was also assessed. All analyses were performed using one-way ANOVA with Dunnett's post-hoc.

Results: WIN 55,212-2 (≥0.3mg/kg, i.p.) reduced Compound 48/80-induced scratching and was blocked by both the CB_1 and CB_2 antagonists. BCP did not reduce 5-HT induced scratching in mice. Surprisingly, BCP (≥25mg/kg, s.c.) administered alone induced scratching.

Conclusions: BCP induces scratching, independent of 5-HT administration. The receptor mechanism through which this pruritic activity occurs is unknown, though future studies will attempt to block both CB_2 and PPAR-γ.

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ACETAMINOPHEN INHIBITS DIACYLGLYCEROL LIPASE A: IMPLICATIONS FOR NOCICEPTION

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Introduction: Acetaminophen is one of the most commonly used medications for pain and fever relief in the world. First synthesized in 1877, its mechanism of action remains unclear. As a consequence, even though acetaminophen liver toxicity causes ~500 deaths each year in the US alone, it has not been possible to design safer alternatives. The endocannabinoid system is an endogenous signaling system consisting of cannabinoid receptors, lipophilic ligands known as endocannabinoids and the enzymatic machinery to produce and break down these lipids. Cannabinoid receptors are widely distributed in the CNS and elsewhere in the body and have been reported to affect pain and inflammation. Endocannabinoids have been proposed to have a role in acetaminophen action.

Methods: In this study, we tested for an interaction between acetaminophen and endocannabinoid signaling in autaptic hippocampal neurons, a well-characterized model of endogenous neuronal cannabinoid signaling as well as lipase activity assays and a hot plate test for nociception.

Results: We now report that acetaminophen inhibits endocannabinoid production in these neurons, doing so at concentrations (as low as 10uM) that fall within the range achieved clinically when acetaminophen is used to treat pain. In lipase activity experiments we find that acetaminophen inhibits the activity of diacylglycerol lipase α (DAGLα) but not DAGLb. This gave rise to the counterintuitive hypothesis that DAGLα *inhibition* may be antinociceptive. In a hot plate test we confirm that the analgesic effects of acetaminophen require CB1 and intriguingly we find that DAGL inhibition by RHC80267 (20mg/kg) is antinociceptive in WT but not CB1 knockout mice.

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

DUAL MAGL AND COX INHIBITION ADDITIVELY ATTENUATES POST-OPERATIVE PAIN IN MICE

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Introduction –Inhibition of either monoacylglycerol lipase (MAGL), the primary enzyme that catabolizes the endocannabinoid 2-arachidonoyglycerol (2-AG) or cyclooxygenase (COX), the enzymes that synthesize prostaglandins, reduces pain and inflammation in various rodent models. We hypothesized that a combination of MAGL and COX inhibitors (i.e., JZL184 and diclofenac sodium), attenuates allodynia caused by hindpaw incision (HPI) a model of post-surgical pain in mice.

Methods – Under isoflurane anesthesia, equal numbers of adult male and female C57Bl/6J mice were subjected to HPI, in which a small incision was made and sutured in the plantar surface of one hind paw. Approximately 24 hours post-surgery, JZL184 (1-40 mg/kg, ip), MJN110 (0.55 – 5 mg/kg), the NSAID diclofenac sodium (1.85-50 mg/kg, ip), the CB_2 agonist, LY2828360 (3 mg/kg, i.p) or vehicle (5% ethanol, 5%, kolliphor EL, 90% saline, ip) was administered. A separate cohort was co-administered JZL184 (1, 40 mg/kg, i.p), diclofenac sodium (1.85, 50 mg/kg, i.p), or both compounds. A separate cohort of mice was administered rimonabant (3 mg/kg, i.p) or SR144528 (3 mg/kg, i.p) prior to JZL184 (40 mg/kg, ip). Mice were also injected repeatedly with JZL184 (8 mg/kg, s.c), cannabidiol (25 mg/kg, s.c), or vehicle before surgery and once daily post-surgery until recovery. Mechanical allodynia was quantified using von Frey filaments.

Results – Approximately, 24 hours post-surgery, acute MAGL inhibition via JZL184 (≥4 mg/kg) or MJN110 (≥5 mg/kg), the CB_2 agonist, LY2828360 (3 mg/kg) or the NSAID diclofenac (≥5.56 mg/kg) each attenuated HPI-induced mechanical allodynia. The combination of subthreshold doses of both diclofenac and JZL184 attenuated HPI-induced allodynia. The CB_2 antagonist SR144528 blocked the anti-allodynic effects of JZL184. Additionally, analgesia was maintained over repeated JZL184 (8 mg/kg) dosing.

Conclusion – In the present study, dual MAGL and COX inhibition attenuated HPI-induced allodynia, indicating an additive drug interaction. The CB_2 antagonist, SR144528 blocked the anti-allodynic effects of JZL184, and the CB_2 agonist LY2828360 reduced HPI-induced allodynia suggesting a CB_2 receptor mechanism. These data support targeting the endocannabinoid and prostanoid enzymes for postoperative pain treatment.

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RELEAF: A PLACEBO-CONTROLLED, RANDOMIZED, DOUBLE-BLINDED TRIAL ASSESSING MEDICAL CANNABIS IMPACT ON OPIOID USE IN CHRONIC PAIN

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Introduction: Chronic non-malignant pain affects nearly 30% of U.S. adults, yet few evidence-based therapies lead to improved patient outcomes. Amidst a growing opioid overdose crisis, new therapeutics are needed to treat chronic pain and reduce opioid use and overdose mortality. No controlled trials have directly examined the impact of medical cannabis on opioid analgesic use among chronic pain patients. Associations between medical cannabis use and adverse events are largely unknown, as prior studies of cannabis-related adverse events have focused on unregulated rather than medical cannabis. To address these research gaps, we are conducting a 14-week, 4-arm, randomized, double-blinded, placebo-controlled trial (ReLeaf) of medical cannabis among 267 adults with chronic pain. ReLeaf's goal is to determine whether medical cannabis reduces prescription opioid use in people with chronic pain; we present here our study design and preliminary results describing the demographic and clinical characteristics of our cohort to date.

Methods: ReLeaf is conducted at Montefiore Medical Center in the Bronx and at Vireo Health medical cannabis dispensary in Queens, both in New York City. Montefiore is the largest healthcare system in the Bronx, with 4 acute care hospitals and >50 ambulatory clinics; in 2017, Montefiore initiated a medical cannabis program in accordance with New York law. ReLeaf began enrolling in October 2020 and has enrolled 142 adults with severe or chronic pain *and* who were prescribed opioids in the past 30 days. To comply with state and federal regulations, participants are randomized at baseline to receive a voucher to purchase one of 4 identical-appearing softgel cannabis capsules: (1) placebo (0mg THC/0mg CBD), (2) high THC:low CBD (4.3mg THC/0.7mg CBD), (3) 1:1 THC:CBD (2.5mg THC/2.5mg CBD), or (4) low THC:high CBD (0.2mg THC/4.8mg CBD) for 14 weeks. After receiving their (blinded) voucher for a specific, heavily discounted, cannabis softgel, participants undergo regular titration evaluations throughout the study period which assess pain and treatment satisfaction and generate dosing recommendations based on an algorithm developed in consultation with our clinical staff and external advisory board. Study enrollees complete 5 in-person or telephone-based research visits over 14 weeks, as well as weekly web-based surveys. Additional data sources include pharmacy, medical, and NYS prescription monitoring program records. Our primary study outcome is cumulative opioid analgesic use by self-report and NYS prescription monitoring program records; we also measure pain, mental health symptoms, and adverse events.

Results: Among 142 participants, mean age is 57±10 y, 66% are female, 43% Black, 45% Latinx, and 82% unemployed. Almost all (>95%) have taken prescribed opioids for pain in the past 30 days, and pain symptoms are severe: mean Brief Pain Inventory score is 7.3 on a 1-10 scale, and 44% endorsed severe pain catastrophizing symptoms. Forty per cent of participants screened positive for moderate or severe anxiety, 45% for moderate or severe depression, 33% for PTSD, and 35% for moderate or severe insomnia. Enrollment is ongoing, and adjustments have been made to the original protocol to account for the COVID-19 pandemic and for changing trends in opioid prescribing.

Discussion: Using an innovative study design and a collegial partnership between an urban academic health center and a medical cannabis dispensary, we are conducting a placebo-controlled, double-blind trial of different cannabis softgel formulations among adults with chronic pain and prescription opioid use. We have successfully enrolled over half our planned cohort; enrolled participants have a high burden of both pain severity and mental health symptoms. Results of this trial will directly inform future recommendations for medical cannabis.

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PERSISTENT EXPOSURE TO Δ^9-TETRAHYDROCANNABINOL DURING ADOLESCENCE DOES NOT AFFECT NOCICEPTIVE RESPONDING IN ADULT MICE

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Introduction: Evidence suggests that Δ^9-tetrahydrocannabinol (Δ^9-THC), the intoxicating component of cannabis, may cause enduring changes in the structure and function of adolescent brain circuits implicated in nociceptive responding. Yet, whether such changes might persistently disrupt nociceptive behaviors remains unknown.

Methods: In the present study, we subjected C57BL6/J mice of both sexes to once-daily injections of Δ^9-THC (5 mg-kg^{-1}, intraperitoneal) or vehicle throughout adolescence (PND 30-43) and, when the animals had reached adulthood (PND 70), assessed nociceptive behavior using the formalin and chronic constriction injury (CCI) tests. We also investigated, using the tail immersion test, the antinociceptive effects of morphine and the development of tolerance to such effects.

Results: The results show that adolescent Δ^9-THC exposure does not significantly impair nociceptive responding or morphine-related antinociception and tolerance in male and female mice.

Conclusion: These findings suggest that daily exposure to an ecologically relevant dose of Δ^9-THC throughout adolescence does not affect nociceptive responding in adult mice.

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RISK OF CANNABIS ABUSE AND DEPENDENCE IN PATIENTS CERTIFIED FOR MEDICAL CANNABIS FOR CHRONIC PAIN

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Background: Although the use of medical cannabis is increasing, data on cannabis abuse and dependence among patients certified for medical cannabis is limited. We aimed to determine if more frequent use of medical cannabis is associated with cannabis abuse or dependence in a cohort of patients who were newly certified for medical cannabis for pain in New York State. Methods: We analyzed data from the first 12 months of the Medical Marijuana and Opioids (MEMO) study, which enrolled patients with chronic or severe pain who reported opioid use and were newly certified for medical cannabis. At baseline and every three months, we assessed DSM-IV Cannabis Abuse and Cannabis Dependence, measured by The Mini-International Neuropsychiatric Interview. Participants were categorized with cannabis abuse or dependence if they met the criteria for any of these outcomes in any study visit. Every two weeks, participants reported in web surveys the frequency of medical cannabis use (0-14 days) and the use of unregulated cannabis (yes/no). We calculated frequencies and used logistic regression to determine the effect of the frequency of medical cannabis use on cannabis abuse or dependence.

Results: The mean age among the 225 participants was 55.1 years and 122 participants (54.2%) identified as female, 79 (35.3%) as non-Hispanic White, 72 (32.1%) as non-Hispanic Black, and 59 (26.3%) as Hispanic. Participants reported medical cannabis use on an average of 5.8 days every two weeks. Only 17 participants (7.5%) met the criteria for cannabis abuse or dependence. There was no significant association between the frequency of medical cannabis use and cannabis abuse or dependence. We additionally found that participants who reported use of unregulated cannabis (n=97) were more likely to meet the criteria for cannabis abuse or dependence.

Conclusions: Cannabis abuse or dependence was rare in this sample of patients newly certified for medical cannabis with chronic pain. Our findings suggest that increased frequency of medical cannabis use is not associated with cannabis abuse or dependence. Given that medical cannabis may be a safer alternative to unregulated cannabis, efforts should be taken to divert patients that are certified for medical cannabis from using unregulated cannabis. One limitation of our study is that the study measures were originally developed for studies of people who use unregulated cannabis and may not be valid for medical cannabis studies.

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 not well 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 nucleus accumbens (NAc) core and shell.

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 hr after the CBD administration, and brains extracted 1.5 hours following the drug-seeking session. Plasma was used to measure endocannabinoid and CBD levels. NAc core and shell tissue was dissected and bulk RNA sequencing performed.

Results: Both doses of CBD attenuated heroin-seeking during the drug-seeking test compared to vehicle controls. Acute CBD treatment increased CBD, 7-OH-CBD, anandamide, and arachidonic acid levels. Bulk RNA sequencing indicated distinct differential gene expression in the NAc core when compared to the shell including biological processes related to morphine addiction and synaptic transmission. Bioinformatic analysis revealed that CBD reversed a number of the metabolic and cell signaling pathway alterations induced by heroin particularly in the NAc shell.

Conclusions: These findings suggest that CBD reduces cue-induced drug seeking behaviors by altering discrete biological pathways impacted by heroin in the NAc core and shell with indication of a greater impact in the NAc shell.

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THE CB2 CANNABINOID RECEPTOR AGONIST LY2828360 SUPPRESSES MECHANISTICALLY DISTINCT FORMS OF NEUROPATHIC PAIN IN RATS

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Introduction: Cannabinoid CB_2 agonists show promise as therapeutic agents because they lack unwanted side effects commonly associated with direct activation of CB_1 receptors. CB_2 receptor activation suppresses pathological pain in animal models. However, the types of pain that best respond to CB_2 agonists are incompletely understood. This gap in knowledge may contribute to failures in clinical translation. Our laboratory previously showed that the G protein-biased CB_2 receptor agonist LY2828360 attenuated the maintenance of inflammatory pain and chemotherapy-induced neuropathic in mice. Whether this finding generalizes to neuropathic pain induced by traumatic nerve injury is not known. Additionally, the ability of LY2828360 to reduce neuropathic in rat models has not been examined. This research is important because although LY2828360 failed in a Phase 2 clinical trial for knee pain due to osteoarthritis, it was shown to be safe to use in humans.

Methods: Neuropathic pain was induced in rats either surgically by performing a spared nerve injury (SNI) or by toxic challenge with the chemotherapeutic agent paclitaxel to induce chemotherapy induced peripheral neuropathy (CIPN) in rats. Paw withdrawal thresholds were measured using an electronic von Frey anesthesiometer before and after induction of neuropathy.

Results: LY2828360 (10 mg/kg i.p.) administered acutely attenuated mechanical allodynia in rats with SNI, whereas lower doses were ineffective (1-3 mg/kg i.p.). By comparison, both 3 and 10 mg/kg LY2828360 (i.p.) administered acutely reversed paclitaxel-induced mechanical allodynia in rats, an effect that was blocked by co-administration with the CB_2 receptor antagonist AM630. In both models LY2828360 retained efficacy following 10-day chronic administration of the effective dose, suggesting that tolerance to the anti-allodynic effect of LY2828360 does not develop in these models. Lastly, prophylactic dosing of LY2828360 (3 mg/kg i.p.) inhibited the development of mechanical allodynia in the CIPN model although this effect was not maintained following cessation of LY2828360 administration. LY2828360 did not alter basal nociceptive thresholds in the absence of the pathological pain state.

Conclusions: Our results provide evidence that LY2828360 suppresses neuropathic nociception caused by SNI injury and the chemotherapeutic agent paclitaxel in rats. Additionally, we showed that chronic administration of LY2828360 in these models does not cause tolerance to the anti-allodynic effects. These findings suggest that the clinical applications of LY2828360 as an anti-hyperalgesic agent should be re-evaluated.

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DO TERPENES REDUCE MECHANICAL ALLODYNIA IN MOUSE MODELS OF CHRONIC PAIN?

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Introduction: In the United States, 20.4% of adults suffer from chronic pain, with a majority reporting unmet or undertreated pain symptoms. Chronic pain reduces physical health, social functioning, work performance, and is associated with co-occurring mental disorders such as anxiety and depression. The average increased expenditure on yearly medical treatments for individuals with chronic pain is $7,726 more than those without. To manage unmet pain symptoms, some people with chronic pain use cannabis to reduce pain and improve quality of life. How cannabis exerts its analgesic properties is unknown, as there are more than 550 chemical compounds including terpenes and more than 100 phytocannabinoids. Preclinical studies have shown terpenes have analgesic potential. This study tests the hypothesis that myrcene, one of eight predominant terpenes identified in cannabis, will alleviate mechanical allodynia associated with nerve injury in a model of chronic neuropathic pain.

Methods: To model chronic pain, we used the Chronic Constriction Injury (CCI) model of neuropathic pain in male mice. Two weeks after surgery, mechanical thresholds were determined in sham (control) and CCI mice prior to and following myrcene (10-200 mg/kg, IP) or vehicle treatment. Mechanical thresholds were determined by Von Frey filaments, using force pressure from a monofilament to record the number of withdrawals out of 10 stimulations. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey post hoc tests at three time points: before surgery, after surgery but before myrcene, and after myrcene administration as well as within subject t-test of placebo vs drug treatment.

Results: Nerve injury increased the number of responses to the mechanical stimulus in CCI mice ($p=0.0012$) but not in shams ($p> 0.05$), demonstrating the presence of mechanical hypersensitivity. Myrcene (100 and 200 mg/kg) compared to placebo significantly attenuated mechanical hypersensitivity, 30 minutes post injection ($p=0.0274$ for 100 mg dose, $p=0.0123$ for the 200 mg dose). Time course data shows maximal effect at 30 minutes post injection and absence of effect by 90 minutes post-injection.

Conclusions: We established an effective model of mechanical hypersensitivity in a neuropathic pain model which was attenuated by myrcene administration. Next steps will be to generate dose response curves and include a cohort of females. We predict to see similar results especially in the female cohort, as females have a more sensitive response to pain and cannabinoids.

ADDITIVE EFFECTS OF SGLT2 TRANSPORTER INHIBITION AND CB1R BLOCKADE AGAINST DIABETIC NEPHROPATHY

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Introduction: Diabetic nephropathy (DN) is essentially characterized by glucotoxicity, altered intra-renal hemodynamics and profound remodeling of the renal matrix leading to tubulointerstitial fibrosis. Despite the use of treatments aimed at blocking the renin-angiotensin system and more recently at inhibiting the sodium-glucose cotransporter type 2 (SGLT2i), many patients continue to progress to severe renal failure. The cannabinoid 1 receptor (CB1R) appears to be an interesting new therapeutic target. Indeed, recent literature suggests that CB1R blockade is beneficial against the development of DN in various animal models and that the mechanisms of action may share commodalities with those of SGLT2i. Therefore, we sought to evaluate whether a combination treatment of a SGLT2i and a CB1R inhibitor could provide additional protection against DN.

Methods: 40 C57BLKS-Leprdb/db mice were fed a high protein diet for 9 weeks. They were then treated daily by gavage with the CB1R antagonist JD5037 (3 mg/kg/day), the SGLT2i empagliflozin (3 mg/kg/day), or a combination of both. In parallel, a group of diabetic mice and a group of healthy mice were exposed to the control vehicle. After 28 days of treatment, we assessed the effect of treatments on parameters used for diagnosis of DN such as albuminuria, glycaemia and urinary albumin to creatinine ratio. In addition, we evaluated the renal status on oxidative stress, inflammation and RAAS activation. Finally, we also evaluated the renal histological remodeling in order to estimate renal damage.

Results: JD5037 and empagliflozin led to a significant improvement of glycemic control concomitant with partial reduction in albuminuria and tubulointerstitial fibrosis. These findings were associated with an inhibition of inflammation and oxidative stress, improvement of intra-renal hemodynamics (reduced angiotensin II activity) and strong inhibition of the TGF-β pathway. For each of these parameters, a significant additive effect of both compounds was observed, leading to normalization of many parameters.

Conclusion: These results suggest that a poly-pharmacological approach based on SGLT2 inhibition and CB1R blockade represents a promising therapeutic strategy for the management of DN.

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PROTECTIVE EFFECT OF PRONOCICEPTIVE AND HEMATOPOIETIC FACTORS AGAINST CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN (CINP) AND DELAY ANTINOCICEPTIVE TOLERANCE TO CP55,940 IN OUR BREAST CANCER CINP MOUSE MODEL

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Introduction: Cannabinoid-based therapies are increasingly being used by cancer patients to treat chemotherapy-induced nausea and vomiting. Our previous work demonstrated that JWH-133, a cannabinoid receptor type 2 (CB2R) selective agonist, stimulates ovarian cancer tumor growth (Blanton et al., 2022). However, preclinical studies have failed to evaluate the effects of cannabinoid compounds on alleviation of pain, development of tolerance, and tumor growth in mice with CINP and cancer. The overarching hypothesis of this study is that breast cancer tumors produce molecular factors that cause a protective effect which suppresses CINP and cannabinoid tolerance.

Methods: The objective of our current work is to identify gene expression changes (RNA-sequencing) in target tissues (tumors, spinal cord and brain) responsible for the protective effects of breast cancer tumors on CINP and cannabinoid tolerance in our newly developed breast (AT3 syngeneic breast cancer cells inoculated at $5x10^6$ cells in mammary fat pad) cancer CINP (cisplatin 5 mg/kg/week; Guindon et al., 2014) mouse model. This will be accomplished using an integrative and comprehensive mechanistic approach that involves behavioral pharmacology, biochemistry, molecular biology and CRISPR techniques.

Results: Our preliminary data demonstrate that injection of AT3 (breast cancer cells) into the mammary fat pads significantly ($P < 0.0001$) decreased CINP in our breast cancer CINP mouse model. Moreover, using RNA-sequencing of breast cancer tumors dissected from CINP mice, we found increased gene expression of **multimerin 1 (Mmrn 1)**, a hematopoietic marker of activated platelets, and a decrease in **neuregulin 3 (Nrg3)**, a membrane-bound protein associated with neurodegenerative and neuropsychiatric diseases that has been shown to exert pronociceptive effects on pain. Furthermore, our results also showed a delay in antinociceptive tolerance to CP55,940 in our breast (AT3) cancer tumor CINP model in comparison to sham CINP mice lacking breast cancer tumors ($P < 0.001$). Therefore, to gain deeper mechanistic insights, we have knocked down Mmrn1 in our breast cancer cell line (AT3) using CRISPR techniques. We used lentiviral delivery allowing higher chromosomal integration of the CRISPR components compared with transfection (Castro-Piedras et al., 2021). Further studies will be looking at the effect of Mmrn1 knock down in breast (AT3) cancer cell line on tumor growth and cannabinoid tolerance in our breast cancer CINP model.

Conclusions: Investigation of these tumor-generated factors and gene expression changes in the tissues responsible for pain processing will enable us to better understand how CINP develops in patients and persists months/years after their last chemotherapeutic treatments, identify detection biomarkers for early onset breast cancer, and improve cannabinoid drug development for treatment/prevention of CINP in cancer patients.

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SEX AND DOSE-DIFFERENCES OF CANNABIDIOL AND AMITRIPTYLINE USING THE FORMALIN INFLAMMATORY PAIN MODEL

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Introduction: The prevalence of chronic pain is projected to increase in coming decades and already affects approximately one-in-five United States adults. These patients frequently suffer a significant reduction in quality-of-life and the estimated cost of their lost productivity has been estimated to be greater than $600 billion each year. Inflammatory pain, a key component of rheumatic diseases, is one of the most common causes of chronic pain and causes pain significant enough to limit patient activity in approximately 40% of rheumatic disease patients. Cannabis has a thousand-year history of use as an analgesic and recent sociocultural and political changes have renewed interest in its antinociceptive properties. Although there are over 550 constituents found in cannabis plants, the actions of cannabis are primarily mediated through its phytocannabinoids Δ9-tetrahydrocannabinol and cannabidiol (CBD). CBD has increasingly been used in the management of acute and inflammatory pain. Amitriptyline is a tricyclic antidepressant that primarily acts through antagonism of serotonin and norepinephrine reuptake. In addition to its anti-depressive properties, amitriptyline is known to have analgesic effects and has been approved by the Food and Drug Administration for use in pain-related syndromes. In this present study, we investigated possible sex differences in CBD and amitriptyline analgesia using C57BL/6j mice using the formalin model of inflammatory pain.

Methods: This study was performed using adult male and female wild-type C57BL/6j mice. Mice were pretreated with either vehicle, CBD (at doses 0.3, 1, 2.5, 10, 30, and 100 mg/kg i.p.), or amitriptyline (at doses 0.1, 0.3, 1, 3, 10, and 30 mg/kg i.p.) and then allowed to adapt for 20 minutes. Mice were then injected with 10 µL of dilute 2.5% formalin s.c. into the left hind paw and their pain behavior was scored for the following hour, with quantification via the composite pain score. Following the formalin test, brain and spinal cord tissue were collected. Once we established the dose-response curve, we determined ED_{50} doses of CBD and amitriptyline for both male and female wild-type mice. Further mice were tested using the ED_{50} doses, both alone and in combination. 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 ($p<0.05$ considered significant).

Results: Both CBD and amitriptyline provided antinociceptive effects in the formalin test during both the acute (phase 1) and inflammatory (phase 2) phases. The dose required to achieve significance in CBD was lower in the inflammatory phase and in males compared to females (female, phase 2: 10 mg/kg and above; male, phase 2: 2.5 mg/kg and above). In amitriptyline, antinociception occurred at lower doses in the inflammatory phase and in male mice (female, phase 2: 1 mg/kg and above; male, phase 2: 0.3 mg/kg and above). Significant sex and sex x dose interaction effects were noted in the inflammatory phase for amitriptyline but only significant sex differences were noted for CBD. ED_{50}s for phase 2 were calculated and found to be 17.783 mg/kg for CBD in females, 9.047 mg/kg for CBD in males, 3.298 mg/kg for amitriptyline in females, and 2.824 for amitriptyline in males. A significant additive effect was noted in male but not female mice when the ED_{50} doses were combined. RT-qPCR analysis will be conducted to evaluate changes in gene expression for cannabinoid receptors (CB1 and CB2), JNK, β-arrestin 1 and 2, and inflammatory markers (IL-1β, Il-6 and TNF-α).

Conclusions: This study demonstrates significant sex differences in amitriptyline analgesia and the need for further research underlying sex differences in both CBD and amitriptyline. Further research is ongoing in terms of changes in gene expressions to better understand the mechanisms of CBD analgesia and CBD/amitriptyline additive effects in the formalin model of inflammatory pain.

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COMPARISON OF CANNABIS-BASED MEDICINAL PRODUCT FORMULATIONS FOR CHRONIC PAIN: A COHORT STUDY

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Introduction: Chronic pain is a complex condition that can negatively impact physical, emotional, and social well-being. However, there is a paucity of evidence on the effectiveness of currently utilised therapeutics used to treat chronic pain. Cannabinoids and cannabis-based medicinal products (CBMPs) have been suggested as a novel therapeutic class for chronic pain, however the applicability of many datasets are limited due to the heterogeneity of CBMPs evaluated. The aim of this study was to analyse the changes in the pain-specific and general patient reported outcome measures, as well as adverse events data in a homogenous treatment cohort with CBMPs.

Methods: This cohort study enrolled patients with chronic pain treated with medium chain triglyceride oils, dried flower or both products (Adven® 20, 50 & EMT, Curaleaf International, Guernsey, UK), who had been enrolled in the UK Medical Cannabis Registry for a minimum of 12 months. Primary outcomes were changes in patient reported outcome measures from baseline, including pain Visual Analogue Scale (P-VAS), Brief Pain Inventory short-form (BPI), Short-form McGill Pain Questionnaire-2 (MPQ2), Single-Item Sleep Quality Scale (SQS), Generalised Anxiety Disorder-7 (GAD-7), EQ-5D-5L and Patient Global Impression of Change (PGIC) scales at 1, 3, 6, and 12 months. Adverse events in the course of treatment were recorded utilising the common terminology criteria for adverse events version 4.0. $p<0.050$ was regarded as statistically significant.

Results: A total number of 672 patients with chronic pain were included in this study. 334 (49.70%), 70 (10.42%) and 268 (39.88%) patients were treated with oil, dried flower or both preparations respectively. Improvements were observed in each group in GAD-7, SQS, EQ-5D-5L, BPI, MPQ2, at 1, 3, 6, and 12 months compared to baseline ($p<0.001$). There was no statistically significant difference between oil (-0.49 ± 1.47), dried flower (-0.87 ± 1.82) and both preparations' (-0.75 ± 1.62; $p=0.059$) mean change in BPI pain severity score at 12 months. However, the BPI interference score was lower in those prescribed both oils and dried flower (-1.10 ± 2.04) or dried flower alone (-1.36 ± 2.00) compared to oils (-0.71 ± 1.98; $p=0.011$). A total of 1856 (276.60%) adverse events were reported by 166 patients (24.89%). The majority (n = 1604, 86.42%) were rated as mild or moderate in severity. Adverse events that occurred in over 100 cases were dizziness (n=101, 5.44%), dry mouth (n=125, 6.73%), fatigue (n=150, 8.08%), headache (n=106, 5.71%), lethargy (115, 6.20%), and somnolence (n=132, 7.11%).

Conclusions: A positive association was identified between CBMP therapy and improvement in pain-specific and general patient reported outcome measures for chronic pain patients. There was no significant difference in outcomes according to pain severity between each formulation, however those treated with dried flower in isolation or in combination with oil products had greater reduction in pain interference. These results must be placed in the limitations of study design, however, and further evaluation through randomised controlled trials is still required.

ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH INFLAMMATORY ARTHRITIS: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

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Introduction: Chronic pain is a cardinal symptom of inflammatory arthropathies, which affect an estimated 2 million individuals in the UK. There are limitations to the utility of currently available analgesia for chronic pain secondary to inflammatory arthritis. Cannabis-based medicinal products (CBMPs) have an increasing pre-clinical and clinical evidence base in treating chronic pain due to inflammatory arthritis. However, there is a lack of research focused on inflammatory arthritis, in particular. The primary aim of this study was to assess changes in validated patient-reported outcome measures in patients with inflammatory arthritis. Secondary aims included an assessment of clinical safety in this population.

Methods: A case series of patients from the UK Medical Cannabis Registry was analysed. Patient demographic details, in addition to pre-existing co-morbidities and drug and alcohol consumption, were assessed. Primary outcomes were changes in the Brief Pain Inventory (BPI)) and McGill Pain Questionnaire (MPQ2) at 1, 3, 6, and 12 months compared to baseline. Secondary outcomes were changes in health-related quality of life measured using the EQ-5D-5L, Generalised Anxiety Disorder-7 (GAD7) questionnaire, and Single-Item Sleep Quality Scale (SQS). Adverse event analysis was performed in accordance with Common Terminology Criteria for Adverse Events v.4.0.. Statistical significance was defined as $p<0.050$.

Results: 82 patients were identified for inclusion with inflammatory arthritis. The mean age of the patients was 47.6 (±14.3) years. 41 (50.0%) patients were male and female respectively. Most patients (n=51; 62.2%) were consumers of cannabis prior to treatment with CBMPs. There were improvements in the MPQ2, BPI pain interference subscale, GAD-7 and SQS at 1 ,3 ,6 and 12 months compared to baseline ($p<0.050$). Improvements were observed in BPI pain severity subscale and EQ-5D-5L index value at 1, 3, and 6 months only ($p<0.050$). There were 230 (280.5%) adverse events recorded by 21 (25.6%) patients. Most adverse events were mild (102; 44.3%) or moderate (97; 42.2%).

Conclusion: These results suggest an improvement in pain associated with inflammatory arthritis following the initiation of treatment with CBMPs. There were also improvements in other patient reported outcome measures recording to health-related quality of life. While causality cannot be assumed in this observational study, the results of this study support the development of randomised controlled trials for symptomatic management of inflammatory arthritis with CBMPs.

COMPARISON OF CANNABIS-BASED MEDICINAL PRODUCT FORMULATIONS FIBROMYALGIA: A COHORT STUDY

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Introduction: Fibromyalgia is a chronic disorder characterised by widespread pain, tenderness on palpation, fatigue, mood disturbance, and cognitive dysfunction. Tricyclic antidepressants are the recommended first line therapy for fibromyalgia, however, recent meta-analyses suggest that benefits do not continue beyond 8 weeks of therapy and adverse events occur in up to 92% of individuals. Cannabis-based medicinal products (CBMPs) have therefore been suggested as a novel therapeutic for fibromyalgia due to the growing clinical evidence for chronic pain disorders. However, there is significant heterogeneity in the aetiologies of chronic pain and type of CBMPs that have been studied to date. This study therefore aims to analyse changes in patient reported outcome measures in patients from the UK Medical Cannabis Registry with fibromyalgia treated with a homogenous group of CBMPs.

Methods: A prospective cohort study of fibromyalgia patients being treated with medium chain triglyceride oils (Adven® 20, 50 and EMT, Curaleaf International, Guernsey, UK), dried flower (Adven®, EMT Curaleaf International, Guernsey, UK) or both products was performed. Participants were excluded if the date of enrollment was <12 months prior to the date of data extraction. Primary outcomes were changes from baseline compared to 1, 3, 6 and 12 months in patient reported outcome measures, including: generalized anxiety disorder -7 (GAD-7), single-item sleep quality scale (SQS), EQ-5D-5L Index values, and the fibromyalgia severity scale (FSS). Secondary outcomes included analysis of adverse events graded according to the CTCAE version 4.0.. $p<0.050$ was regarded as statistically significant.

Results: On January 8th 2023, 148 participants were identified as meeting the study inclusion criteria. 77 (52.0%), 14 (9.5%) and 57 (38.5%) individuals were prescribed oils, dried flower or both formulations respectively. Comparing outcomes at 1, 3, 6, and 12 months following initiation of CBMP treatment, improvements in GAD-7, SQS, FSS and EQ-5D-5L Index values were observed ($p<0.050$). There was no significant difference between each cohort in patient reported outcome measures at 12 months, except the mean improvement in EQ-5D-5L was greater in the oils and flower cohort (0.14 ± 0.25) than those prescribed oils only (0.03 ± 0.12; $p=0.005$). 36 (24.32%) patients experienced a total of 648 (437.83%) adverse events. The most common adverse events were fatigue (n=47; 31.76%), headache (n=46; 31.08%), dry mouth (n=44; 29.72%), somnolence (n=44; 29.72%), and lethargy (n=43; 29.05%).

Conclusion: Improvements were observed across all primary outcomes at up to 12 months of therapy with CBMPs. There were no differences those treated with different formulations of CBMPs, except those prescribed dried flower had larger improvements in health-related quality of life. Due to the observational nature of the study further research to evaluate if this association is caused by CBMPs or is due to factors that cannot be adequately controlled for in the present study design.

CBD INTERACTIONS WITH MORPHINE IN MICE

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Introduction: Previous studies found that greater doses of midazolam, fentanyl and propofol were required for adequate sedation for endoscopic procedures in cannabis users versus non-users.[1] Previous data from our lab in mice suggest that CBD may be responsible for this effect for propofol, and also found synergistic effects between CBD propofol and midazolam.

Methods: C57BL/6 male and female mice were administered 200 mg/kg cannabidiol (CBD), vehicle (20% sesame oil: 8% Tween-80: 82% ddH_2O), and/or 10 mg/kg morphine sulfate (in ddH_2O) i.p. at 0.01 ml/g. In synergy experiments, CBD was given 1 hour before 10 mg/kg morphine. In desensitization experiments (including only males so far), mice received CBD daily on days 1-3 then morphine on day 5. Change in body temperature was assessed with an IR thermometer and antinociceptive effects were assessed via the latency to tail withdrawal from a water bath set to 53°C before and 1 hour after drug administration. All data were analyzed by 2-way ANOVA (sex vs treatment) at $\alpha = 0.05$.

Results: Single treatments with CBD or morphine alone each caused changes in body temperature of -1.5 to -1.9°C with no difference between sexes. The combination of CBD and morphine caused a change of -3.5 to -3.7°C in females and males, respectively, which showed a significant difference from vehicle or morphine alone, but no difference between sexes. Morphine alone (but not CBD) produce a %MPE in the tail withdrawal test of 71% in male and 36% in female mice and the combination of CBD and morphine produced 49% and 36% MPE in male and female mice, respectively. While morphine showed a significant effect versus vehicle or CBD, the effect of CBD plus morphine was not significantly different from morphine alone and there were no sex differences. Preliminary data in males suggest that 3-day pre-treatment with CBD may desensitize mice to morphine's effects.

Conclusions: CBD synergistically enhanced the hypothermic but not the antinociceptive effect of morphine. Data on whether CBD desensitizes mice to morphine are preliminary.

[1]Twardowski, M. A., Link, M. M. and Twardowski, N. M. (2019), *J Am Osteopath Assoc*. 19(5): 307-311.

MEDICAL CANNABIS AND OPIOID USE AMONG ADULTS WITH CHRONIC PAIN: PRELIMINARY RESULTS FROM AN 18-MONTH LONGITUDINAL STUDY

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Introduction: Chronic pain affects over 30% of adults and has led to marked over-prescribing of opioid analgesics in the U.S. Despite recent reversals to this trend, opioid use disorder diagnoses and overdoses are increasing. To determine the role of medical cannabis as an alternative treatment to prescription opioids, we are conducting MeMO (Medical Marijuana and Opioids), an 18-month longitudinal cohort study of medical cannabis use among 225 adults taking prescription opioids for chronic pain. We report here baseline demographic and clinical characteristics of our study cohort, and describe patterns of medical cannabis use, pain, and prescription opioid receipt in the first 3 months of the study.

Methods: MeMO participants were recruited from Montefiore Medical Center in the Bronx, NY and from 4 cannabis dispensaries in NY City. MeMO began recruiting in September 2018, and reached its enrollment goal of 225 in December 2021. Participants were adults with severe or chronic pain who reported taking prescription opioid medications from any source in the 30 days before enrollment and had recently been certified for medical cannabis. MeMO included 7 quarterly research visits over 18 months, plus web-based questionnaires every 2 weeks, to assess detailed medical cannabis use (including THC/CBD content, and number of days of use in the past 2 weeks). To assess pain, participants completed the Pain, Enjoyment, and General Activity Scale ([PEG]; 0-10; continuous) every 2 weeks, and reported pain location, pain severity, and pain interference at baseline and every quarterly on the Brief Pain Inventory ([BPI]; 0-10; continuous). To assess prescription opioid use, we determined active morphine milliequivalents (MME) on the date of each web-based questionnaire, using available opioid prescription data from the NY State prescription monitoring program; a prescription was considered active if its duration included the date of the web-based survey. To examine changes from baseline to 3-months, we used t-tests to examine how PEG score, pain severity, and interference differed from baseline to 3-months.

Results: Among 225 MeMO participants, mean age at enrollment was 54 y, 54% were female, 35% non-Hispanic White, 32% non-Hispanic Black, 26% Hispanic, 78% unemployed, and 56% living below the national poverty level. The most common pain locations were neck or back (76%) and limb (79%); 79% had pain at multiple body sites. Psychiatric symptoms were common, including symptoms of: moderate/severe anxiety (37%), moderate/severe depression (46%), PTSD (38%) and ADHD (40%). One hundred and eighteen participants (52%) used prescription opioids daily, 49 (22%) reported regular unregulated cannabis use, 57 (25%) used sedatives in the past 30 days, and 75 (33%) used tobacco. Mean baseline PEG score was 7.08 (standard deviation [SD] 1.9), mean BPI severity score was 6.61 (SD 1.82), and mean BPI interference score was 6.79 (SD 2.06). Mean active MME was 117 and 113 participants had an active MME at enrollment. To date, we have analyzed the first 3 months of web survey data from 140 participants (total: 1,377 web-based surveys). Among these, 29% of surveys reported use of predominantly high-THC products during the preceding 2 weeks, 30% reported use of other medical cannabis products, and 41% reported no medical cannabis use. Over 3-months, we observed increases in both high-THC product use (from 22% to 33%) and any medical cannabis use (49% to 63%). MeMO participants had decreased pain: PEG score ($p=0.003$) and BPI pain interference decreased ($p <0.001$) over 3 months. The number of MeMO participants with active MMEs dropped from 113 to 86.

Discussion: In the first 3 months of the MeMO study, participants experienced significant reduction in pain and fewer participants were taking opioid medications 3 months after baseline. MeMO is collecting robust data about medical cannabis use in a diverse population. Our findings add to existing literature that supports medical cannabis use for pain and adds promising evidence that medical cannabis use can reduce opioid use in this population.

MEDICAL CANNABIS USE AND ADHD SYMPTOMS IN ADULTS WITH CHRONIC PAIN

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Introduction: Adults with attention deficit-hyperactivity disorder (ADHD) have disproportionate chronic pain. As use of medical cannabis (MC) expands for management of chronic pain, it is important to understand its impact on ADHD symptoms, but prior research is extremely limited. In a cohort of adults with chronic pain and certified for MC, we examined: 1) whether MC use (any MC and "high THC" MC [≥2:1 THC:CBD]) is associated with change in ADHD symptoms, and 2) whether these associations persist in patients with ADHD symptoms who are prescribed stimulants. We hypothesized that MC use will be associated with worsened ADHD symptoms, that high THC MC (vs. <2:1 THC:CBD MC) will worsen ADHD symptoms, and that dispensed stimulants will mitigate these associations.

Methods: This is a secondary analysis of an 18-month longitudinal cohort study of adults with chronic pain who are prescribed opioids and newly certified for MC, restricted to those who have completed 12-months of study visits. Data sources were quarterly surveys (0, 3, 6, 9, 12 months), every-two-week web-based surveys, and prescription monitoring program data (0-12 months). The primary independent variable was MC use (vs. no use; web-based surveys). The secondary independent variable was high THC (≥2:1 THC:CBD on most days; web-based surveys) MC use (vs. <2:1 THC:CBD MC). The dependent variable was change in ADHD symptoms from 0-12 months (Adult ADHD Self-Reporting Rating Screening Scale for DSM-5 score, continuous, 0-25; ≥14 moderate/severe ADHD symptoms; quarterly surveys). We used prescription monitoring program data to determine stimulant prescription (dichotomous, yes/no). Other variables were baseline daily past 30-day opioid use (dichotomous, yes/no), pain severity (0-10, continuous), pain interference (0-10, continuous), insomnia (Insomnia Severity Index, 0-28, dichotomous, moderate/severe ≥15), health related quality of life (EuroQol 5-Dimension Scale, -0.59-1, continuous), anxiety symptoms (Generalized Anxiety Disorder-7, 0-21, dichotomous, moderate/severe ≥10), depressive symptoms (Patient Health Questionnaire-9, 0-27, dichotomous, moderate/severe ≥10). We conducted t-test, chi-square, and linear regression (covariates: anxiety, depression, and opioid use) analyses to examine the association between independent variables and change in ADHD symptoms. We repeated analyses restricted to participants with moderate/severe ADHD symptoms at baseline, and stratified analyses by stimulant use.

Results: Of 225 participants, 54% were female and mean age at baseline was 54 years. Participants with moderate/severe ADHD symptoms (vs. minor/no ADHD symptoms) were younger (50.4 vs 56.7 years, $p<0.001$), more likely to be Non-Hispanic White ($p=0.04$), reported higher pain interference ($p=0.01$) and insomnia scores ($p<0.001$), and lower health-related quality of life scores ($p<0.003$). Neither MC nor high-THC MC use was associated with change in ADHD symptoms in bivariate analyses, when controlled for covariates, or after stratifying by stimulant prescription. In participants with baseline moderate/severe ADHD symptoms (n=61), MC use (vs. no use) was associated with an increase in ADHD symptoms ($p=0.04$), even when controlling for covariates ($p=0.02$); the increase was greater in those not prescribed stimulants ($p=0.02$). Among participants with moderate/severe ADHD who use MC (n=42), high THC MC use (vs. <2:1 THC:CBD MC) was associated with a decrease in ADHD symptoms ($p=0.04$), even after controlling for covariates ($p=0.03$); the decrease was more pronounced in those not taking stimulants ($p=0.02$)

Conclusion: In a cohort of adults with chronic pain prescribed opioids and newly certified for MC, MC use (vs. no use) was associated with worse ADHD symptoms in those with moderate/severe ADHD symptoms, and high THC MC use (vs. <2:1 THC:CBD MC) was associated with reduction in ADHD symptoms, especially in those not taking stimulants. These findings were unexpected, and require future, more nuanced, studies to more completely understand how cannabis impacts ADHD symptoms in adults.

BETA-CARYOPHYLLENE FOR THE TREATMENT OF PAIN AND INFLAMMATION IN EXPERIMENTAL INTERSTITIAL CYSTITIS

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Introduction: Interstitial cystitis (IC) is an inflammatory condition of the bladder that is associated with chronic lower abdominal pain. At present, there are no effective long-term treatments available for IC. Beta-caryophyllene (BCP) is a sesquiterpene found in plants such as cannabis and cloves. BCP preferentially activates cannabinoid receptor 2 to reduce pain and inflammation. The aim of this study is to evaluate the effects of a novel oral BCP formulation in experimental IC.

Methods: Mice were pre-treated with BCP (100 mg/kg, orally). IC was induced via intravesical administration of lipopolysaccharide (LPS; 0.375 mg/kg, intraurethrally) held in the bladder for 30 minutes. 24 hours later, mice were evaluated for changes in behaviour and evoked pain tolerance. Intravital microscopy was used to evaluate changes in leukocyte activation and capillary perfusion in the microcirculation of the bladder wall. Bladder tissue samples were taken to assess changes in histology and local cytokine levels.

Results: Intravesical LPS administration induced significant inflammation of the bladder as shown by increased leukocyte activation within the bladder and resulted in marked pain in experimental animals. Randomized treatment experiments using oral BCP vs. vehicle in animals with experimental IC are ongoing. Preliminary results will be presented.

Conclusions: Administration of 0.375 mg/kg LPS held in the bladder for 30 minutes is an appropriate experimental model to study novel formulations of BCP as a treatment for IC.

OPIOID AND CANNABINOID INTERACTIONS: A STRATEGIC APPROACH TO PREVENT OPIOID INDUCED PERSISTENT APNEA

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Introduction: Opioids are excellent analgesics; but also, the driving cause of over half a million overdose fatalities, resulting in an overall decline in life expectancy. Opioids activate inhibitory **mu opioid receptors (MORs)** in the **breathing central pattern generator (bCPG)**, to cause dose-dependent slowed and shallow breaths until breathing stops; known as **opioid induced persistent apnea (OIPA)**. I recently demonstrated a **brain penetrant (BP) CB2R** agonist, AM2301 **(BP CB2R agonist),** or a **peripherally restricted (PR)** CB1R agonist, PrNMI **(PR CB1R agonist)**, coadministered with morphine in awake animals mitigated **morphine-induced respiratory depression (MIRD)**. However, if these cannabinoids would be effective against fentanyl remains to be elucidated. I will use a model that reliably allows us to evoke an OIPA to investigate the efficacy of each synthetic cannabinoid, and the combination of the two, to increase the dose of fentanyl necessary to induce a persistent apnea.

Methods: Recordings of respiratory rate, tidal volume, and minute volume were collected before and after pretreatment with one, or both, cannabinoids i.p. (BP CB2R agonist, AM2301 10 mg/kg and PR CB1R agonist, PrNMI 0.3 mg/kg), and again following intraventricular administration of fentanyl, at a rate of 100 ng/min, until the onset of persistent apnea defined as the absence of respiration for longer than one minute, from urethane anesthetized mice.

Results: Administration of either synthetic cannabinoid did not alter respiratory rate on their own. Pretreatment with either the BP CB2 agonist, AM2301 10 mg/kg, or the PR CB1R agonist, PrNMI 0.3 mg/kg, increased the total fentanyl dose to cause OIPA compared to vehicle. Additionally, the PR CB1R agonist, PrNMI 0.3 mg/kg, was more efficacious at increasing the lethal dose of interventricular fentanyl before resulting in OIPA than the BP CB2R agonist, AM2301 10 mg/kg.

Conclusions: These studies revealed dual mechanisms, via the endocannabinoid system, able to increase the lethal dose of fentanyl to result in OIPA, via administration of an i.p. injection of the BP CB2R agonist, AM2301, or the PR CB1R agonist, PrNMI, in mice. These findings could lead to the discovery of a safe opioid, void the potentially fatal side effect of OIPA, and save thousands of lives.

CANNABIS SUBSTITUTION FOR OPIOID USE AMONG PEOPLE WHO USE UNREGULATED DRUGS IN VANCOUVER, CANADA

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Introduction: The intersection of cannabis policy reforms with the opioid overdose has sparked scientific interest into the effects of cannabis use on the use of prescription and unregulated opioids, particularly among people who use unregulated drugs (PWUD). Our objective was to investigate the association between cannabis substitution for opioids and the frequency of unregulated opioid use among structurally marginalized PWUD.

Methods: The data for this analysis was collected from three prospective cohorts of PWUD in Vancouver, Canada. Logistic regression was used to analyze the association between socio-demographic variables and cannabis use behaviours with unregulated opioid use.

Results: A total of 205 people who use opioids were enrolled in the present study from December 2019 to November 2021. Daily cannabis use was reported by 88 (43.1%) participants, and 118 (57.6%) reported cannabis substitution for unregulated opioid use. In the multivariable analysis, female sex (adjusted Odds Ratio [aOR] = 2.55 ,95% confidence interval [CI]: 1.22, 5.35), daily cannabis use (aOR = 2.11, 95%CI: 1.11, 4.02), use of high THC cannabis (aOR = 2.06, 95%CI: 1.04, 4.08) and using cannabis to reduce opioid cravings (aOR = 2.59, 95%CI: 1.34, 5.02) were associated with decreased opioid use during periods of cannabis use.

Conclusions: These findings indicate that substitution for opioid use is a prevalent motivation for cannabis use among people who use unregulated drugs. With accumulating evidence indicating that cannabis substitution is often used as a harm reduction strategy among PWUD, increasing the accessibility of cannabis products for therapeutic use may be a useful supplementary strategy to mitigate exposure to unregulated opioids and associated harm during the ongoing overdose crisis.

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A BEHAVIOURAL ASSESSMENT OF REDUCED EFFORT EXPENDITURE AND REWARD SENSITIVITY IN CANNABIS USE DISORDER

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Introduction: Cannabis use disorder (CUD) is a psychiatric disorder characterized by alterations in motivation. With increasing cannabis legalization worldwide, understanding the drug's effects on motivation is crucial. Applying a neuroeconomic lens to assess decision-making can operationalize and quantify motivation into its basic elements. Behavioural studies of effort-based decision-making show that, with increasing levels of effort, healthy individuals choose high effort expenditure tasks less often. With increasing reward levels, conversely, individuals choose tasks producing high rewards more often, suggesting adapting choice behaviour for reward maximization. Despite these established patterns of choice behaviour, behavioural investigations of effort-based decision-making in disorders characterized by low motivation remain scarce. This study applied the Effort Expenditure for Rewards Task (EEfRT), a validated measure of effort-based decision-making, in individuals with CUD to quantify motivation and capture group differences in effort-expenditure for rewards.

Methods: Data come from a larger ongoing functional magnetic resonance imaging (fMRI) study. Forty-one participants completed 2 runs of the EEfRT while undergoing fMRI (CUD n =21; controls n=20). Group differences were tested in i) the number of accepted trials, ii) the number of accepted trials across effort levels, and iii) the number of accepted trials across reward magnitudes, using MANOVAs. Repeated-measures ANOVAs were used to analyse within-group differences between EEfRT runs. All analyses were considered significant at $p<0.05$.

Results: Collapsed across groups, there was a significant decrease in the number of accepted trials between both runs of the EEfRT. As effort levels increased, there was a significant decrease in high-effort choices and a significant decrease in the number of accepted trials during Run2. As reward magnitude increased, there was a significant increase in high-reward choices and a significant decreased in the number of accept low-reward trials during Run2. Although groups did not significantly differ in the number of accepted trials across effort levels and runs, individuals with CUD accepted trials with high rewards (>$3.50) significantly less often than controls, particularly during Run1 of the EEfRT.

Conclusion: Our results replicate patterns of choice behaviour previously outlined in effort-based decision-making studies using healthy controls. Extending behavioural assessments of effort-based decision-making to individuals with CUD demonstrates that individuals with CUD may be less sensitive to reward magnitudes than controls. This work can clarify behavioural mechanisms underlying the onset and maintenance of cost-benefit decision-making alterations in CUD.

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SQUARAMIDE-BASED ALLOSTERIC MODULATORS OF CB_1 RECEPTOR ATTENUATE CUE-INDUCED REINSTATEMENT OF COCAINE-SEEKING BEHAVIOR

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Introduction: The cannabinoid 1 receptor (CB_1) plays an important role in many physiological processes including reward circuits in the central nervous system. Our group has been developing negative allosteric modulators as an alternate approach to modulate CB_1R that may avoid serious psychiatric side effects of orthosteric CB_1R inverse agonist/antagonists. We have now expanded the chemical space by exploring substitution of the urea moiety present in traditional CB_1R allosteric modulators such as PSNCBAM-1 and RTICBM-74 with a squaramide, a vinylogous amide motif often used as bioisosteric replacements of functional groups such as amides and ureas.

Methods: A series of squaramide-based CB_1R allosteric modulators were designed, synthesized, and characterized by ^1H and ^{13}C NMR, LCMS, and HPLC. All target compounds were evaluated for their CB_1R modulating activity in calcium mobilization assays using CHO-RD-HGA16 overexpressing human CB_1R or CB_2R and [^{35}S]GTPγS binding assays using CB_1-expressing HEK293 cell membranes. Promising compounds were then assessed for metabolic stability against rat liver microsomes. One compound (RTICBM-262) was evaluated for its efficacy to attenuate cue-induced reinstatement of cocaine-seeking and its effect on locomotor activity in male Sprague-Dawley rats.

Results: Most compounds possessed nanomolar IC_{50} values at CB1R without any significant activities at the CB2R. These compounds reduced the E_{max} of the orthosteric CB_1R orthosteric agonist CP55,940 in calcium mobilization assays, consistent with negative allosteric modulation. In general, for most compounds in the series, the potencies in the calcium mobilization assay correlated with those of the [^{35}S]GTPγS binding assays. RTICBM-262 possessed good metabolic stability ($T_{1/2}$ = 40 min) and effectively reduced cue-induced reinstatement of cocaine-seeking at 5.6 mg/kg without affecting locomotor activity.

Conclusions: This work expanded the chemical space of CB1R allosteric modulators and characterized a series of squaramides that may be further developed as potential medications for the treatment of drug addiction and other CB1R-mediated conditions.

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PATIENT PERCEPTIONS ON EVALUATING CANNABINOIDS AS NOVEL CANCER THERAPIES PRIOR TO CLINICAL TRANSLATION

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Introduction: There is growing evidence of the cytotoxic effects of cannabinoids and other cannabis-derived compounds in pre-clinical studies. Pre-clinical cancer research is an important component for clinical translation of treatments for cancer. However, there are no published reports on public perceptions of research in this setting. This study therefore aimed to analyse public perceptions on different pre-clinical cancer models for the assessment of cannabinoids.

Methods: A survey was distributed to attendees of a public engagement event at Imperial College London. Questions elicited opinions on the perceived efficacy and cost-effectiveness of different pre-clinical models for evaluating cancer treatments using a 5-point Likert scale (1–most effective; 5–least effective) and analysed using the mean score ± standard deviation.

Results: Twenty-one participants (age: 30.3±15.7 years) ranked the following models from most to least effective: phase I clinical trials (1.43±0.87), animal models (1.95±0.67), patient-derived organoids (3.33±1.07), 2D co-culture models (3.67±0.58), and 2D cancer models (4.62±0.87). The models were ranked as follows on cost-effectiveness: patient-derived organoids (2.76±1.04), phase I clinical trials (2.81 ± 1.69), animal models (2.86±1.46), 2D co-culture models (3.29±1.01), and 2D cancer models (3.29±1.77). All participants (n=21; 100%) agreed in principle to donate excess tissue from a cancer resection for the generation of patient-derived organoids.

Conclusions: In this limited sample, members of the public highlighted that phase I clinical trials are the most effective method of assessing effectiveness of the medication compared to *in vitro* and *in vivo* models. However, patient-derived organoid cultures were deemed to be the most cost-effective method of assessing the effects of cannabinoids on cancer. These results, highlight public perceived differences in cost-effectiveness which may help to guide study design and funding during evaluation of novel cannabinoid therapeutics for cancer.

A SIGNIFICANT IMPROVEMENT IN SYMPTOMS, QUALITY OF LIFE AND FUNCTIONAL STATUS IN A BREAST CANCER SURVIVOR FOLLOWING CANNABIDIOL ADMINISTRATION: CASE REPORT

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Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) affects up to 80% of breast cancer survivors and is challenging to treat. A registry is on-going where breast cancer survivors suffering from CIPN are first given duloxetine (60 mg/day) (phase 1). If duloxetine fails to improve pain scores by ≥50% after 4 weeks, patients are randomized to receive a treatment with a CBD-isolate or an exercise program for two months (phase 2).

Case presentation (Methods & Results): A 52-year old Caucasian woman with a history breast cancer, in remission for over five years, presented with CIPN in her feet and neuropathic pain around the R axilla and R shoulder. She had previously received neoadjuvant chemotherapy in the form of paclitaxel, trastuzumab and pertuzumab, followed by pertuzumab-trastuzumab, followed by breast surgery and adjuvant radiation. Her comorbidities include arthritis and lymphedema. ***At baseline***, she was moderately active (score=22) according to the Godin-Shephard leisure-time physical activity (GSLPA) Her worst pain and average pain were classified as 9 and 4, respectively, on the painDETECT questionnaire (1=none and 10=max pain). Her white blood cell (WBC) count ($3.1x10^6$/mL) and percent lymphocytes (32.53%) were normal, but her C-reactive protein (14mg/L) was elevated. ***In Phase 1***, a starting duloxetine dose of 30 mg/daily produced excessive sweats and was discontinued by the patient after 4 weeks. Her relative lymphocyte percentage was elevated (45.20%) and her WBC count was normal ($2.8x10^6$/mL). Her physical activity status improved to active (score=24). Her worst pain score decreased by 30% (score=6) and her average pain score remained unchanged (score= 4). ***In phase 2***, the patient was able to take the maximum dose of CBD (300mg/daily) after 6 weeks of its gradual increase. All previously elevated parameters (C-reactive protein, %lymphocytes) normalized. But here WBC count was elevated ($5.4x10^6$/mL). The participant was more active (score=40, 45% increase in activity from baseline). She experienced a clinically meaningful improvement (CMI) in quality of life determined through the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity scale (=12 points score improvement). Her worst and average pain were scored at 4 (= 50% reduction since baseline) and at 3 (10% reduction since baseline), respectively. Several functional tests (Short Physical Performance Battery-SPPB, Hand Grip Strength, 30 second single leg stance) were performed to test mobility, strength and balance. From baseline, she experienced improvements by 16.7% in the SPPB, by 15.7 % in the Gait Speed Test, by 24.3 % in the 5x Chair stand test and by 17.24% in upper body strength. She only reported mild/temporary diarrhea as a side effect while transitioning from 100 mg to 200 mg of daily CBD and liver enzymes remained within normal limits.

Conclusion: This case report shows that CMIs in CIPN symptoms, quality of life and functional status can be achieved through a safe administration of 300 mg/day of CBD. CBD is also not associated with bothersome effects associated with duloxetine use, such as lymphocyte profiles' perturbations and hyperhidrosis. More real-world data are being collected to confirm these observations.

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IMPACT OF FETAL BOVINE SERUM ON CANNABINOID EFFICACY AGAINST CANCER CELLS

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Introduction: Studies have reported that cannabinoids, in particular Δ^9-THC and CBD, significantly reduce cancer cell viability *in vitro*. Unfortunately, treatment conditions vary significantly across reports. In particular, a majority of reports utilize treatment conditions with reduced serum concentrations (0%-3%) that may themselves compromise the growth of the cells, as well as the observed results. The present studies were designed to test the hypothesis that, based on their known protein binding characteristics, cannabinoids would be less effective in the presence of fetal bovine serum (FBS). We wished to determine if the cannabinoid treatments served to be cytotoxic or cytostatic under these conditions. Moreover, we explored whether the low serum conditions significantly impacted cell growth.

Methods: Six cancer cell lines, representing two independent lines of three different types of cancer (glioblastoma, melanoma, and colorectal cancer) were treated with 10 μM pure Δ^9-THC, CBD, KM-233, and HU-331 for 48h (in the presence or absence of FBS). Cell viability was assessed with the MTT assay (that measures mitochondrial activity). Dose response curves were then generated comparing the potencies of the four cannabinoids under the same conditions.

Results: We found that serum-free medium alone produces cell cycle arrest for colorectal cancer cells and slows cell growth for the other cancer types. The anti-neoplastic effects of three of the four cannabinoids (Δ^9-THC, CBD, and KM-233) increase when serum is omitted from the media. Additionally, dose response curves for these drugs demonstrated lower IC_{50} values for serum-free media compared to media with 10% serum in all cell lines. The fourth compound, HU-331 (a known potent topoisomerase inhibitor), was equally effective under both conditions. A further confound we observed is that omission of serum dramatically enhances binding of Δ^9-THC and CBD to plastic.

Conclusions: Treatment of cancer cells in the absence of FBS appears to enhance the potency of cannabinoids as has been reported by numerous research groups. However, (1) omission of FBS itself compromises cell growth and represents a less physiological condition. Indeed, colorectal cancer cells stopped proliferating in the absence of serum. (2) In addition, in the absence of FBS, cannabinoids bind to the plastic of the tissue culture plate. Therefore, given the knowledge that cannabinoids are 90-95% protein bound and have well known affinities for plastic, it may be ill-advised to treat cells under conditions where the cells are not growing optimally and where known concentrations cannot be assumed (*i.e.*, FBS-free conditions).

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THE METASTATIC UVEAL MELANOMA CELL LINE OMM2.5 DISPLAYS SELECTIVE SENSITIVITY TO THE SYNTHETIC CANNABINOID HU-210

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Introduction: This research evaluates the relevance of cannabinoid receptors in uveal melanoma (UM) and the therapeutic potential of synthetic cannabinoids in UM cell lines and in *ex vivo* orthotopic patient-derived xenografts (OPDX). UM is the most common intraocular malignancy in adults. Up to 50% of patients develop liver metastases with overall survival ranging from 4-15 months.

Methods: Cannabinoid receptor gene expression in 80 primary UM samples within The Cancer Genome Atlas was analysed for association with disease-free and overall survival. Immunoblotting analysed CB_1 receptor expression. *In vitro* assays utilised Mel285 and OMM2.5 human UM cell lines derived from tumours of the eye and a liver metastasis, respectively; and normal human epidermal-derived melanocytes (HEM). Cell viability was indirectly examined by measuring metabolic activity. Colony formation assays assessed long-term cell proliferation. Multiplex ELISA quantified secreted levels of inflammatory factors in OMM2.5 cells. Proteomic profiling of OMM2.5 cells treated with 20 μM HU-210 was performed by mass spectrometry.

Results: Kaplan-Meier curves demonstrate a significant correlation between high CB_1 expression and lower disease-free survival ($p=0.027$) or high CB_2 expression and lower overall survival ($p=0.005$) in UM patients. CB_1 expression was confirmed in both UM cell lines. 10 μM or 20 μM HU-210, a CB_1/CB_2 agonist, was required to significantly reduce cell viability in HEM or in Mel285 cells, respectively. However, HU-210 shows a selective effect in OMM2.5 where concentrations ranging 100 fM to 20 μM significantly reduce cell viability. In contrast, 150 μM of CB_2 agonist JWH-133 was required to significantly reduced viability by 80% ($p=0.0001$) in both UM cell lines. 20 μM HU-210 significantly reduces long-term proliferation of Mel285 ($p=0.005$) and OMM2.5 ($p<0.0001$) cells and significantly alters secretion of 17 out of 54 inflammatory factors analysed in OMM2.5 cells. Proteome profiling of OMM2.5 cells treated with 20 μM HU-210 identified 55, 41 and 193 proteins significantly differentially expressed after 4,8- or 24-hours treatment, respectively. These are being validated by immunoblotting.

Conclusions: Significant correlations occur between high CB_1 or CB_2 expression and survival in UM patients. The potent cannabinoid HU-210 reduces viability and clone proliferation of UM cell lines and significantly alters the secretion of inflammatory factors in OMM2.5. Future directions will evaluate the molecular mechanisms of HU-210 action in UM cells. We are currently analysing the secretome of OPDX samples obtained from xenografts of UM tumours.

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CANNABIDIOL INDUCES CYTOTXOCITY VIA A CERAMIDE SYNTHASE 1, GRP78, ATF4 AND CHOP MECHANISM IN PANCREATIC DUCTAL ADENOCARCINOMA

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most aggressive malignancies with a median 5 year-survival rate of 8%. Cannabidiol (CBD) has been found to exhibit antineoplastic potential and may potentiate the anticancer effects of gemcitabine. The biological mechanism for this synergy remains elusive but in other tumour types, CBD therapy has been linked to *de novo* synthesis of ceramide and induction of ER (endoplasmic reticulum) stress. The sphingolipid ceramide is a potent tumour suppressor lipid with roles in apoptosis and autophagy. The aim of this work was to establish the downstream cytotoxic mechanism of action of CBD in PDAC, evaluate the potential synergy with gemcitabine therapy and determine cell cycle effects and receptor involvement.

Methods: Pancreatic cancer cell lines; Panc03.27, Panc1 and murine-3275 were screened for their IC_{50} values following treatment with CBD and gemcitabine. siRNA transfections, q-PCR and western blots were performed to evaluate expression levels of CerS1, GRP78, ATF4 and CHOP. Cell cycle and death analysis was performed using flow cytometry and Annexin/PI.

Results: The findings point to evidence of a putative ceramide synthase 1 (CerS1) dependent pathway driven by CBD in activating endoplasmic reticulum (ER) stress target; GRP78. Specifically, in gemcitabine resistant cells, Panc1, the activation of the GRP78/ATF4/CHOP arm of the unfolded protein response (UPR) pathway was observed. In gemcitabine sensitive cells, Panc03.27, CerS1 showed enhanced upregulation of CerS1, GRP78, CHOP targets in combination with gemcitabine. The involvement of a cannabinoid receptor was analysed by viability and protein expression which showed evidence of CB_2 and GPR55 expression in both human and murine cells. Cell cycle analysis showed arrest in G0-G1 phase and an increase in apoptotic cells via annexin/PI studies, indicating cell death specific effects of CBD in pancreatic cancer cells.

Conclusions: We show in this work, dose-dependent and time-dependent cytotoxic effects of CBD in both human and murine pancreatic cancer cells. CBD arrests pancreatic cancer cells in the G0-G1 phase indicating apoptotic route of mechanism of cytotoxicity, supported by annexin/ PI analysis. Gemcitabine and CBD in combination upregulate CerS1 greater in gemcitabine sensitive cells and in resistant cells, CBD alone can upregulate CerS1 equally. The downstream effects of CerS1 upregulation induce ER stress which activate the GRP78/ATF4/CHOP arm of the UPR response in gemcitabine resistant cells, through a possible CB_2 or GPR55 receptor mediated mechanism. These effects provide an avenue of exploratory mechanism to evaluate in 3D models of pancreatic cancer such as organoids and co-cultures to recapitulate the tumour microenvironment.

ACUTE-TO-CHRONIC KIDNEY DISEASE PROGRESSION REVEALS DYNAMIC AND SPATIAL CHANGES IN THE ENDOCANNABINOID SYSTEM

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INTRODUCTION: Acute Kidney Injury (AKI) is a common complication in hospitalized patients and a major source of morbidity and mortality. aWhile the endocannabinoid system (ECS) contributes to various forms of renal injury, a thorough understanding of its role in the AKI-to-CKD continuum is lacking. This study provides a temporal, dynamic, and spatial mapping of the ECS response as AKI progresses to CKD.

METHODS: The murine model of folic-acid (FA)-induced AKI (240 mg/kg FA, single dose, i.p.) was utilized to evaluate the dynamic changes as the acute injury, observed at 2-days post injection, progresses to CKD, 14-days post injection. The different disease states were characterized by evaluating renal dysfunction, injury, fibrosis and unbiased high-throughput metabolomic profiling. Renal endocannabinoid (eCB) changes were evaluated using both quantitative measurements (LC-MS/MS) and spatial imaging (DESI-MS) techniques. Cannabinoid receptors (CBRs) and eCB metabolic enzymes transcriptional and translational expression levels were analysed by RT-PCR and Western blotting.

RESULTS: Though kidney disease was evident at both time-points, the acute and chronic stages displayed differential phenotypic and metabolomic signatures, with elevation in renal dysfunction markers characterizing the acute stage versus renal fibrosis and systemic disruptions specific to the chronic stage. At the acute stage of the injury there was a drastic decrease in anandamide, with levels remaining below normal in the chronic stage. Whereas 2-AG was decreased at the acute stage, it was significantly elevated (up to 30% higher than pre-injury levels) in the chronic stage. Utilizing DESI-MS, we observed the spatial location of these temporal eCB fluctuations amongst the renal architecture. These alterations in eCB 'tone' were coupled with changes in the expression and activity of the eCB synthesizing (DAGLa, DAGLb, NAPE-PLD) and metabolizing (MAGL, FAAH) enzymes. Interestingly, CB1R and CB2R expression was only elevated in the chronic stage.

CONCLUSIONS: The pathogenesis of AKI and subsequent maladaptive repair exhibit distinctive metabolomic and disease phenotypes, with temporal and dynamic changes in the ECS. This study supports further research on the specific roles eCBs play at the different disease states and guides the therapeutic development of ECS-targeting drugs in the AKI-to-CKD continuum.

CANNABINOID RECEPTOR 1 (CB_1R) IN ALVEOLAR MACROPHAGES REGULATES THE DEVELOPMENT OF PROFIBROTIC MACROPHAGES DURING PULMONARY FIBROSIS

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease with a poor prognosis. Among different pathogenic mechanisms, overactivity of the endocannabinoid/cannabinoid receptor 1 (CB_1R) system plays a central role in the development of IPF in humans (*Cinar, JCI Insight 2017 2(8):e92281*). To understand the cell-specific role of CB_1R in the fibrotic lung microenvironment, we generated myeloid cells-specific CB_1R knockout mice (My-*Cnr1*$^{-/-}$) and found My-*Cnr1*$^{-/-}$ mice showed significant inhibition of pulmonary fibrosis (PF) development. Interestingly, CB_1R expression significantly increased only in the alveolar macrophages (AMs) among all myeloid populations of the fibrotic mice lungs. Further elucidating the regulatory role of CB_1R in AMs during fibrosis may offer new approaches to therapeutically mitigate PF development and progression.

Methods: To study the role of CB_1R at the cellular level, we used a murine AMs cell line, MH-S, and primary AMs (pAMs). Mimicking the *in vivo* fibrosis model, both AMs, MH-S and pAM, were exposed to bleomycin (1mU/mL) to characterize the phenotypic and genotypic changes caused by bleomycin. To delineate CB_1R-specific effects, bleomycin-exposed AMs were also pre-treated (30 mins) with rimonabant (1 μM), a CB_1R inverse agonist.

Results: Bleomycin treatment caused higher expression of CB_1R in the AMs and higher levels of anandamide (AEA) in the culture supernatant after 72- and 96-hr treatment. Corroborating with the overactivity of the endocannabinoid system, macrophage polarization towards profibrotic M2 macrophages (CD206) was also noticed. Rimonabant treatment significantly reduced the development of profibrotic macrophages. We also observed a radical increase in the population of M1 (CD80) and M2 macrophages expressing CB_1R after 72- and 96-hr of bleomycin treatment. Rimonabant treatment was also able to reduce the expression of CB_1R in both M1 and M2 AMs. Similar observations with the hybrid macrophage phenotype (dual CD80 and CD206 +ve) further revealed the regulatory role of CB_1R in the AMs during fibrosis induction. The CB_1R specific phenotypic changes in the AMs by rimonabant resulted in significantly reduced expression of profibrotic regulators of fibrosis, viz., *Il6* and *Ccl7*.

Conclusions: AMs showed a phenotypic shift after bleomycin treatment to mimic the *in vivo* fibrosis murine model. CB_1R expressing profibrotic macrophage population was increased due to bleomycin treatment and reduced when treated with the CB_1R inverse agonist rimonabant confirming the dominant role of CB_1R in the AMs during the development of PF.

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ABNORMAL CANNABIDIOL DERIVATIVES POSSESS ANTI-*CANDIDA* PROPERTIES

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Introduction: Abnormal cannabidiol (AbnCBD) was first discovered as a by-product of CBD synthesis. Previous research showed the cardioprotective and anti-inflammatory potentials of AbnCBD. With the recent increase in cases of *Candida* infections and anti-fungal drug resistance, the development of novel treatments is imperative. Therefore, we evaluated the anti-fungal effect of AbnCBDs on *Candida* species.

Methods: AbnCBDs were synthesized by acid catalysis-induced coupling. Log phase *C. albicans* strains and other *Candida species (C. parapsilosis, glabrata, auris)* were incubated with several concentrations of AbnCBDs or Fluconazole (FLC). The susceptibility of *Candida* to treatment was evaluated by spectrophotometry (OD600nm) and by colony-forming units (CFU) counts. The effects of the compounds on the formation and the eradication of *C. albicans* matured biofilm was assessed by culturing log-phase yeast and already formed biofilm respectively with different concentrations of the AbnCBDs. MTT assay was used to assess the viability of the biofilms. The antifungal effect of AbnCBD was assessed in a vulvovaginitis (VVC) mice model by analyzing fungal clearance from vaginal tissue sections (H&E) and CFU in the vaginal lavage. ANOVA and Tukey's Post hoc test were used to determine statistical significance.

Results: Growth of *C. albicans* (SC5314) was significantly inhibited by the AbnCBD and some of the derivatives in a dose-dependent fashion and optimally at a concentration of 16 µg/ml (10.4± 1.1 viability for AbnCBD, 27.3 ± 4.0 for FLC and 99.2 ± 1.5 for 0.5% DMSO, $P<0.05$ vs 0.5% DMSO and FLC). Similar results were observed also with *C. glabrata* (13.1± 0.5 viability for AbnCBD, 27.3 ± 4.0 for FLC and 101.5 ± 8.1 for 0.5% DMSO, $P<0.05$ vs 0.5% DMSO and FLC) and *C. parapsilosis* (42.4 ± 0.9 viability for AbnCBD, 36.5 ± 2.5 for FLC and 100.0 ± 2.6 for 0.5% DMSO, $P<0.05$ vs 0.5% DMSO) but not with *C. auris.* Furthermore, Abn-CBDs inhibited *C. albicans* biofilm formation and disrupted already formed biofilms (16.45 ± 4.8 viability for AbnCBD, 69.6 ± 0.7 for FLC and 103.0 ± 8.1 for 0.5% DMSO, $P<0.05$ vs 0.5% DMSO and FLC), and was effective even in FLC-resistant strains (VC007, VC009, and VC015). In the VVC model, Abn-CBD at 100 mg/kg significantly reduced the fungal burden and exhibited high fungal clearance compared to the vehicle-treated mice.

Conclusions: Abn-CBD derivatives are promising compounds for treating *Candida* infections as detected by their ability to inhibit the proliferation of *Candida*, by preventing its transition to the infectious hyphae stage, by disrupting its biofilm and by the in-vivo antifungal effect in vulvovaginal model. Importantly these Abn-CBD properties are also present in some FLC-resistant strains. We will further evaluate its effects on the systemic *Candida* model in immunocompromised mice.

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DOES CANNABIGEROL CHANGE SPHINGOLIPID DEPOSITION IN THE LIVER OF INSULIN-RESISTANT RATS?

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Introduction: Insulin resistance is an important health problem in Western society, and its occurrence is strongly associated with obesity. The elevated levels of circulating free fatty acids reaching the liver are stored as triacylglycerols. This lipid fraction may be esterified to other more biologically active lipids such as sphingolipids. The main representative of sphingolipids is ceramide (CER) which may be formed from the de novo pathway, hydrolysis of sphingomyelin, and salvage pathway from sphingosine (SFO). On the other hand, ceramide may be catabolized to SFO and later to sphingosine-1-phosphate (S1P). Increased deposition of sphingolipids may interact with various signaling pathways such as the insulin pathway. Thus, regulating its content in the cell is of prime importance. In the present study, we hypothesized that natural compounds like cannabigerol (CBG) may affect the sphingolipid pathway. Our study aimed to evaluate the effect of CBG on sphingolipid accumulation in the liver and plasma of high-fat-high-sucrose-induced insulin-resistant animals.

Methods: The experiments were conducted on male Wistar rats receiving standard rat chow or high-fat-high-sucrose diet (HFHS) for 6 weeks. In each experimental group, half of the animals were obtaining intragastrically CBG (30 mg/kg-1 body weight) or its vehicle for the last 14 days of the experiment. The plasma samples were taken from dormant animals. Moreover, the liver was isolated and snap frozen in liquid nitrogen and stored at−80°C. The Western Blot technique was used to determine the expression of enzymes from the sphingolipid metabolism pathway. Sphingolipids content was assessed by high-performance liquid chromatography. Data were analyzed by two-way ANOVA followed by an appropriate post-hoc test ($P < 0.05$ considered significant).

Results: In our study, we indicated a considerable increase in sphinganine (SFA) and S1P concentrations in the liver after CBG as well as CBG and HFHS treatment compared to the control and HFHS groups respectively. Furthermore, we observed similar results in the SFA content in plasma samples. However, the content of S1P in plasma was decreased in the group treated with HFHS and CBG. A similar decrease was observed in the liver and plasma content of sphinganine-1-phosphate in the HFHS+CBG group. Liver CER content was not affected by CBG treatment in the HFHS group but was increased in the plasma. The expression of enzymes from the ceramide de novo synthesis pathway was not affected by CBG treatment, however, the expression of the enzymes responsible for ceramide catabolism was increased in the HFHS+CBG group.

Conclusions: Our data clearly showed that the main sphingolipid metabolism pathway facilitated by CBG under the high availability of fatty acids and sucrose was the ceramide catabolism pathway. As a result, in the liver increased deposition of S1P was observed, without any significant changes in the ceramide accumulation. We suspect that synthesized ceramide undergoes two routes: the first is catabolism into S1P and the second is transporting into plasma where an increased concentration of CER was observed. We may conclude that CBG caused the redirection of sphingolipid metabolism into catabolism which is the livers' protective mechanism against excessive deposition of other more biologically active lipids.

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HEAVY ALCOHOL DRINKING INDUCED REGULATION OF ENDOCANNABINOID/CB_1R SYSTEM IN LUNGS OF MOUSE

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Introduction: Heavy alcohol drinking induces oxidative stress, tissue remodeling, and alters the function of immune cells in the lungs. Accordingly, chronic alcohol drinking makes the lungs susceptible to inflammatory conditions. Overactivation of cannabinoid receptor 1 (CB_1R) was shown to promote lung inflammation and fibrosis. Furthermore, decline in pulmonary function was negatively correlated with increased level of anandamide (AEA) in bronchoalveolar lavage fluid (BALF) in pulmonary fibrosis (PF) patients as well as animal model of PF (*Cinar et al., JCI Insight 2017 2(8):92281*). Although proinflammatory and profibrotic role of CB_1R in lungs described in different inflammatory and fibrotic lung diseases, up to date, regulation of endocannabinoid/ CB_1R in lungs by chronic alcohol drinking has not been explored.

Method: To explore effect of heavy alcohol drinking on endocannabinoid/CB_1R system, we employed the NIAAA alcohol diet model (*Bertola et al., Nature Protocols 2013 8(3) 627-637*), which is known to cause significant liver injury and alcoholic steatohepatitis as well as lung inflammation (*Appolonia et al, Front Physiol. 2022 13:860449*) in mice with 10 days of a Lieber-DeCarli liquid diet containing 5% ethanol followed by a single ethanol binge (5 g/kg).

Results: We found that chronic ethanol and single binge increased gene expression of *Cnr1*, encoding CB_1R. Additionally, endocannabinoid AEA but not 2-AG significantly increased in both lungs and BALF in mice after ethanol drinking. We also checked gene expression of anabolic and catabolic enzymes of endocannabinoids to understand regulatory role of alcohol drinking on elevated AEA level in lungs. Gene expression of AEA synthetic enzyme *Napepld* was unchanged, whereas expression of AEA degrading enzyme *Faah* decreased about 60% in lungs by ethanol ingestion. On the other hand, gene expressions both 2-AG synthetic and degrading enzymes *Daglb1* and *Mgll* were decreased similarly about half by ethanol compared to the control group.

Conclusion: In conclusion, chronic alcohol and binge drinking activates endocannabinoid/CB_1R system in lung microenvironment. Alcohol drinking may decrease activity of FAAH enzyme that could be responsible for the AEA increase in lungs and BALF. We will explore potential pro-inflammatory role of endocannabinoid/CB_1R system in alcohol-induced lung inflammation and injury in future studies.

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THE CANNABINOID RECEPTOR 2 ANTAGONIST, SR144528 BLOCKS LPS/IFN-γ-INDUCED MICROGLIAL ACTIVATION IN A CB2 INDEPENDENT MANNER

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Introduction: Cannabinoid receptor 2 (CB2) modulates microglial response to inflammatory stimuli. Our previous studies demonstrated that genetic deletion of CB2 inhibits microglia activation during inflammatory stimulation with toll-like receptor (TLR) ligands (Schmöle et al., 2022). However, we cannot exclude the developmental effects of CB2 constitutive knockout ($CB2^{-/-}$), which could have triggered a compensatory outcome in $CB2^{-/-}$ mice. Therefore, in the present study, we aimed to test whether acute pharmacological inhibition of the CB2 receptor has a similar effect on microglial activation as in $CB2^{-/-}$ in response to inflammatory stimulation.

Methods: We pretreated primary microglia and organotypic hippocampal slices (OHSCs) with the CB2-specific antagonist SR144528 at 1μM concentration or lower for 15 minutes, followed by stimulation with LPS/IFN-γ for 16 hours. The secretion of pro-inflammatory cytokines, Iba1 intensity, CD68 levels, and microglial morphology were evaluated using ELISA, immunohistochemistry, and 3D reconstruction analysis.

Results: Our data showed that SR144528 suppressed LPS/IFN-γ-stimulated cytokine secretion at 1 μM but not lower concentrations (1 nM-100 nM). Surprisingly, this anti-inflammatory effect of 1 μM SR144528 was not dependent on CB2 receptors, as this effect was also observed in $CB2^{-/-}$ microglia after TLR4 stimulation. We further showed that 1 μM of SR144528 significantly reduces activated microglial morphology, Iba1, and CD68 staining intensities independent of the CB2 receptor and exceeded the Ki on CB2 receptors by more than a thousand-fold.

Conclusions: We present that the cannabinoid antagonist, SR144528 at 1 μM, does not act through CB2 receptor to modulate TLR4-induced microglial activation. Thus, pharmacological inhibition of CB2 receptors with the lower SR144528 concentrations (1nM-100nM) does not mimic the anti-inflammatory effects observed in the $CB2^{-/-}$ microglia after TLR4 stimulation. Therefore, we propose that the permanent lack of CB2 in mice ($CB2^{-/-}$) probably triggered a compensatory mechanism that makes them less responsive to TLR4 stimulation, hence the different outcomes.

NOVEL INSIGHTS INTO CANNABIGEROL ACTION: DOES IT AFFECT SPHINGOLIPID METABOLISM IN THE SKELETAL MUSCLES OF INSULIN-RESISTANT RATS?

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Introduction: Obesity is considered to be one of the most serious health concerns and is associated with a significant susceptibility to many comorbidities, such as insulin resistance (IR). A diet high in fatty acids (FAs) and sugar leads to excessive lipids deposition in adipocytes and subsequently in insulin-sensitive tissues, including the skeletal muscle. The oversupplied FAs are stored primarily in triacylglycerols (TAG) as well as in other biologically active fractions such as sphingolipids. Of these, ceramide (CER) is a central metabolite and plays a pivotal role in a variety of cellular responses, such as the insulin signaling pathway. In addition, numerous studies demonstrated that a class of sphingolipids regulates processes related to cell growth and apoptosis. Hence, it seems reasonable to control the content of sphingolipids in tissues, and scientists are still looking for compounds that can regulate their metabolism. Over the last several years, attention was paid to phytocannabinoids due to their beneficial properties and increasing evidence supports the therapeutic potential of cannabis-derived cannabigerol (CBG). Therefore, the present study aimed to evaluate the effect of CBG on sphingolipid deposition and metabolism in the skeletal muscle of rats with insulin resistance induced by a high-fat-high-sucrose (HFHS) diet.

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 (white and red *gastrocnemius muscle)* were collected and immediately frozen in liquid nitrogen and stored at −80°C. Sphingolipid content was evaluated using high-performance liquid chromatography (HPLC). The expression of proteins involved in the sphingolipid pathway was determined by the Western blot technique. Data were analyzed by one-way ANOVA followed by an appropriate post-hoc test ($p < 0.05$ considered significant).

Results: The present study demonstrated a significant increase in sphinganine (SFA) content in the HFHS group in the red gastrocnemius muscle, which was further reduced by two-week CBG treatment. Subsequently, in HFHS and HFHS+CBG groups we observed markedly elevated CER and sphingosine (SFO) concentrations compared to the control group in the same muscle type. Furthermore, the content of sphingosine-1-phosphate (S1P) was substantially increased after CBG treatment of HFHS-fed animals in both white and red skeletal muscles. Additionally, the muscular expression of enzymes involved in the ceramide de novo synthesis and its catabolism pathway reflected changes in the concentrations of the above-mentioned bioactive compounds.

Conclusions: Our data clearly indicated that CBG under the conditions of an increased supply of FAs and sucrose in the diet has a regulatory effect on the sphingolipid metabolism pathway. CBG inhibits the de novo CER synthesis pathway and promotes its catabolism into S1P. Moreover, we indicated pronounced changes in the content of sphingolipids in skeletal muscles depending on their metabolism. This indicates that CBG due to the wide spectrum of biological activity seems to be a promising compound for use in treating obesity and related disturbances.

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THE INTOXICATION EQUIVALENCY OF 11-HYDROXY-Δ^9-TETRAHYDROCANNABINOL (11-OH-THC) TO Δ^9-TETRAHYDROCANNABINOL (THC)

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Introduction: Δ^9-Tetrahydrocannabinol (THC) is a psychoactive phytocannabinoid found in the *Cannabis sativa* plant. THC is primarily metabolized into two different compounds, 11-OH-THC and COOH-THC, that have some affinity for cannabinoid receptors CB1 and CB2. However, there is very little research-based evidence for the pharmacokinetics (PK) of 11-OH-THC as an individual compound or its pharmacodynamic effects.

Methods: To fill this knowledge gap, we treated male C57BL/6 mice with 10 mg/kg 11-OH-THC via three routes of administration (*i.p., i.v., p.o..)* and quantified whole blood compound levels to determine the T_{max}, C_{max}, and monitored behaviour in the tetrad battery of responses as compared to THC. In addition, mice were treated with either THC or 11-OH-THC *i.v.* (0.3, 1, 3, 10 mg/kg) and assessed in the tetrad battery.

Results: For the PK profile, we found that 11-OH-THC had a 30 min T_{max} for *i.p. and p.o.* administration and no difference in C_{max} between *i.v.* and *i.p.* (1,153 ± 74; 978 ± 105 ng/mL, respectively), whereas the *p.o.* C_{max} was only 104 ± 48 ng/mL. THC had a 10 min T_{max} for i.*p.* and 60 min for *p.o.* with a significant reduction in C_{max} for *i.p.* (377 ± 208 ng/mL) and *p.o.* (150 ± 113 ng/mL). When accounting for T_{max} blood concentrations, catalepsy was greater in male mice injected with 10 mg/kg *i.v.* 11-OH-THC compared to THC (80% *versus* 60% MPE). Catalepsy was also observed in *i.p.*-treated 11-OH-THC mice compared to vehicle. Anti-nociception was greater in the tail-flick assay in 11-OH-THC *i.v.*- and *i.p.*-injected mice compared to THC but not mice receiving 11-OH-THC by oral gavage. Decreased body temperature was also greater in 11-OH-THC *i.v.*- and *i.p.*-injected groups , compared to THC *i.v.*-injected mice.

Conclusions: Our findings demonstrate that the THC metabolite, 11-OH-THC, was able to produce *in vivo* tetrad effects to a greater degree than the parent drug, THC, even when accounting for potential PK differences.

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INVESTIGATING THE EFFECTS OF SEX AND CB2R ON NEUTROPHIL FUNCTION IN MICE WITH SYSTEMIC *CANDIDA ALBICANS* INFECTIONS

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Candida albicans, an opportunistic pathogen found in our normal flora, is the fourth most common nosocomial infection in the United States. ICUs across the U.S report *C. albicans* responsible for 10% of blood infections encountered. Incidentally, individuals seeking symptomatic relief resort to the use of marijuana and its compounds such as THC and CBD. These marijuana compounds interact with the individuals' endocannabinoid system (ECS). The ECS includes cannabinoid receptors (CBRs). The best known CBRs are the central and peripheral cannabinoid receptors, CB1R and CB2R, respectively. CB1R is predominantly expressed in the CNS while CB2R is mostly expressed in cells of the immune system. Thus, it is important to understand the role of CB2R and how it may affect fungal resistance. Another factor affecting susceptibility to fungal infections is sex. It is known that males tend to be more susceptible to fungal infections compared to females.

To investigate the role of sex and CB2R in the resistance to *C. albicans* infection, CB2R knockout (CB2R-/-) and CB2R +/+ male and female mice, were intravenously injected with 5 uL/g of $7.5x10^6$ *C. albicans* cells/mL. Kidneys, blood, spleen, and bone marrow (BM) were collected 3 days after the infection. Kidneys were analyzed for fungal load and cytokine (IL-6 and TNF-α) levels were assessed from kidneys and blood serum. The spleens and bone marrow were pooled by sex and genotype (F +/+, F-/-, M+/+, M-/-). Splenocyte cytokine production was determined by Enzyme Linked Immunosorbent Assays (ELISA). Neutrophil production was determined from bone marrow cells using flow cytometry. IL-6, and IL-1β gene expression was determined using RT-qPCR.

There was no difference in kidney fungal load between the sexes nor genotypes investigated. Serum IL-6 was absent in uninfected mice, but present in all infected mice. IL-6 levels were similar between sexes and genotypes. Male **kidneys had higher IL-6 levels** compared to the other groups, but all groups had similar TNF-α levels. The percentage of BM neutrophils was higher in CB2R+/+ infected mice when compared to the uninfected CB2R+/+ mice. However, there was no differences in BM neutrophils levels CB2R-/- infected and uninfected mice regardless of sex. There were no differences between infected male and female BM neutrophil levels. Uninfected male CB2R-/- had higher BM neutrophil levels compared to uninfected CB2R+/+ males and females but was not different from CB2R-/- uninfected female mice. It has been previously established that IL-6 plays a role in BM neutrophil mobilization via IRF-4 mediated CX3CR1 expression.

Taken together, the lack of change in BM neutrophil production in the CB2R -/- groups suggests that CB2R may play a role in neutrophil recruitment. We hypothesize that IL-6 and CB2R play a role in expression of neutrophil chemo-attractants. While possible mechanisms are still being determined, a link between soluble IL-6 and CX_3CR1 expression may be at play.

TARGETING BIOACTIVE LIPIDS IN GASTROINTESTINAL CANCER: A POSSIBLE CROSS-TALK BETWEEN PRO-RESOLVING LIPID MEDIATORS AND ENDOCANNABINOID SYSTEM

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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. Gastrointestinal (GI) tract, massively exposed to the external stimuli, necessarily depends, more than other tissue districts, on these specific lipid pathways important in pro-resolving and homeostatic activities to tolerate insults and to pursue their balance. GI cancers exhibit a chronic low-grade inflammatory state and are still in need of better prognostic measures due to their poor patient survival statistics. Recently, SPMs has been described as potent anti-angiogenic mediators in GI tract cancers [1]. The eCB system has also been postulated as a modulator of tumour angiogenesis, being implicated in anti-angiogenic action by decreasing the survival and migration of endothelial cells and/or reducing the expression of proangiogenic factors in several types of carcinomas (particularly lung, breast, skin, and brain) [2]. eCB levels are 3- and 2-fold higher in adenomas and colorectal cancer (CRC) than normal mucosa, and endogenous eCBs – anandamide (AEA) and 2-arachidonoylglycerol (2-AG) potently inhibit CRC cell proliferation [3]. The anti-angiogenic potential of eCBs in GI cancer and the underlying molecular mechanisms with respect to SPM system have not been investigated yet. We hypothesize that, in the GI tract, these bioactive lipids can synergically operate exerting a strong anti-cancer potential on shared molecular pathways: cannabinoid signalling might determine an anti-cancer response by sustaining a pro-resolving program and vice versa. We aimed at examining eCB/SPM receptor involvement focusing on the synergistic operation of their molecular pathways against tumour growth and spreading.

Methods: Quantitative determination of eCB and SPM receptors (i.e. CB1, CB2, TRPs, GPR55, GPR18 and FPR2) in GC (namely, AGS and MKN45) and CRC (namely, HCT116 and HT-29) cell lines was performed by qPCR, WB and FACS analysis. The effect of SPMs [Resolvin D1 (RvD1) and Lipoxin B4 (LxB4) (100 pM – 10 nM)] and eCB [ACEA and JWH133 (100nM - 10μM)] on cell viability was tested by SRB assay. Targeted LC-MS lipidomic analysis and EIA assays were utilised to detect the SPM-mediated production of eCBs and the eCB-mediated production of SPMs, respectively. ELISA and qPCR assays were performed to study the effect on angiogenic factors.

Results: All GC and CRC cell lines under study showed basal expression of eCB (CB1 and CB2) and SPM (GPR32, GPR18, GPR55, FPR2) receptors. Treatment with one class of lipids modulated the receptor expression of the other class of lipids. CRC cells showed a marked increase in GPR32 mRNA expression upon dose-dependent eCB treatment (5-fold and 3-fold in HT29 with AEA and JWH-133, respectively; 1.5-fold and 8-fold in HCT116 with AEA and JWH-133, respectively), which was confirmed by increased GPR32 protein expression (WB). Similar experiments are ongoing in GC cells and with SPM treatments. With respect to cell proliferation, among the entire cell panel under study, only AGS cells exhibited small changes in cell viability upon eCB or SPM treatments indicating that these bioactive lipids must function via a different pathway to bring about GI tumour amelioration. ACEA (0.1 and 1□M, 24h) or JWH-133 (1 and 10□M, 24h) led to 2-fold higher expression of enzymes responsible for endogenous SPMs synthesis (ALOX5, ALOX15) and significantly increase the release of the two SPMs RvD1 and LxB4, in GC (MKN45) and CRC (HT-29) models. The eCB-mediated production of SPMs, was dependent on CBR-dependent mitogen-activated protein kinase MAPK activation, as demonstrated by WB analysis and a specific inhibitor. Similar eCB treatments significantly lowered the mRNA expression of several angiogenic factors (CXCL1, VEGFB and VEGFC) in both MKN45 and HCT116 cells. Preliminary data indicate that in CRC models the pro-resolving pathway sustained by the known SPMs, RvD1 and LxB4, is crucial in restraining cancer progression by inhibiting the angiogenic response via pharmacologic inhibition or genetic deletion of formyl peptide receptor 1 (FPR1), with activation of FPR1 mediating opposite effects. We are now determining whether eCB treatment regimen can reproduce similar actions and how FPR1 ligands can modulate CBR activation.

Conclusion: The study indicate a clear induction of molecular biosynthetic pathway of SPMs upon CBR activation, thus facilitating marked reduction in angiogenic and inflammatory markers in such GI cell lines. Experiments are ongoing to investigate whether exogenous SPMs could resolve inflammation and reduce angiogenesis by raising the endogenous eCB production and how the two lipid classes (and their receptors) are interconnected.

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SUPPLEMENTS BASED ON CANNABIMIMETIC PLANT EXTRACTS OR OMEGA-3 FATTY ACIDS AMELIORATE INTESTINAL AND BEHAVIOURAL PARAMETERS IN A DSS-INDUCED MOUSE MODEL OF COLITIS IN CONJUNCTION WITH ALTERATIONS OF THE GUT MICROBIOME

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Introduction: Inflammatory bowel diseases (IBDs) are associated with alterations of various members of the endocannabinoidome (eCBome) and gut microbiome, both of which hold therapeutic potential for the treatment of inflammation and affective disorders associated with IBDs, such as ulcerative colitis. The endocannabinoidome and the gut microbiome are strongly influenced by diet and thus may be targeted with nutritional supplements.

Methods: We assessed the effects of: 1) a nutritional supplement comprised of a proprietary blend of cannabimimetic plant extracts, or 2) omega 3 fatty acid-rich fish oil or seal oil, on the development of inflammation, intestinal permeability, anxiety, the gut microbiome and intestinal and brain eCBome in a DSS-induced mouse model of colitis. Mice were pretreated with supplements for 2 weeks prior to induction of a 5-day DSS-treatment protocol; behavioural assessments were performed 7 and 8 days after initiation of DSS and mice were sacrificed and samples harvested on day 9.

Results: While all treatments rendered DSS-induced weight loss in mice insignificant, none affected colon length:weight ratio. However, the cannabimimetic formulation and seal oil both significantly reduced DSS-induced increases in intestinal permeability. Furthermore, all treatments reduced anxiety-like symptoms in DSS-treated mice as measured by the open field test, while no effects could be detected in the elevated plus maze. DSS induced noticeable alterations in the overall gut microbiome community (beta diversity) of mice. While none of the treatments significantly affected this parameter, alterations within specific taxa were observed. All treatments, but particularly the administration of oils, resulted in the inhibition of the increase in abundance of the bacterial family *Eryspelatoclostridiaceae* and decrease in the abundance of the genus *Family XIII UCG001*, alterations of which have been associated with colitis. Detailed analysis of markers of intestinal and neural inflammation, intestinal permeability and effects on the eCBome are ongoing.

Conclusions: Targeting the eCBome with a proprietary blend of cannabimimetic plant extracts or omega 3 fatty acid-rich seal oil resulted in significant improvement of increased intestinal permeability and anxiety-like behaviour in mice with DSS-induced colitis in association with changes in specific bacterial taxa. Whether or not these changes are due to the capability of these treatments to alter the eCBome and inflammation is currently under investigation.

CANNABIGEROL AS A NEW POTENTIAL STRATEGY FOR THE TREATMENT OF FIBROSIS IN PRIMARY RAT HEPATOCYTES CULTURED IN PALMITATE AND FRUCTOSE MEDIA

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Introduction: Hepatic fibrosis can be defined as partially reversible excessive scar tissue formation that is a consequence of persistent liver injuries. There is overproduction and accumulation of extracellular matrix (ECM) proteins with simultaneous disruption of matrix turnover processes that are under the regulation of matrix metalloproteinase (MMP) and their tissue inhibitor (TIMP). However, transforming growth factor beta 1 (TGF-β1) plays an overriding role in fibrogenesis. So, it is important to search for a promising agent with anti-inflammatory properties that alleviate the development of hepatic fibrosis. The latest data indicate that cannabigerol (CBG; *Cannabis sativa* derivative) may have a useful influence on lipid metabolism-related processes in the liver tissue with a lack of psychoactive effect. The effect of CBG on fibrosis is still unclear. Thus, we assessed the impact of cannabigerol on ECM synthesis and degradation in primary rat hepatocytes in a dosage-dependent manner.

Methods: Primary hepatocytes were isolated from rat's liver using the two-step perfusion technique (with collagenase and EDTA), and then were cultured in standard media (Control group) or cultured with the solution of 0.4 mM palmitic acid and 10 mM fructose (PA-F group) for 48h. Half of hepatocytes from the Control and PA-F groups were incubated with 1 μM, 5 μM or 10 μM cannabigerol solution for 48h. After all incubations, cells were washed with PBS, collected and homogenized by ultrasonification in ice-cold RIPA buffer with protease and phosphatase inhibitors. In cell lysates the expression of selected ECM proteins, i.e., collagen (COL-1α1, COL-3α1), MMP-2, MMP-9, TIMP-1, TIMP-2, TGF-β1, were determined by the Western blot method. The data were analyzed by two-way ANOVA followed by a proper post-hoc test (significant difference was set as $p<0.05$).

Results: In the PA-F group we observed an impairment in the intracellular TGF-β1 expression with simultaneous an increase in the expression of COL-1α1, and COL-3α1 as a confirmation of fibrosis development. In the same group, TIMP-1 expression was decreased. In palmitate and fructose conditions, treatment with 1 μM CBG induced a decrease in COL-1α1, COL-3α1, TIMP-2, and TGF-β1 expression (*vs.* PA-F group). Exposition to 5 μM CBG with PA and F reduced the expression of COL-1α1, COL-3α1, and MMP-9 with concomitantly enlarged MMP-2 and TIMP-2 expression (*vs.* PA-F group). Moreover, the incubation of hepatocytes with 10 μM CBG, PA and F caused a diminishment in the expression of COL-1α1, COL-3α1, MMP-9, and TIMP-2 and also an increment in MMP-2 and TIMP-1 expression (*vs.* PA-F group).

Conclusions: The present data suggest that cannabigerol may affect the expression of ECM components. The fibrotic changes in primary hepatocytes were attenuated by the treatment with cannabigerol. We can assume the hypothesis that cannabigerol at a 1 μM concentration is the most effective in reducing fibrosis development in primary hepatocytes exposed to palmitate and fructose media by a significantly decreased level of factor TGF-β1.

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WITHDRAWN

ACUTE SUBJECTIVE EFFECTS OF TETRAHYDROCANNABINOL (THC): A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: With an increasing number of legal markets and greater acceptance in healthcare, there may be a further increase in cannabis use. To provide guidance to users and encourage safe use, there has been a recent proposal to develop a standard cannabis unit like the standard units used for alcohol. This approach recommends a standard 5mg delta-9-tetrahydrocannabinol (THC) unit, based on previous ecological and experimental evidence. However, it is currently unknown what effects are experienced when such a dose is administered, particularly across routes of administration. We aimed to synthesise the available evidence to outline the subjective effects of THC across routes of administration and their relationship with dose.

Methods: MEDLINE, EMBASE and PsycINFO were searched until 9th February 2022. Studies were considered for inclusion if they were randomised, double-blind and placebo-controlled studies which reported acute subjective effects of THC in healthy infrequent cannabis users. Pairwise meta-analysis was conducted stratified by route of administration. The meta-analytical effect-size measure was mean difference (MD). If significant heterogeneity was present with greater than five studies contributing data, meta-regressions of dose were performed. As THC doses are not comparable across different routes of administration, meta-regressions were stratified by route of administration.

Results: 1992 articles were screened with 37 studies (47 samples) meeting eligibility criteria. Subjective effect outcomes extracted were for high, anxiety, alertness, tiredness, calm and contentedness.

Preliminary results show that THC significantly increased high ($k=47$, $MD=39.4$, $p<0.0001$), anxiety ($k=17$, $MD=15.67$, $p<0.0001$), tiredness ($k=21$, $MD=27.27$, $p<0.0001$), while significantly reducing alertness ($k=13$, $MD=-18.04$, $p<0.0001$) and contentedness ($k=5$, $MD=-12.29$, $p<0.0001$) compared to placebo. Inhaled dose showed a significant positive association with high ($b=1.05$, $p<0.001$) and a negative association with calmness ($b=0.002$, $p=0.03$). No significant effects were seen on any other outcomes ($p>0.05$). From these preliminary results, inhaling 1 THC unit (5mg) would result in the following effects on a 100-point scale: high:30.9, anxiety:20.4, alertness:12.07, calmness: -0.65, tiredness:23.63, contentedness:0.92.

Discussion: Using the available evidence, we can estimate the subjective effect profile of a standard THC unit. These results will be able to provide guidance for people who use cannabis and health policy surrounding medicinal and recreational cannabis.

THE EFFECT OF CHRONIC ORAL ADMINISTRATION OF THC AND CBD ON OBESITY AND RELATED COMORBIDITIES

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Introduction: Obesity has become a serious health care concern reaching pandemic proportions and contributes to 4 million deaths per year globally. It is well established that the endocannabinoid system (ECS) is involved in both the regulation of energy balance and the development of obesity, making it a compelling target for obesity treatment. Paradoxically, prevalence of obesity and metabolic diseases are lower in cannabis users compared to none-users. Indeed, several studies have shown the anti-obesity properties of CBD and THC, the major constituents of the plant *cannabis sativa,* however limited studies have examined chronic *per-os* administration. Therefore, our objective was to investigate the effect of chronically administered *per-os* THC and CBD, on obesity-associated metabolic disorders and modulation of the peripheral endocannabinoid tone.

Methods: C57BL/6 male mice were fed an HFD or chow diet for 14-weeks, then received 10 mg/kg *per-os* treatment of THC or CBD for 5 weeks followed by an increased dose of 30 mg/kg for an additional 5 weeks. We investigated the effect on cumulative food intake, weight gain, glucose-tolerance, adipocyte hypertrophy, inflammatory markers in white adipose tissue (WAT), liver triglycerides, and endocannabinoid levels in epididymal fat and liver.

Results: Mice treated with purified THC showed a biphasic effect in weight gain and glucose-tolerance at the different dose-concentrations. THC administered at 10 mg/kg level resulted in weight gain and impaired glucose-tolerance while treatment with 30 mg/kg led to a significant reduction in weight gain and markedly improved glucose-tolerance, followed by distinct improvement in steatosis markers, however no improvement was shown in WAT inflammation markers. Treatment with CBD dose-dependently improved glucose-tolerance regardless of weight gain and elevated steatosis markers. Improved metabolic parameters in both treatment groups were associated with down-regulation of the endocannabinoid 2-arachidonoylglycerol (2-AG) in the liver.

Conclusions: Chronic oral consumption of sufficient concentrations of THC, but not of CBD, ameliorates diet-induced obesity and metabolic parameters directly affecting associated metabolic disorders, possibly through, but not exclusively, via downregulation of the endocannabinoid 2-AG in the liver, the endogenous full agonist for the CB1 and CB2 receptors.

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THE *IN VIVO* INTOXICATION EQUIVALENCY OF CANNABIGEROL AND Δ^9-TETRAHYDROCANNABIPHOROL COMPARED TO Δ^9-TETRAHYDROCANNABINOL

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Introduction: Since the introduction of the Cannabis Act in 2018, overall cannabis use has increased among the general Canadian population, extending a trend that existed before legalization. However, cannabis use among youth during this time has not increased. Cannabis holds therapeutic potential via it's agonism at cannabinoid receptor type 1 (CB1R) and type 2 (CB2R); however, activation of CB1R via Δ^9-tetrahydrocannabinol (THC) is also responsible for the characteristic "high" induced by cannabis. The plant-derived (phyto) cannabinoid, THC has a well-established pharmacology and is known to be a CB1R partial agonist. The pharmacology of the less-abundant phytocannabinoids – such as cannabigerol (CBG) and Δ^9-tetrahydrocannabiphorol (THCP) – are less established.

Methods: Using THC as a reference compound, we have worked to determine the acute intoxication equivalency index of CBG in C56BL/6 mice, with THCP assays ongoing. THC, CBG, and THCP are being assessed for their pharmacokinetic profiles, as well as cataleptic, hypothermic, and anti-nociceptive effects following oral *(p.o.),* intraperitoneal *(i.p.),* and intravenous *(i.v.)* administration in mice.

Results: Our data thus far reveal *in vivo* responses associated with CB1R activation following THC administration, but not CBG. We expect further experiments will show that CBG is not intoxicating, even when compared at the same routes of administration and plasma concentrations, whereas THCP will be intoxicating.

Conclusion: These experiments will provide valuable data on how phytocannabinoid pharmacology differs from THC and whether this difference is of medicinal importance or significant in the context of harms reduction.

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INVESTIGATING THE EFFECTS OF ACUTE THC VAPOUR EXPOSURE ON AUDITORY FEAR CONDITIONING AND FEAR EXTINCTION

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Introduction: In the clinical setting, management of post-traumatic stress disorder (PTSD) symptomology in patients is often listed as a primary motivation behind cannabis use, yet the understanding of how acute exposure to cannabis modulates the neurobehavioural response to negatively valenced stimuli, like fear, is not well characterized. Preclinical research has demonstrated mixed effects of tetrahydrocannabinol (THC), much of which is suggestive of dose-dependent effects; however, the predominance of this work has employed an injection method to administer THC. 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. With the recent development of rodent vapour-based delivery systems for cannabis extracts, we can now establish the impact of acute, inhaled cannabis extracts on the processing of fear in a highly translational manner. The current study aims are twofold: to examine the impact of acute, controlled, passive delivery of THC vapour on 1) the acquisition and 2) the extinction of fear memories during a fear conditioning paradigm.

Method: Male and 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 underwent auditory fear conditioning, extinction training, and extinction retrieval. Experiments were separated into two distinct aims to investigate the effects of THC on both fear conditioning and fear extinction. For study 1 (THC effects on fear conditioning acquisition), thirty-two rats were exposed to THC or vehicle ***prior*** to auditory fear conditioning. The next day rats underwent extinction, followed by two extinction retrieval tests twenty-four hours and seven days later. For study 2 (THC effects on fear extinction), fifty rats underwent auditory fear conditioning the day ***before*** THC or vehicle vapour exposure. The following day rats were exposed to either THC or vehicle vapour and subsequently underwent extinction and an extinction retrieval test twenty-four hours later. In both studies, passive (freezing) and active (darting) conditioned responses were quantified.

Results: For study 1, exposure to THC had no impact on the acquisition of conditioned fear but did result in THC-exposed female rats performing significantly better than vehicle-exposed females during extinction training. Male and female THC-exposed rats performed significantly better during a seven-day extinction retrieval test, showing less freezing than vehicle-exposed male and female rats. For study 2, exposure to THC prior to extinction training produced diverging sex differences. While THC prior to extinction training did not alter fear extinction in males, it did, however, impair fear extinction in females.

Conclusion: This research suggests that THC prior to fear memory acquisition is sufficient to produce long-term changes in fear memory recovery in both males and females, as shown by a two-week extinction retrieval task. These findings also provide evidence to support that acute exposure to THC vapour has sex-dependent effects on the extinction of fear memories.

THE ENDOCANNABINIOD SYSTEM AS AN INTEGRATOR OF COLD AND DIET INFLUENCES ON BIOENERGETICS

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Introduction: Northern populations are exposed to large thermal fluctuations and this extreme environmental condition affects metabolic health. The endocannabinoidome plays a significant role centrally and peripherally in energy balance regulation, and dysregulation of this system is linked to various pathological conditions such as obesity, as well as metabolic syndrome. This study aimed to investigate the regulation of endocannabinoidome in epididymal white adipose tissue (eWAT), gastrocnemius muscle (GAS), and liver following exposure to cold as well as a high-fat diet.

Methods: C3H/HeJ mice were fed either a high-fat diet or a low-fat diet for 8 and 4 weeks, respectively at room temperature. Following that, mice were divided into two groups; half being maintained at 30˚C and half moved to 10˚C for a further 4 weeks. After scarifying, tissues were collected and mRNA profiling of endocannabinoidome synthesis, degradation, and receptor genes in eWAT and GAS muscle was studied by quantitative PCR. The endocannabinoid system mediators and related bioactive lipids including N-acylethanolamines, 2-monoacylglycerols, and fatty acid oxygenated metabolites in the liver and GAS muscle, were quantified using liquid chromatography–mass spectrometry technique. Data analysis was performed using two-way ANOVA followed by Tukey post-hoc tests. Statistically significant differences between the groups were identified for P-values less than 0.05.

Results: The results indicated that cold exposure upregulated the gene expression of endocannabinoidome biosynthetic enzymes in eWAT with Gde1 showing the highest changes; while, it hardly affected the muscle tissue. A high-fat diet showed the strongest effects on Abhd12, Daglb, Gdpd1, Inpp5d, Pla1a, and Ptpn22 genes in eWAT, and Fam213b gene in GAS muscle tissue. While cold exposure only increased the concentration of a few endocannabinoids in GAS muscle tissue, the concentrations of lipid mediators such as PEA, LEA, OEA, SDA, EPA, and DHA were remarkably elevated in the liver of cold-exposed mice fed a low-fat diet but not a high-fat diet.

Conclusions: Adipose tissue as well as the liver were significantly more affected by diet and exposure to cold compared to GAS muscle. Cold exposure and a high-fat diet regulate endocannabinoidome tone in a tissue-specific manner.

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ROLE OF THE GUT-BRAIN ENDOCANNABINOID SYSTEM IN FOOD REWARD

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Introduction: The obesity epidemic is largely a result of overeating tasty, high-fat and high-sugar diets combined with a sedentary lifestyle. Dysregulation of several neural and molecular pathways has been identified that contribute to obesity and overeating, including the gut-brain endocannabinoid (eCB) system. The eCB system plays a major role in food intake, energy homeostasis, and reward. It is largely unknown, however, if gut-brain eCB signaling controls brain reward mechanisms, and elucidating roles for this system in these processes is the focus of this project. Our lab reported that rodents, when given a choice, display a strong preference for a Western-style diet (WD, high fat and sucrose) versus standard rodent chow (SD, low fat and no sucrose). Importantly, this preference was attenuated in mice treated with a global antagonist for cannabinoid subtype-1 receptors (CB_1Rs) and in mice with conditional deletion of CB_1Rs specifically in the gut lining (IntCB1-/-) (Avalos, et al. 2020), which suggests that eCB signaling in the intestinal epithelium controls dietary preferences. It is unclear, however, if CB_1Rs in the intestinal epithelium mediate gut-brain neurotransmission that controls dopaminergic signaling associated with palatable food preference.

Methods: In this study, we began to explore roles for CB_1R in the gut in these processes by examining dopaminergic signaling in the nucleus accumbens during an acute dietary preference test for WD or SD. The genetically encoded fluorescent Grab DA dopamine sensor (AAV-hSyn-GRAB_DA2m) was injected into the nucleus accumbens via stereotaxic surgery along with a unilateral optical fiber implant for recording dopamine activity via fiber photometry. On the day of testing, mice were administered the peripherally-restricted CB_1R neutral antagonist, AM6545 (10 mg per kg), and *in vivo* dopamine signaling was recorded as the mice underwent the preference test for two hours in an open field. GrabDA signals were processed using a custom-written MATLAB script adapted from Barker et al. (2017) and the TDT MATLAB Offline Analysis Tools.

Results: Mice treated with AM6545 had a significant reduction in WD preference and caloric intake compared to the vehicle-treated mice. Both the vehicle- and AM6545-treated mice displayed a brief spike in dopamine release in the nucleus accumbens when approaching the WD. This DA release was maintained as the vehicle-treated mice consumed the WD, while DA release was attenuated in the AM6545-treated mice. Additionally, mice treated with AM6545 displayed a lower number of peaks during WD consumption, but not during SD consumption. AM6545 had no significant effect on the maximum amplitude.

Conclusions: These studies suggest that preferences for high-energy palatable foods, and associated dopaminergic signaling in the nucleus accumbens, are controlled by a mechanism that includes eCB signaling in the periphery. Roles for CB_1Rs in the intestinal epithelium in gut-brain mediated food reward are currently under investigation.

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TARGETING THE BASOLATERAL AMYGDALA GLUTAMATERGIC SYSTEM TO REVERSE THE LONG-TERM IMPACTS OF PRE-NATAL THC EXPOSURE ON ANXIETY AND AFFECTIVE PATHOPHENOTYPES

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Introduction: Prenatal cannabis exposure (PCE) can harm fetal brain development and increase the risk of affective disorders and emotional dysregulation. Fetal exposure to Δ9-THC impairs mesocorticolimbic (MCL) circuit function, but the impacts of PCE on pre-natal amygdala development is poorly understood. The basolateral amygdala (BLA) plays a central role in the MCL circuit and requires functional balance between dopamine (DA), GABAergic, and glutamatergic inputs for emotional regulation. We are exploring the effect of PCE on the BLA using behavioral, electrophysiological, molecular, MALDI IMS, and mechanistic assays. Our results demonstrate profound dysregulation of normal glutamate function in the BLA. We assessed two mechanistic approaches to ameliorate this phenotype: direct BLA microinfusions of GLUTergic compounds to restore normal glutamate activity states and systemic administration of L-theanine during adolescence, a treatment we have recently shown to normalize adolescent THC-induced dysregulation of glutamate/GABA balance and reduce anxiety-like phenotypes.

Methods: Pregnant Wistar rats were administered VEH or 3mg/kg THC (daily, *i.p.*; n=7 dams/treatment; n=4 progeny /sex/dam) from gestational day (GD) 7 to GD22. Between post-natal (PD) 70-85, progeny (n=20/treatment/sex) were assessed for anxiety, sociability, prepulse inhibition, and contextual fear. Between PD90-120, *in vivo* electrophysiology was used to assess activity of DA, glutamate, and GABA neurons in the VTA-BLA-NAc network. At PD120. Molecular protein assays and mass spectrometry imaging via MALDI IMS were performed for neurotransmitter marker analyses. BLA protein analyses revealed significant disruption of several glutamatergic, GABAergic, and dopaminergic markers. Namely, males exhibited significant increases in levels of vGLUT1/2, GAD67, D1R, GABA-Aa1, mGLUR2, GLUR2, while females displayed significantly greater levels of vGLUT2, GABAARa1, GABA-Ay2, and GLUR2. Accordingly, we are conducting two mechanistic approaches to ameliorate this pathophenotype. A second cohort of progeny (n=3 dams/treatment) received intra-BLA cannulations with either NMDA antagonist DL-AP5 (1mg/kg) or VEH; the behavioural phenotype was then assessed. A third cohort (n=4 dams/treatment) following PCE, were randomly assigned to L-theanine (10mg/kg, *i.p.*,) or VEH treatment. Behavioural, electrophysiological, MALDI IMS, and the molecular phenotype will be assessed.

Results: Consistent with our previous research, male PCE progeny exhibited an anxiety-like phenotype, as well as increased freezing behavior, a distinct molecular, and neural phenotype, including significant decreases in NAc GABA and increases in glutamate and GABA neuron activity in the BLA. To address this pathophenotype, we administered DL-AP5 via BLA cannulation to reduce glutamate activity. However, this treatment did not prevent the behavioural phenotype. L-Theanine assessments are ongoing.

Conclusions: Our findings indicate that BLA disruptions may be responsible for the anxiety-like deficits in male progeny, while female progeny are protected from BLA-related pathology. While DL-AP5 did not prevent the phenotype, adolescent L-theanine may mitigate the pathophenotype observed in adulthood.

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CHARACTERIZATION OF CANNABIGEROL (CBG) EFFECTS ON NOCICEPTION, INFLAMMATION AND HYPERALGESIA, APPETITE, ATTENTION AND MEMORY

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Introduction. Cannabigerol (CBG) is a minor cannabinoid that is highly expressed in cannabis and some commercial hemp varieties. Its acid, CBGA, serves as the substrate for the synthesis of multiple cannabinoids including CBD and THC. CBG is most often sold as an oral formulation, and touted as a therapeutic for pain, anxiety, and appetite stimulation, and to increase 'alertness' or 'focus'.

Methods: Male and female adult Sprague-Dawley rats assigned to different groups (n=10-12 per sex) to assess: 1) the standard cannabinoid tetrad of behaviors (antinociception, rectal temperature, catalepsy, and motor activity), 2) food-maintained operant responding, 3) sustained attention in the rodent psychomotor vigilance task (rPVT), 4) visuospatial memory in the Y-maze task and 5) Carrageenan-induced inflammation and hyperalgesia. CBG was dissolved in USP sesame oil and administered via oral gavage (1ml/kg). Doses ranged 10-600 mg/kg/ml. Effects were compared with those of THC and for experiment 5, with ketoprofen as well.

Results: High doses of CBG (300-600 mg/kg/ml) produced mild increases in motor activity in females but had negligible effects on other behaviors in the cannabinoid tetrad. CBG did not increase food-maintained responding. Intraplantar injection of carrageenan produced transient inflammation (paw swelling) and hyperalgesia. At the doses tested (10-60 mg/kg), CBG pretreatment did not improve carrageenan-induced inflammation or hyperalgesia. In contrast, pre-treatment with ketoprofen (10-20 mg/kg) mitigated carrageenan-induced hyperalgesia and decreased paw edema. THC (3-10 mg/kg) pretreatment prevented hyperalgesia but effects on paw edema were minimal. High doses of CBG caused impairments in sustained attention, which were comparable to those of 10 mg/kg THC; visuospatial working memory was not affected.

Conclusions: Taken together, these data do not support the use of CBG to improve focus or attention, treat pain, hyperalgesia, and inflammation, or to stimulate appetite.

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STRESS-INDUCED ANXIETY AND ANHEDONIA SYMPTOMS ARE REVERSED BY THE INHIBITION OF FATTY ACID BINDING PROTEIN-5 VIA CB2 AND GPR55 SIGNALLING MECHANISMS

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Introduction: Currently prescribed medications for anxiety disorders and depression have limitations in their efficacy and tolerability, which is a pharmacotherapeutic obstacle that is yet to be overcome. A promising neurobiological system in which to target the development of novel pharmacotherapies for neuropsychiatric disorders is the endocannabinoid system (eCB), which has been shown to modulate emotional behaviour and neuronal transmission patterns in both humans and rodents. Fatty acid binding protein-5 (FABP-5) is a chaperone protein in the eCB system, responsible for the intracellular transport of anandamide for degradation by fatty acid amide hydroxylase. Previously, we showed that pharmacological inhibition of FABP-5 within the prelimbic cortex of rats induced an anxiolytic behavioral phenotype and an altered neurophysiological activity in a CB2 receptor dependent fashion. In this study, we aimed to examine the effects of systemic FABP-5 inhibition on anxiety- and depression-related behaviours.

Methods: Following a 2-week long unpredictable stress paradigm, we assessed whether an acute intraperitoneal injection of SBFI-103, a selective inhibitor of FABP-5, could reverse stress-induced anxiogenic and depressive-like phenotype in Sprague-Dawley rats. Also, we investigated mRNA expression levels and activity and proteins in key signaling pathways of the eCB system within the limbic regions of the rat brain using RT-qPCR and Western blotting, respectively.

Results: Our results of anxiety-like behaviour using elevated plus maze and light-dark box tests indicate that both low dose (2mg/kg) and high dose (20mg/kg) SBFI-103 ameliorate stress-induced anxiogenesis. Furthermore, using sucrose preference, novelty-suppressed feeding and forced swim tests, we found that that high dose SBFI-103 restores stress-induced anhedonia and depression-like behaviour. Neither dose of SBFI-103 influences cognition or memory. Our molecular findings indicate that SBFI-103-induced behavioural effects are possibly modulated by altered transcription of CB2 and GPR55 receptors as well as by altered phosphorylation of Erk1-2 and p70S6 kinase, key proteins that are heavily associated with depressive behaviour, in the ventral hippocampus.

Conclusions: Our data reveals a promising role for FABP-5 inhibition as a novel pharmacotherapy of anxiety disorders and depression. According to our proposed mechanism of action, FABP-5 inhibition elevates anandamide availability, which triggers a CB2R- and/or GPR55-dependent signaling cascade involving Erk1-2 and p70S6 kinase to control emotional behaviour.

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BIDIRECTIONAL EFFECT OF 2-AG ON HYPERDOPAMINERGIC STATES: IMPLICATIONS FOR 2-AG MODULATION IN DOPAMINE PATHOLOGIES

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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. Before wide therapeutic use, it's imperative to understand the effect of MAGLi in vulnerable populations, and whether decreasing 2-AG is therapeutic. A subpopulation with dysregulated 2-AG may be vulnerable to psychiatric effects of MAGLi. Therefore, we assessed pre-clinical effects of MAGLi (increase 2-AG) and DAGLi (decrease 2-AG) in two models of hyperDA; based on well-established associations between psychopathologies and increased subcortical dopamine.

Methods: Genetic (adult DAT-knockout (DATKO) and pharmacological (C57Bl/6J with amphetamine) models of hyperDA were treated acutely with a MAGL (MJN110, 5mg/kg) or DAGL (DO34, 30mg/kg) inhibitor, and tested on behavioural assays. Lipidomic and molecular analysis was completed (striatal brain samples), and partial correlation networks were generated. Data were analyzed with three-way ANOVA (behaviour) and Student's t-test for lipidomics.

Results: DATKO present with subcortical hyperDA; exploratory hyperactivity, impaired sensorimotor gating, blunted response to psychostimulants, and disrupted lipid profiles. MAGLi exacerbated hyperlocomotion, sensorimotor deficits, and further disrupted lipid networks in DATKO. MAGLi increased reward association in DATKO, but not WT, suggesting a worrisome addiction liability. MAGLi effects weren't limited to DATKO; it exacerbated psychostimulant responses in C57BL/6J. Data suggests that increasing 2-AG via MAGLi exacerbates states of hyperdopaminergia, mediated by CB1. Interestingly, decreasing 2-AG (via DAGLi) presented opposite effects on all measured hyperdopaminergic behavioural outputs in both DATKO and C57BL/6J.

Conclusion: Our data highlight a potential therapeutic avenue for novel, dopamine-indirect, treatments that target lowering 2-AG in vulnerable hyperDA populations. In parallel with behaviour analysis, understanding the profound brain-region specific remodelling of 2-AG using molecular and lipidomic assays under hyperDA conditions has enormous potential to identify yet unrecognized lipid biomarkers and targets that could constitute novel mechanisms to target high DA pathologies.

THE EFFECT OF ACUTE DELTA-9-TETRAHYDROCANNABINOL IN MALE HIV-1 TRANSGENIC RATS ON COGNITIVE FUNCTION

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Background: People with HIV-1 (PWH) smoke cannabis at higher rates than the general population, possibly for self-medication purposes given that both improved and worsening clinical outcomes have been observed. Cannabis effects on PWH could be domain specific but determining directionality of effects is impossible. The HIV-1 transgenic (TG) rat is a reliable model of neuroHIV in the current age of combination antiretroviral therapy, where viral replication is suppressed while chronic low-level infection persists. Hence, the impact of acute delta-9-tetrahydrocannabinol (THC) on HIV-relevant cognitive behaviors was tested.

Methods: Male HIV-1TG rats and wildtype (WT) controls were tested in a battery including the Iowa Gambling Task (IGT; to measure risky decision-making), the Probabilistic Reversal-Learning Task (PRLT; to measure learning and cognitive flexibility), and the Progressive-Ratio Breakpoint Task (PRBT; to measure motivation). After baseline the rats were retested following treatment with 0, 0.3, or 3 mg/kg THC administered intraperitoneal (n=6-7/group).

Results: At baseline, HIV-1TG rats exhibited more premature responding in the IGT compared to controls, suggesting heightened motoric impulsivity, although no effect on risky choice was observed. There was a trend for high dose THC to decrease premature responding ($F(2,32)=2.2, p<0.11$), with TG rats continuing to exhibit more premature responses ($F(2,31)=3.8, p<0.06$). Acute THC dose-dependently reduced trials in WT but not TG rats ($F(2,32)=5.5, p<0.01$). High dose THC slowed mean choice latency ($F(2,31)=6.5, p<0.004$), but sped mean reward latency ($F(2,31)=8.45$, $p<0.001$) across genotypes. At baseline in the PRLT, there were no genotype effects on trials, reversal switches, or learning rate. Acute THC did not affect the number of switches across genotype, though low dose THC tended to increase the learning rate in HIV-1tg only ($F(2,21)=2.5, p=0.1$), suggesting possible precognitive effects. There was no genotype effect on breakpoint in the PRBT at baseline, while high dose THC reduced breakpoint across genotypes ($F(2,32)=12.1, p<0.001$).

Conclusions: HIV-1TG rats exhibited heightened motor impulsivity (premature responses) relative to WT rats, though performed similarly in other measures. THC reduced premature responses in both genotypes but reduced total trials in WT rats only, consistent with previous reports of impairing sensorimotor gating in WT but not TG rats. THC also enhanced the PRLT learning rate in TG rats only. These results support the premise that the cognitive effects of THC on cognition in PWH may be task-dependent, with evidence for possible precognitive effects. Ongoing experiments include increasing sample size, adding female rats, and testing the impact of chronic administration of THC.

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EXPRESSION AND FUNCTION OF DAGLa IN CEREBELLAR PURKINJE CELLS

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Introduction: The cerebellum is a highly compacted and compartmentalized cortical structure, slightly smaller in area as compared to the cerebral cortex but containing more that 50% of all the neurons in the human brain. Endocannabinoid (eCB) signaling plays a key role in cerebellar learning and synaptic plasticity. For example, learning and adjusting skilled movements depends on eCB-dependent long-term depression (LTD) in parallel fiber to Purkinje cell (PC) synapses. In addition, eCB signaling plays a key role in short-term cerebellar synaptic plasticity in both excitatory and inhibitory synapses (i.e., depolarization-induced suppression of excitation (DSE) and inhibition (DSI)). Diacylglycerol lipase alpha (DAGLα), a prominent enzyme involved in the synthesis of 2-arachidonoylglycerol (2-AG), a major neuronal eCB, is highly expressed in PCs. However, levels of DAGLα expression and activity in different cerebellar compartments and in individual PCs have not been characterized, and it is not yet known if DAGLα is required for PC-derived eCB regulation of cerebellar synaptic plasticity.

Methods: To elucidate the role of DAGLα in the regulation of cerebellar synaptic plasticity, we generated PC-specific DAGLα conditional knockout mice. Electrophysiology in cerebellar slices was used to assess DSI and DSE in DAGLα-null PCs. Immunohistochemistry and 3D volumetric reconstruction were used to elucidate changes in synaptic morphology in DAGLα-null PCs.

Results: DAGLα expression in PCs obeys functional stripe domains. DAGLα-null PCs exhibit shifts in excitability and in structural properties of synapse terminating onto them.

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

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ENDOCANNABINOID SYSTEM MODULATION AFFECTS LOCOMOTORY EPISODE STRUCTURE

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Introduction: In our previous studies we have shown bidirectional effects of selective inhibitors of enzymes responsible for endocannabinoid degradation on locomotor activity in mice. We observed that inhibition of Fatty Acid Amide Hydrolase (FAAH) and Monoacylglycerol Lipase (MAGL) caused, respectively, inhibition and elevation of locomotor activity. Here we evaluated whether these compounds affected the microstructure of locomotory episodes.

Methods: To elevate signaling of endocannabinoids 2-Arachidonoylglycerol (2-AG) and Anandamide (AEA) we used selective inhibitors (MJN110 and PF3845, respectively) of enzymes responsible for their degradation: MAGL and FAAH, respectively. High-speed, high-resolution marker-based 3D motion capture system (Qualisys) was used to track movement (3D trajectories and speed of markers) during voluntary locomotor tasks: open field exploration and vertical climbing, in C57BL/6 male mice (n=10 per group). CP55,940 was used as a positive control.

Results: We have found that every locomotory episode (defined by maintained displacement of the mouse hip at speeds over 40 mm/s) is composed of smaller activity bouts ("locobouts") during which the mice accelerate and decelerate in a repetitive fashion. Unlike locomotory episodes, these locobouts were mostly invariant across drug treatments and time. Also, the interval between locobouts was not affected. PF3845 (30 mg/kg) significantly decreased the total number of locomotory episodes compared to vehicle ($p=0.013$) and MJN110 ($p=0.0026$) and increased the length of pauses between them. PF3845 also caused a significant decrease in the acceleration of the locobouts ($p<0.001$) but the duration of locobouts and intervals between them were not affected. MJN110 (2.5 mg/kg) did not change the number of locomotory episodes compared to vehicle and did not affect locobout acceleration and duration, or their intervals, despite increasing the distance traveled and time spent mobile. Interestingly, CP55,940 at a low dose of 0.3 mg/kg, which produces a slight decline in general activity, without a significant decline in the total number of locomotory episodes, affected the duration of pauses between locomotory episodes but not acceleration and duration of the locobouts or their interval.

Conclusions: While the number of locomotory episodes reflects the overall locomotor activity level, the microstructure of locomotory behavior remains largely unchanged. The presence or absence of changes in the locomotory episode microstructure can be informative about the mechanisms underlying observed behavioral effects.

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VOLUNTARY CHRONIC ALCOHOL DRINKING INCREASES ENDOCANNABINOIDS AEA AND 2AG IN BRONCOALVEOLAR LAVAGE FLUID FROM NONHUMAN PRIMATES

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Introduction: Chronic alcohol drinking predisposes lungs to development of acute tissue injury, pneumonia, and alcoholic lung disease. It is known that chronic alcohol ingestion alters the function of alveolar macrophages (AM) via dysregulation of chemokines and cytokine levels. Eventually, this can result in a defective immune response during host defense reactions. Additionally, all these changes make the lungs susceptible to inflammatory and fibroproliferative disorders in the case of a secondary hit of tissue injury. We recently found that the endocannabinoid and cannabinoid receptor 1 (CB_1R) system is significantly upregulated in inflammatory and fibroproliferative conditions in multiple cell types in the lung. Increased level of anandamide (AEA) in bronchoalveolar lavage fluid (BALF) was negatively correlated with pulmonary function decline in patients with pulmonary fibrosis (PF) as well as animal model of PF (*Cinar et al., JCI Insight 2017 2(8):92281*). This demonstrated inflammation and fibrosis promoting role of endocannabinoids and CB_1R system in lung microenvironment. However, the status of the endocannabinoid/CB_1R system has not yet been explored in lungs in experimental models upon voluntary chronic alcohol drinking which mimic the human voluntary drinking.

Method: To address this, we measured levels of endocannabinoids by liquid chromatography mass spectrometry in BALF from rhesus monkeys at baseline and after ethanol induction followed by voluntary chronic ethanol drinking. we employed 6 months chronic voluntary alcohol drinking model (*Baker et al., Alcohol Clin Exp Res. 2017 41(3) 626-636*), which requires induction period consist of 2 months of water induction, a month of 0.5 g/kg/day ethanol followed by a month of 1.0 g/kg/day and then a month of 1.5 g/kg/day ethanol. After the induction period follows 6 months of voluntary 22h open access ethanol drinking.

Results: We found that levels of anandamide in BALF significantly increased 2-fold by ETOH induction compared to the baseline measurement, whereas 2AG level was unchanged. Furthermore, voluntary ethanol drinking for additional 6 months dramatically increased both AEA and 2-AG levels about 22-fold compared to the baseline in BALF.

Conclusion: In conclusion, voluntary chronic alcohol drinking significantly increases endocannabinoids in lung microenvironment in monkeys, suggesting an activation of endocannabinoid/CB_1R system may involve alcohol induced lung injury, which warrants further studies.

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EFFECT OF ACUTE VAPOURIZED CANNABIS ON MICROGLIA IN THE MOUSE PREFRONTAL CORTEX

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Introduction: Microglia, the brain's resident immune cells, are increasingly becoming recognized for their physiological, as well as immunological, roles. Microglia possess cannabinoid receptors and respond to cannabinoids, such as phytocannabinoids. Administration of the primary phytocannabinoids, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), typically leads to anti-inflammatory responses, but this is dependent on the route (i.e., generally injection or *ex vivo*) and duration of administration, and the compounds delivered. Our goal is to understand how another relevant route of administration, inhalation, affects microglial physiological functions.

Methods: Whole cannabis plant was administered to adult, male, C57BL/6J mice for 15 min (one 15 second puff every 5 min; 3 puffs total; 0.15 g flower/puff). Four groups were utilized, mice that received control air vapor, and mice that were exposed to either: high CBD/low THC [CBD], high THC/low CBD [THC], or balanced THC/CBD [Balanced] cannabis strains. Brains were isolated 30 min post-cannabis administration onset, when THC levels peak in the brain. We stained the tissue with antibodies against IBA1 (microglia and macrophages) and TMEM119 (more specific for microglia). We looked at IBA1+ cell density, nearest neighbor distance and spacing index (changes in number and distribution), in regions important for cognition, memory, and emotional regulation, specifically focusing on the prefrontal cortex. We also investigated IBA1 and TMEM119 colocalization, as well as IBA1+ cell morphology.

Results: Our preliminary data indicates that microglial distribution and spacing, as ascertained by nearest neighbor distance, was modified by cannabis exposure in the prefrontal cortex of male mice, and differently in the infra- and prelimbic cortices. Specifically, IBA1+ cells in the infralimbic area, but not prelimbic cortex, were altered in CBD containing strains *versus* others. In the prelimbic cortex, all IBA1+ cells analyzed colocalized with TMEM119, indicating these cells were likely all resident/homeostatic microglia. Furthermore, in the prelimbic area, although there were no changes in density or distribution, microglial morphology was affected. In the balanced strain exposed group compared to control air exposed group, microglial fractal dimension was reduced, indicating a less complex morphology; and microglial lacunarity was increased, indicating altered shape. Furthermore, the distribution of microglial morphology in all cannabis strains was significantly different compared to microglia from the control group, even if there was not an overall significant in the average fractal dimension and lacunarity—potentially indicating the emergence of different microglial states in response to acute cannabis exposure.

Conclusions: Our preliminary data indicates that acute cannabis exposure modifies microglial morphology in the prelimbic cortex. We will next use electron microscopy to investigate at the nanoscale potential changes in microglial organelles and interactions with parenchymal elements. This work will lay the foundation for understanding how vaporized cannabis exposure changes microglial form and function, and determining how these parameters change with chronic exposure and in response to stress, infection or disease. We are also investigating potential sex differences in the response of microglia to acute cannabis exposure.

UP-REGULATION OF CANNABINOID RECEPTOR 2 (CB_2) AND FATTY ACID AMIDE HYDROLASE (FAAH) IN THE RETINA OF A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Introduction: Accumulated evidence suggests that Alzheimer's disease (AD) develops extra-cerebral manifestations in the retina, which is then considered a "window to the brain". Recently, the dysregulation of the endocannabinoid (eCB) system in AD has been highlighted. Here, we aimed at exploring for the first time the onset of gliosis and the possible alterations of eCBs metabolism/signaling in the retinas of a mouse model of AD.

Methods: Twelve month-old heterozygous Tg2576 transgenic mice, characterized by the over-expression of APP, were used for the purpose of the study and were compared to age-matched wild type (WT) mice. Gliosis was investigated on retinal cryosections of Tg2576 in terms of microglia number (IBA1 + cells) and astrocytes/Müller cells reactivity (GFAP immunostaing). As a first step, in this study we focused the analysis on the cannabinoid receptor 2 (CB_2) and on the fatty acid amide hydrolase (FAAH) - which bind to and degrade anandamide (AEA) respectively – since they were previously demonstrated to play a relevant role in AD inflammatory events. CB_2 and FAAH protein levels were then quantified through Western blotting on retinal lysates and fluorescence intensity analysis on retinal cryosections through plot profile graphs. Statistical analysis was performed by SigmaPlot software using the Student's *t*-test. The correlation between CB_2 and FAAH protein levels for individual animals was investigated through linear regression analysis.

Results: The retinas of Tg2576 showed a significant increase of IBA1 (+) cells compared to WT ($p=0,002$ - TG: n=5, WT:n=4), and GFAP expression was up-regulated throughout the retinal layers, suggesting Müller cells reactivity. CB_2 protein levels were significantly up-regulated in Tg2576 retinas compared to WT (1,5 folds over WT; $p=0,032$ - TG: n=8, WT:n=5). Also, FAAH was up-regulated in TG mice compared to WT littermates (1,4 folds over WT; $p=0,1$ - TG: n=8, WT:n=5), but the difference was not statistically significant due to a higher variability in the TG group compared to the WT group. Interestingly, the linear regression analysis showed a significant correlation between CB_2 and FAAH protein levels ($p=0,02$ - TG: n=8). Plot profile graphs on retinal cryosections were consistent with the Western blot results and an increase of the fluorescence intensity was observed throughout the retinal layers in TG *versus* WT mice.

Conclusions: Overall, our findings suggest that (i) the retina of the Tg2576 mouse model of AD exhibits gliotic events similar to the AD brain, and (ii) the up-regulation of CB_2 and FAAH occurs in the retinas of the same model. These findings suggest that the eCB system may play a role in AD-associated retinal inflammation as observed in AD brain, and further supports the retina as a window to the brain also at a molecular level. Future studies are needed to expand the knowledge on additional receptors and enzymes of the eCB system that may be potentially dysregulated in AD retinas.

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INHIBITION OF FATTY-ACID-BINDING PROTEIN-5 IN THE BASOLATERAL AMYGDALA INDUCES ANXIOLYTIC EFFECTS AND MODULATES FEAR MEMORY PROCESSING

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Introduction: The endocannabinoid (eCB) system is a crucial regulator of emotion within the brain, exhibiting control over anxiety and fear-related behaviours. Given that eCB ligands, such as anandamide, are water-insoluble lipids with limited ability for intracellular movement, their termination of action requires chaperone proteins such as Fatty Acid Binding Proteins (FABPs). Here, we investigated the effects of a novel FABP5 inhibitor (**SBFI-103**) when infused locally to the basolateral amygdala (BLA) of adult rats on anxiety and fear-related behaviours, along with neurophysiological and localized molecular signaling analyses. Further, we investigated the role of the anandamide ligand and the CB1 and CB2 receptors in the observed effects.

Methods: We investigated the effects of intra-BLA FABP5 inhibition at behavioural, neuronal, and molecular levels in adult male Sprague Dawley rats. We tested two doses of SBFI-103 and co-administered SBFI-103 with endocannabinoid receptor antagonists. Further, we co-administered SBFI-103 (5 μg) with an inhibitor of NAPE-PLD to block anandamide synthesis to investigate the role of the anandamide ligand. We surgically implanted guide cannulae targeting the BLA region to enable local acute administration of treatment drugs prior to running anxiety, fear memory, and control behaviour test paradigms. After, brains were collected for molecular analysis of various anxiety and fear-memory-related biomarkers. We performed *in vivo* electrophysiology on a separate cohort of rats, where we infused treatment drugs directly to the BLA and recorded single- and multi-neuron activity in the prefrontal cortex (PFC).

Results: Upon acute intra-BLA administration of SBFI-103, we observed strong anxiolytic effects across multiple behavioural domains. Furthermore, animals exhibited acute and long-term accelerated fear memory extinction following intra-BLA FABP5 inhibition. On a neuronal level, we found BLA FABP5 inhibition induced strong modulatory effects on putative PFC pyramidal neurons along with significantly increased gamma oscillation power. Finally, we observed local BLA changes in the phosphorylation activity of various anxiety- and fear memory-related molecular biomarkers in the PI3K/Akt and MAPK/Erk signaling pathways. The functional effects of SBFI-103 at all three levels depended on availability of the anandamide ligand. Further, we observed a functional role for CB2 receptors in the modulation of putative PFC pyramidal neurons by intra-BLA FABP5 inhibition. We now plan to investigate the role of CB1 an CB2 receptors in our behavioural and molecular testing and hope to present these additional findings at ICRS 2023.

Conclusions: These findings demonstrate a novel intra-BLA FABP5 mechanism regulating anxiety and associative-fear memory behaviours, neuronal activity, and molecular pathways working via anandamide modulation and the CB2r system.

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CHANGES IN BDNF FRONTO-CEREBELLAR LEVELS FOLLOWED BY CHRONIC SYSTEMIC ADMINISTRATION OF SYNTHETIC CANNABINOID WIN55,212-2 TREATMENT IN ADOLESCENT RATS

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Cannabinoids are ligand molecules that bind to endocannabinoid receptors (eCBRs) CB1 and CB2, abundantly present in the central and peripheral nervous system. The synthetic CB1 /CB2 receptor agonist WIN55,212-2 (WIN) acts as a dual modulator by emulating the effects of delta-9-tetrahydrocannabinol (THC), the psychoactive component of cannabis, and cannabidiol (CBD)known for its therapeutic anti-inflammatory properties. Interestingly, endocannabinoids (eCBs), anandamide (AEA) and/or 2-arachidonoyl glycerol (2-AG), and neurotrophins, particularly brain derived neurotrophic factor (BDNF) play key roles, immune and endocrine homeostasis, stress/anxiety response, and neuroplasticity. Changes in either BDNF or endocannabinoid signaling is associated with an overlapping set of neurologic and psychiatric diseases. The chronic use of cannabinoids during adolescence, a vulnerable stage for brain development may affect or alter the homeostasis and neuroplasticity dependent on BDNF. Therefore, we evaluated the effect of adolescent exposure to WIN on blood and the brain dorsolateral periaqueductal gray (PAG), prefrontal cortex (PFC), hippocampus (HIP), and cerebellar vermis (CV) concentration levels of BDNF. Methods: adolescent rats received 5 twice-daily injections of saline (1 mL/kg i.p.) or WIN55,212-2 (0.8 mg/kg i.p.) every other day. Brain tissue and truncal blood were collected, and ELISA immuno-specific assay was used to determine pro and mature BDNF levels. Results: One way ANOVA revealed that WIN increased proBDNF [$F_{1,11} = 12.57$, $p<0.05$]and mBDNF levels [$F_{1,11} = 2.63$, $p<0.05$], in the dorsolateral PAG and periphery [$F_{1,11} = 5.15$, $p<0.05$], increased mature BDNF levels 2-fold in the HIP [$F_{1,10}=8.85$, $p<0.01$] and proBDNF in the cerebellum [$F_{1,11}=5.77$, $p<0.05$]. Discussion: The chronic exposure of synthetic cannabinoid WIN during adolescence modifies the proBDNF/mBDNF ratio in the brain PAG, HIP, CV, and blood periphery, suggesting that proBDNF/mBDNF ratio are involved in endocannabinoid-mediated adolescence brain plasticity.

CHARACTERIZING THE NEURODEVELOPMENTAL, ENDOCRINE, IMMUNE, METABOLIC, AND BEHAVIOURAL OUTCOMES OF INHALED CANNABIS EXPOSURE DURING PREGNANCY IN MALE AND FEMALE RATS

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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 growth retardation, increased incidence of autism or social behaviour deficits, immune changes, and cognitive dysfunction. Nevertheless, little is actually known about the mechanism through which PCE may alter neurodevelopment and subsequent behaviour. Further, while clinical studies are confounded by unknown timing and exposure level, animal models can control for these variables while examining mechanism. Therefore, our studies aim to characterize the effects of inhaled cannabis exposure during pregnancy on a wide range of domains including: structural brain development, endocrine and immune system functioning, social behaviour, stress-reactive behaviour, and glucose metabolism and feeding. Moreover, as cannabis exerts its effects through acting on the endocannabinoid (eCB) system, which is involved in many processes of brain development, 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 beginning on gestational day (GD) 1. In aim 1, dams and their fetuses were euthanized on GD15, 17, or 19 to examine fetal brain development. In aim 2, dams gave birth and a cohort of their offspring were euthanized on postnatal day (PD) 0 and 5 to examine postnatal brain development. Maternal blood and spleen, placenta, and fetal brains were collected via caesarean section surgery from aim 1 and postnatal brains were collected from aim 2 – tissues were analyzed for levels of THC and metabolites, eCB levels, eCB and immune-related gene expression, and cytokine levels. In aim 3, separate cohorts of offspring were tested across the lifespan for the following measurements: 1) cytokine levels after an acute immune challenge on PD14; 2) structural brain development via MRI imaging on PD25-30; 3) social play behaviours on PD33-PD37; 4) anxiety-like behaviour assessed in the elevated plus maze and acute stress response following 30-minute restraint stress in adulthood; and 5) glucose metabolism and feeding patterns following 4-months of high fat diet exposure in adulthood.

Results and Conclusions: Results from aim 1 and 2 have found no impact of PCE on fetal brain eCB levels or cannabinoid receptor gene expression. Analysis of eCB and immune-related gene expression and cytokine levels are ongoing. Results from aim 3 have found no impact of PCE on anxiety-like behaviour or stress-response following restraint. Analysis of social play behaviour, MRI imaging, and cytokine levels following immune challenge are ongoing. Interestingly, PCE-alone increased chow intake in adulthood and improved glucose metabolism, whereas PCE combined with high-fat diet exposure impaired glucose metabolism in males. In conjunction with ongoing research, our results may help determine the mechanism through which PCE alters neurodevelopment and help determine the safety of cannabis consumption during pregnancy.

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A COHORT STUDY OF MEDICAL CANNABIS FOR ANXIETY PATIENTS WITH AND WITHOUT CO-MORBID SLEEP DISTURBANCE

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Introduction: Pre-clinical evidence suggests that major cannabinoids have anxiolytic effects mediated by the endocannabinoid system. Evidence demonstrates that generalized anxiety disorder (GAD) is one of the most common conditions for which cannabis-based medicinal products (CBMPs) are prescribed. However, there is limited evidence on which patients are most likely to benefit from therapy. Sleep disruption has been shown to have a bidirectional relationship with GAD, increasing the persistence of one another. The endocannabinoid system has been implicated in the regulation of sleep-wake cycles. The present study therefore aims to compare the patient reported outcome measures (PROMs) of patients prescribed CBMPs for GAD, between those with impaired sleep quality and those without.

Methods: A cohort study of patients with GAD enrolled on the UK Medical Cannabis Registry was conducted. Primary outcomes were changes in select PROMs from baseline at 1, 3, 6, and 12 months: GAD-7, Sleep Quality Scale (SQS) and EQ-5D-5L between those with impaired sleep (baseline SQS $\leq$3) or unimpaired sleep (baseline SQS $\geq$4). Multivariate logistic regression was utilised to compare factors associated with a clinically significant improvement in GAD-7 at 12 months. Secondary outcomes included the incidence and frequency of adverse events. Statistical significance was defined as $p < 0.050$.

Results: 302 patients met the inclusion criteria for this study. Compared to baseline (13.25 ± 6.02), the mean GAD-7 score improved at 1 (8.52 ± 5.77), 3 (8.42 ± 5.79), 6 (8.90 ± 6.36), and 12 (10.21 ± 6.48; $p<0.001$) months. Improvements were also identified in SQS and EQ-5D-5L index scores at 1, 3, 6, and 12 months ($p<0.001$). Improvements in each PROM were largest in in those with co-morbid sleep impairment at each time period ($p<0.050$). On multivariate logistic regression, there was no difference in the likelihood of experiencing a clinically significant improvement in GAD-7 score in those with or without sleep impairment (odds ratio: 1.214; 95% confidence interval: 0.559 - 2.634; $p=0.625$). GAD severity was the only variable associated with an increased likelihood of observing a clinically significant improvement in anxiety ($p<0.001$). 707 (234%) adverse events were reported by 55 (18.21%) participants – the majority of which were categorised as mild or moderate (n=628, 208%).

Conclusions: This study observed an association between CBMP treatment and improvements in anxiety, sleep quality, and general health-related quality of life in patients with GAD. Whilst patients with co-morbid sleep disruption had greater improvements in anxiety, the differences were not maintained in a multivariate analysis. Those, with more severe GAD were more likely to have an improvement, indicating that CBMPs may have greater effects in those most affected by symptoms of anxiety. Further assessment in randomised controlled trials will ultimately be necessary to explore this relationship further.

COMPARISON OF CANNABIS-BASED MEDICINAL PRODUCT FORMULATIONS FOR ANXIETY: A COHORT STUDY

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Introduction: Anxiety disorders are the most common mental health problem in the UK. Cannabis-based medicinal products (CBMPs) have been cited as potential therapeutic agents for generalized anxiety disorder (GAD) due to the effects shown in pre-clinical studies of major cannabinoids on fear circuitry and serotoninergic pathways within the brain. However, there is a paucity of high-quality evidence on the outcomes of patients prescribed CBMPs for GAD. A key reason for this is the heterogeneity of CBMPs evaluated to date. This study aims to analyse changes in health-related quality of life and safety in patients with GAD prescribed a homogenous selection of CBMPs.

Methods: Patients who had started CBMP therapy for GAD prior to January 2022 were identified from the UK Medical Cannabis Registry prescribed medium chain triglyceride oils, dried flower or both products (Adven® 20, 50 & EMT, Curaleaf International, Guernsey, UK). Primary outcomes were changes in patient reported outcome measures (PROMs) from baseline which included Generalised Anxiety Disorder-7 (GAD-7), Sleep Quality Scale (SQS), and EQ-5D-5L questionnaires. PROMs were administered at baseline and 1, 3, 6, and 12 months from initiation of treatment Adverse events were recorded using CTCAE version 4.0.. Statistical significance was defined as $p<0.050$.

Results: 120 patients were identified for inclusion, of which 74 (61.7%) were male and 46 (38.3%) were female. The mean age of participants was 39.38 (±12.5) years. 38 (31.7%), 52 (43.3%), and 30 (25.0%) individuals were prescribed oils, dried flower, and both formulations of CBMP respectively. 59 (49.2%) patients consumed illicit cannabis at the point of enrolment with 44 (74.6%) of this group reporting daily use. CBMP therapy initiation was associated with statistically significant improvements in GAD-7, SQS and EQ-5D-5L at 1, 3, 6 and 12 months compared to baseline ($p<0.010$). Individuals prescribed dried flower only has a greater reduction in GAD-7 score at 12 months (-4.1 ± 6.3) compared to those prescribed oils only (-1.5 ± 3.8; $p=0.046$). However, there were no other significant differences change in PROMs at 12 months between each cohort. There were 24 (20.0%) patients who reported 442 (368.3%) adverse events, most of which were mild (n=184, 41.6%) and moderate (n=197, 44.6%).

Conclusion: Participants within each treatment category reported an associated improvement in PROMs following initiation of CBMPs. The cohort prescribed the dried flower only formulation of CBMPs reported a larger reduction in anxiety at 12 months compared to those prescribed oils only, otherwise the magnitude of change was consistent between all treatments. Whilst the study design limits interpretation of these findings, the results provide further evidence to support evaluation of dried flower formulation of CBMPs for GAD within the setting of randomised controlled trials.

THE POTENTIAL EFFECTIVENESS OF MEDICAL CANNABIS IN THE TREATMENT OF WELLNESS OUTCOMES FOR VETERANS WITH DEPRESSION

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Introduction: Depression is a pervasive mental illness with a prevalence of ~9% of the Canadian population (Ameringen et al., 2008). Depression commonly presents with a number of chronic symptoms that can severely impact quality of life and mental well-being, such as intrusive thoughts, flashbacks, irritability, anxiety and sleep disturbances(Sharpless & Barber, 2011). Military veterans notably present with much higher rates of depression compared to civilians(Veterans Affairs Canada, 2019). There is no single effective treatment and persons with depression might receive multiple modes of treatment in combination (Ameringen et al., 2008). To date, few studies have characterized the demographic characteristics of medical marijuana patients or assessed for pre-post changes in well being. To better understand therapeutic benefits for patients, a mixed methods study was conducted. Here, we aimed to describe physician authorization patterns of medical cannabis products and observe the self-reported effectiveness and wellness outcomes (depression and anxiety) of medical cannabis among Veterans from Avail Cannabis Clinic.

Methods: Total of 34 patients were recruited for retrospective chart review. researchers compared outcomes using the The Patient-Health Questionnaire (PHQ-9) for Depression wellness scale . On intake and assessment doctors interview patients on their lived experience and the questions were similar to the survey questions with more opportunities for open-ended responses. Patients were asked to describe pre -conditions and treatment goals.

Results: The average score before the administration of the treatment was 14.6, which according to the scoring guide denotes moderate to moderately severe depression severity. After the treatment was administered the average PHQ-9 total score decreased to 10.5, which according to the scoring guide highlights mild to moderate depression symptoms. A linear regression was also conducted to determine whether dosage is a good predictor of the change in depression. The r2 value shows that the model explains roughly 23% (r^2-0.236) of the data. When asked to provide a percentage to characterize change in symptoms; an average decline of 42% was verbally indicated by participants when asked to report on the percentage of decreased pain. A similar self-reported improvement was seen with increased activity where 38% of participants increased activity.

Conclusion: The interview and survey results indicate that patients are seeking medical cannabis for the relief of medical ailments and reported better improved quality of life , mobility and decreased pain. This finding aligned with the survey study by Crowell (2017) which also found increased overall condition and energy as the greatest perceived benefits. The findings may provide further clinical evidence to support the use of medical cannabis for Depression symptoms and to support larger research studies in future.

MOTIVATIONS FOR CANNABIS USE IN INDIVIDUALS WITH SOCIAL ANXIETY DISORDER (SAD)

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Introduction: Social Anxiety Disorder (SAD) represents a considerable risk (7-fold) for developing Cannabis Use Disorder (CUD). However, most previous studies on this topic focused on examining early adult populations using correlational research methods; thus, it is currently unknown what the motivations for cannabis use are in the general adult population with SAD and why these individuals would initiate, continue, and maintain cannabis use. Understanding these reasons could help us improve cannabis-use prevention efforts in SAD. The primary objective of our research was to understand the difference between motivations for cannabis use in adults with and without SAD and determine the timeline trajectory of cannabis use motivations. Our exploratory analysis aimed to investigate the primary coping strategies in adults with SAD.

Methods: We have employed the mixed-methods approach. Twenty-six adults (27.9 ± 7.3 mean age 54% F) with moderate to severe SAD and 26 cannabis users without psychiatric history, except for CUD (27.4 ± 6.7 mean age; 50.0% F), were administered Marijuana Motives Measure (MMM). In addition, motivations to initiate, continue and maintain cannabis use in SAD (12/24, 50.0% F) and their primary coping strategies were assessed using in-depth interviews.

Results: Curiosity, peer pressure, enhancement, and coping were the top four motivations for initiating cannabis use in those with SAD, whereas, at the continuing stage, the motivations were enhancement, coping, relaxation, and expansion. Compared to individuals without SAD, social ($F = 4.48$; $p = 0.04$) and coping ($F = 8.07$; $p = 0.005$) motivations were significantly higher in the SAD group, whereas conformity motivations were low in both groups ($F = 0.018$; $p = 0.893$). The primary SAD-coping strategy was cannabis use.

Conclusion: In line with the biopsychosocial model of social anxiety and substance use, our research suggests that individuals with SAD are more likely to use cannabis for social facilitation and to cope with their symptoms. Cannabis seems to be the primary SAD-coping strategy. The time trajectory of motivations for cannabis use indicates that initial exposure to cannabis is primarily due to curiosity. After the initial exposure, those with SAD continue to use cannabis after discovering its intoxicating and self-medicating properties, which pose a risk of developing CUD.

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THE EFFECTS OF CANNABIDIOL ON SLEEP DISTURBANCES: A DOUBLE-BLIND, RANDOMIZED PLACEBO CONTROLLED TRIAL

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Introduction: In the United States, millions suffer from the effects of insomnia, broadly defined as disturbances in sleep (e.g., difficulty falling or staying asleep; Zeng et al., 2020). Notably, sleep disturbances are associated with negative physical (e.g., hypertension, diabetes) and mental (e.g., depression, suicidal ideation) health outcomes (Wu et al., 2023). Cannabidiol (CBD), a non-intoxicating molecule derived from the *Cannabis sativa* L. plant, has garnered recent interest as a potential therapeutic for sleep disturbances. Existing preclinical (Chagas et al., 2013; Hsiao et al., 2012) and human subjects (Chagas et al., 2014; Notcutt et al., 2004; Shannon et al., 2016; Shannon et al., 2019) work suggests that CBD may alleviate sleep disturbances. To date, no study has investigated the effects of repeated CBD administration on sleep disturbances in a randomized, control trial.

Methods: The current study examined the effects of 300mg CBD, 50mg CBD, and placebo administered daily for 14 days on sleep disturbances as a secondary analysis of data drawn from a larger study that was designed to examine the effects of CBD on worry among a sample of high trait worriers. Participants (N = 63; M_{age} = 29.27; SD_{age} = 9.58) were elevated in trait worry, meeting a comprehensive list of eligibility criteria (e.g., no past month CBD or THC use). Participants were randomly assigned to condition (300mg CBD, 50mg CBD, placebo) and reported sleep disturbances (PROMIS Sleep Disturbance Scale [PROMIS-Sleep]; Buysse et al., 2010) at baseline (prior to randomization), and day 14.

Results: Mixed effects models revealed a significant time by condition interaction for 300mg vs 50mg (β = 5.49, t = 2.59, $p < 0.05$, η^2 = 0.11) and 300mg vs placebo (β = 4.44, t = 2.09, $p < 0.05$, η^2 = 0.11). Simple slope tests revealed significant decreases in PROMIS-Sleep scores from day one to week two among the 300mg (β = -7.53, CI[-10.57, -4.50]) and placebo (β = -3.10, CI[-6.07, -0.12]), but not 50mg (β = -2.05, CI[-5.02, 0.93) condition. Post hoc pairwise trend comparisons revealed the 300mg condition reported significantly greater decreases in PROMIS-Sleep scores vs the 50mg condition (β = -5.49, t = -2.59, $p < 0.05$, d = 1.14), but not placebo (β = -4.44, t = -2.09, p = 0.10, d = 0.92). The trend of PROMIS-Sleep score within the 50mg condition did not significantly differ from placebo (β = 1.05, t = 0.50, p = 0.872, d = 0.22).

Conclusions: These data suggest 300mg of CBD reduces sleep disturbances compared to 50mg of CBD and placebo after two weeks of administering CBD. The current study extends the small, but growing empirical literature regarding the effects of CBD on sleep-related outcomes. These results are drawn from a secondary analysis of data drawn from a larger study that was designed to examine the effect of CBD on worry among a sample of high trait worriers. Therefore, future work should further explore the effects of CBD on sleep in a randomized, placebo control trial specifically designed to assess the effects of CBD on insomnia.

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A SURVEY STUDY EXAMINING THE SUBJECTIVE EFFECTS OF HEXAHYDROCANNABINOL COMPARED TO Δ-9-TETRAHYDROCANNABINOL, Δ-8-TETRAHYDROCANNABINOL, AND CANNABIDIOL

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Introduction: Hexahydrocannabinol (HHC), an intoxicating cannabinoid of the *Cannabis sativa* L. plant (cannabis), is one of many cannabinoids that has become commercially available with the rapidly increasing cannabis marketplace. Previously, many individuals used cannabis products that primarily contained Δ-9 tetrahydrocannabinol (Δ9-THC) or cannabidiol (CBD); however, as a result of the 2018 Farm Bill, increases in minor cannabinoids, such as delta-8-tetrahydrocannabinol (Δ8-THC) and HHC have been observed. Given the increasing accessibility and use of HHC, it is important to compare the subjective effects of HHC to other commonly used cannabinoids (i.e., Δ9-THC, Δ8-THC, CBD), in order to further characterize potential benefits and drawbacks of HHC use.

Methods: Participants (*N*=114) self-reported HHC use at least once in the past six months recruited from Prolific, a crowdsourcing platform. Qualifying participants completed a 20-minute survey that asked them about their HHC and other cannabinoid use. Participants were compensated $4.20 for study completion.

Results: A series of paired sample t-tests revealed meaningful differences in subjective effects across cannabinoids (HHC, Δ9-THC, Δ8-THC, CBD). Ratings for overall drug effect were meaningfully higher for Δ9-THC (*M*=55.30, *SD*=38.31) compared to HHC (*M*=46.34, *SD*=35.89; *t*(89)=-2.83, *p*=0.006, *d*=0.30), were relatively equal for Δ8-THC (*M*=44.95, *SD*=34.60) compared to HHC (M=45.82, *SD*=36.43; *t*(76)=0.27, *p*=0.79, *d*=0.03), and were meaningfully lower for CBD (*M*=19.46, *SD*=24.56) compared to HHC (*M* = 48.92, *SD* = 36.47; *t*(82)=7.39, *p*=<.001, *d*=0.81). Ratings for feeling high were meaningfully higher for Δ9-THC (*M*=75.88, *SD*=25.76) compared to HHC (*M*=66.31, *SD*=29.44; *t*(90)=-3.23, *p*=<.001, *d*=0.34), were relatively equal for Δ8-THC (*M*=60.49, *SD*=29.96) compared to HHC (*M*=66.15, *SD*=30.10; *t*(78)=1.47, *p*=0.15, *d*=0.17), and were meaningfully lower for CBD (*M*=22.39, *SD*=29.92) compared to HHC (*M*=66.22, *SD*=29.77; *t*(83)=9.99, *p*=<.001, *d*=1.09). Subjective drug effect ratings were analyzed across multiple indications (e.g., anxiety, munchies), as well as adverse effects and will be included on the poster.

Conclusions: Findings suggest that, when comparing subjective effects of HHC, users reported dampened effects compared to Δ9-THC, similar effects compared to Δ8-THC, and heightened effects compared to CBD. Laboratory-based work is needed to clarify the differences in subjective effects across cannabinoids.

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MOTIVATIONS, PERCEPTIONS AND EFFECTS OF CANNABIS USE IN INDIVIDUALS WITH MOOD AND ANXIETY DISORDERS

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Introduction: Cannabis use is common in individuals with mood and anxiety disorders, the most prevalent mental health conditions in Canada and worldwide. In the era of cannabis legalization, medical cannabis program and a variety of available cannabis products in Canada, there is controversy and uncertainty about the potential risks and benefits of cannabis use in these individuals. Thus, we conducted a systematic review of the current scientific literature and a mixed methods study evaluating perceptions, motivations, knowledge, and effects of cannabis use in individuals with mood and anxiety disorders.

Methods: For the review, PubMed database was searched and PRISMA guidelines were followed. The mixed methods study included an anonymous survey and in-depth interviews focusing on perceptions, motivations, knowledge, and effects of cannabis use in individuals with mood and anxiety disorders. Participants of the mixed methods study were recruited at the Centre for Addiction and Mental Health in Toronto.

Results: A total of 73 peer-reviewed articles met eligibility criteria and were summarized qualitatively. 36 adult participants with a mood disorder, anxiety disorder, OCD, or PTSD and current cannabis use (CU) completed qualitative virtual interviews. 209 participants completed the survey (130 current cannabis users, 41 past users, and 38 non-users). Preliminary findings from the survey and the interview indicate that about half of individuals reported age <18 years when initiating CU, with curiosity, peer pressure and acceptance, but also treatment of mental health symptoms and sleep problems among the most common motives. Nearly 50% of cannabis users in the survey reported CU for medical and - at the same time - recreational purposes. It was further observed in the interview that with respect to using cannabis for medical reasons, only about 20% reported this being recommended and/or prescribed, and less than 15% obtained information about cannabis from a medical professional. In addition, the majority of participants reported using inhalation as their preferred consumption method and obtaining products from non-regulated sources. Commonly reported concerns and negative effects associated with CU (from interview, survey and review) included worsening of cognitive functioning (memory, concentration), energy, motivation, productivity, and depressive symptoms. Reported motivations, explanations and reasons for CU included coping motives, insomnia, managing side effects of pharmacological treatment or CU being perceived as safer than alcohol use. Studies in PTSD included in the review indicated additional reasons for CU, such as confronting sources of trauma. Participants in the interview found cannabis products with high CBD helpful for sleep problems and pain, including migraines. High THC content was perceived to enhance creativity, focus, and libido and as helpful to overcome to inhibitions and be more extroverted.

Conclusion: Initial results from this work reveal motives and reasons for initiating and maintaining CU in individuals with mood and anxiety disorders and indicate some perceived benefits of cannabis and specific cannabis products and/or cannabinoids. However, negative effects related to CU are also regularly reported. CU for medical reasons concurrent with recreational CU, use of non-prescribed cannabis for medical reasons, inhalation as preferred method for consumption, and use non-regulated cannabis products were commonly reported. While this work will improve our understanding of reasons and motives for CU in individuals with mood and anxiety disorders, it also highlights areas of concern that we think will be relevant to inform policy, prevention strategies, and clinical practice.

PART I: AN OPEN LABEL CLINICAL STUDY TO EVALUATE THE SAFETY AND EFFICACY OF MEDICANE'S BALANCED T3:C3 MEDICAL CANNABIS OIL FOR TREATMENT OF AGITATION AND DISRUPTIVE BEHAVIORS IN SUBJECTS WITH DEMENTIA

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Introduction: Behavioral symptoms are considered core features of dementia. Agitation and disruptive behaviors, expressed as excessive fidgeting, restlessness, pacing, shouting, uninhibited behaviors and aggression, occur in over half of the subjects suffering from dementia during the course of their illness. These are the most challenging behaviors to treat and are associated with significantly increased subject suffering, caregiver burden, increased treatment costs and institutionalization rates. The pharmacological interventions currently used for the treatment of agitation and disruptive behaviors, including off-label use of antipsychotics, sedatives/hypnotics, anxiolytics, acetylcholinesterase inhibitors, and antidepressants, are associated with limited efficacy and an increased risk of side effects, morbidity, and mortality. Here, in Part I of the clinical study, we sought to investigate the safety and efficacy of MediCane's marketed, balanced T3:C3 oil as an add-on therapy to standard of care, in reducing agitation and disruptive behaviors in subjects with dementia.

Methods: The study is designed as a two-part study, Part I an Open-label; and Part II a Randomized double-blind, placebo-controlled study to evaluate the safety and efficacy of MediCane's balanced T3:C3 medical cannabis oil for treatment of agitation and disruptive behaviors in subjects with dementia.

Results: The open label phase of the study was initiated by M.H MediCane Ltd. in the two largest medical centers in Israel and began recruiting in October 2022. To date, nine subjects have been enrolled and are in different stages of the study.

Conclusions: Characteristics of the Investigational Medicinal Product, the study design, and initial insights will be presented.

EFFECTS OF SYNTHETIC CANNABIDIOL, DELTA-9-TETRAHYDROCANNABINOL, AND A COMBINATION OF BOTH, ON AMYLOID PATHOLOGY IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

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Introduction: Considering epidemiologic data, the search for novel therapies for Alzheimer's disease (AD) is utterly urgent. In this context, the use of phytocannabinoids for the treatment of several symptoms of AD (such as nocturnal agitation) has been proposed for several decades. Specifically, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have been proposed as putative therapeutics for AD. THC exhibits several properties, such as reduction of inflammation, induction of hippocampal neurogenesis, and enhancement of beta amyloid (Aβ) removal, that are promising in the context of AD. On the other hand, CBD has been shown to be a potent antioxidant and anti-inflammatory, and to induce neuroprotection, both *in vitro* as well as *in vivo*. In the context of AD, CBD has proven beneficial effects in neuronal cell lines, glial cells, and in animal models of this disease. The present studies have been designed to explore the potential anti-inflammatory and neuroprotective effects of synthetic cannabinoids (CBD, THC and a combination of both) in the 5xFAD mouse model of AD.

Methods: Eight-month-old male hemizygous mice (N=16 per treatment group) co-expressing five familial Alzheimer's disease mutations (5xFAD) and their wildtype (WT) littermates were used. Synthetic CBD, THC, or CBD:THC were supplied by Purisys LLC (Athens, Georgia, USA), made up in vehicle (VEH; Cremophor®:Ethanol:saline in a ratio of 1:1:18). Mice were treated for 28 consecutive days. Each mice received a daily i.p. injection containing CBD (0.273 mg/kg), THC (0.205 mg/kg), or CBD:THC (0.273mg/kg:0.205 mg/kg) and subjected to behavioral tests, including Barnes maze, elevated plus maze (EPM), tail suspension (TS), open field and rotarod. Brain samples were used to quantify the expression of several neuroinflammation-related parameters.

Results: THC enhanced anxiety and depression (EPM and TS tests), while CBD and THC showed different effects when administered alone than in combination (Barnes maze test). All treatments with these synthetic cannabinoids led to an increase in the insoluble form of Aβ. Importantly, these effects were not accompanied by significant changes in molecular parameters of inflammation, at the mRNA or protein levels.

Conclusions: The exposure to synthetic cannabinoids modifies behavior in 5xFAD mice and alters amyloid dynamics.

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ASSOCIATION OF G-PROTEIN COUPLED RECEPTOR 55 (GPR55) SINGLE NUCLEOTIDE POLYMORPHISMS AND ALZHEIMER'S DISEASE PHENOTYPES

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Introduction: The G-protein-coupled receptor 55 (GPR55) is a lysophosphatidylinositol-sensitive receptor. Various cannabinoid molecules – e.g., cannabidiol and THC– modulate GPR55 signalling. *GPR55* mRNA is expressed in several brain areas including the hippocampus and cerebellum. GPR55 signalling and single nucleotide polymorphisms (SNPs) have been indicated in modulating inflammation, homeostasis and has potential as a therapeutic target for neurodegenerative diseases with neuroinflammatory backgrounds such as Alzheimer's disease (AD). However, foundational knowledge on functional GPR55 SNPs and AD phenotypes is lacking. We hypothesised that genetic variation in GPR55 SNPs may be associated with differential changes in AD biomarker, brain atrophy and cognitive decline.

Methods: This retrospective, longitudinal, observational study used data from the Alzheimer's Disease Neuroimaging Initiative (ADNI; n=1019). Clinically normal patients without AD whom were amyloid beta-42 (Aβ42) PET negative (n=444) and patients with biological AD hence Aβ42 PET positive at MCI or mild AD stages (ADMCI; n=574) were sampled from the three phases of ADNI. GPR55 SNPs representative of clusters in linkage disequilibrium were screened with the following inclusion criteria: (i) minor allele frequency greater than 10%; (ii) significant differences between major allele homozygotes and minor allele carriers (dominant model) in two or more AD phenotypes (AD biomarker abnormalities, neurodegeneration and cognitive decline). Cerebrospinal phosphorylated tau (p-tau) for AD biomarkers, MRI brain volumes for neurodegeneration, Mini-Mental State Exam (MMSE), Logical Memory Delayed Recall Test and Cognitive Subscale Alzheimer's Disease Assessment Scale (ADASQ4) were selected to evaluate overall cognitive status, executive function and memory. Longitudinal associations were assessed as interactions between SNP and visit (baseline, 24 and 48 months) in linear mixed effects models, controlling for sex, age, baseline MMSE, APOE e4 and baseline intracranial volume.

Results: Screening resulted in three SNPs fulfilling the criteria: rs2969126G/C, rs3111776C/T and rs7574470T/C. ADMCI rs2969126 minor-C allele carriers showed significantly greater declines in cerebrospinal p-tau concentrations ($F_{(1,68.47)}$=3.99, β=-0.11, p=0.049) and inferior temporal cortex volumes ($F_{(1,91.32)}$=6.1, β=-25.77, p=0.015). ADMCI rs311176 T-allele carriers showed greater deterioration in cerebellum ($F_{(1,90.82)}$=4.94, β=-39.22 p=0.028) and word recognition ($F_{(1,132.19)}$=4.4, β=0.02, p=0.038). ADMCI rs754470 C-allele carriers showed increased thalamus volume ($F_{(1,132.44)}$=5.42, β=11.53, p=0.021) and MMSE decline ($F_{(1,158.44)}$=5.1, β=-0.05, p=0.025). In healthy rs754470 C-allele carriers, slower deterioration in whole-brain ($F_{(1,169.87)}$=5.35, p=0.022) and fusiform ($F_{(1,176.32)}$=5.21, β=202.95, p=0.024) was observed, with improvement in memory ($F_{(1,115.50)}$=4.83, β=0.05, p=0.029).

Conclusions: Genetic variation in GPR55 may play a role in cognitive decline and cerebral atrophy in AD. Opposite relationships in amyloid negative cognitively normal people for rs754470 (TT vs TC/CC) suggest potential interactions between cannabinoid signalling and AD pathogenesis. These preliminary findings prompt replication studies across the stages of AD progression, to understand the potential role of GPR55 and its ligands in brain functionalities and therapeutics.

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

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

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 1 Hz paired-pulse stimulation of the Schaffer collaterals or by exposure to the metabotropic glutamate receptor agonist DHPG.

Results: We examined the role of eCB signaling in LTD by recording fEPSPs in the CA1 stratum radiatum in hippocampal slices from juvenile mice. Significant LTD (~50% decrease from baseline) could be induced by either 15 min of 1 Hz electrical stmulation or 10 min of exposure to DHPG. The magnitude of both forms of LTD was significantly reduced by blocking cannabinoid receptor activation with the CB1 receptor antagonist NESS-0327. The roles of the endogenous ligands 2-AG and anandamide were examined by using selective inhibtors of DAG-lipase and NAPE-PLD, respectively. Electrical stimulation-induced LTD was significantly reduced by the NAPE-PLD inhibitor LEI-401, but was not affected by the DAG-lipase inhibitor DO34. DO34, however, significantly reduced DHPG-induced LTD.

Conclusions: These results indicate that both stimulation-induced LTD and DHPG-induced LTD require activation of CB1 receptors. Interestingly, the endogenous cannabinoid anandamide is required for sitmulation-induced LTD. 2-AG does not appear to contribute to stimulation-induced LTD, but is required for DHPG-induced LTD.

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THE ROLE OF MICROGLIA IN 5XFAD/FAAH$^{-/-}$ MICE: AN *IN VIVO* MULTIPHOTON MICROSCOPY AND MOLECULAR STUDY

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Introduction: Alzheimer's disease (AD) is the most common form of dementia. Neuroinflammation in AD is triggered by the formation of neurofibrillary tangles of hiperphosphorylated tau protein, together with the deposition of extracellular 1-42 and 1-40 beta amyloid peptides (Aβ) that form neuritic plaques in the brain parenchyma. Although neuroinflammation has a prominent role in the pathogenesis of AD, its specific role in the progression of AD is still controversial. Recently, we performed a Gen Set Enrichment Analysis (GSEA), confirming a paradoxical effect observed in previous *in vitro* and *in vivo* studies in 5xFAD mice lacking the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH); these data showed an exacerbated inflammatory phenotype associated to a neuroprotective effect, including recovery of synaptic dysfunction, dendritic spine preservation and improvement of several behavioral parameters.

Methods: We performed a time course analysis in 5xFAD/Cx3cr1$^{+/GFP}$ and 5xFAD/FAAH$^{-/-}$/Cx3cr1$^{+/GFP}$ mice by *in vivo* multiphoton microscopy in order to understand better the role of microglial cells. Mice were subjected to surgery to implant a cranial window, granting direct access for the observation of the brain parenchyma. After 4 weeks of recovery, mice were exposed to PLX5622 (a CSF1R antagonist) in the diet for 28-days to cause the pharmacological ablation of microglia. Images were obtained once per week. Afterwards, mice were switched to control diet for 7 additional days to allow microglia re-population. In addition to image analysis, a molecular analysis (mRNA and protein levels) was performed in Cx3cr1$^{+/GFP}$, 5xFAD/Cx3cr1$^{+/GFP}$, FAAH$^{-/-}$/Cx3cr1$^{+/GFP}$ and 5xFAD/FAAH$^{-/-}$/Cx3cr1$^{+/GFP}$ mice fed with PLX5622 chow ad libitum for 4 weeks.

Results: After one week of exposure to PLX5622, and as expected, we found an almost complete disappearance of brain microglia; cessation of the treatment allowed a quick re-population of the brain by microglial cells. Our data showed that the genetic inactivation of FAAH altered the neuroinflammatory and microglial profiles in the brains of 5xFAD mice. Furthermore, we found that some altered parameters were found selectively in microglial cells, as the treatment with PLX prevented those changes.

Conclusions: These results may indicate that the genetic inactivation of FAAH would lead to a significant modification of the microglial activity profile in the context of AD.

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CANNABINOID TYPE 2 RECEPTORS MODULATE MICROGLIA FUNCTIONS IN A MOUSE MODEL OF ALZHEIMER DISEASE

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Microglia, as resident immune cells in the central nervous system (CNS), is involved in a wide variety of pathophysiological processes. Its ability to dynamically shift between different polarization states is thought to contribute to the initiation and evolution several neurodegenerative diseases. Alzheimer's disease (AD) is characterized by an intense and chronic neuroinflammation derived from the aggregation of amyloid peptide (Aβ) in the brain parenchyma, as well as the formation of neurofibrillary tangles inside the neurons. Microglia cells seem to play a relevant role in the amyloid-triggered neuroinflammation.

Cannabinoid type 2 (CB_2) receptor is primarily expressed by microglia, exclusively under neuroinflammation conditions and appears to be involved in the modulation of microglial phenotypes. To study the role of CB_2 in adult microglia in the neuroinflammatory context derived from Aβ plaques, we used the mouse model $5xFAD^{+/-}/CB_2^{EGFP/f/f}/Cx3cr1^{CreER}$, recently validated by our group. This conditional, microglia specific CB_2KO model, inducible by tamoxifen administration, allowed us to eliminate CB_2 receptor exclusively in microglia and in adulthood. To find out whether CB_2 deletion still improved Alzheimer's disease-related behavioral deficits, we analyzed spatial memory using the Barnes maze, anxiety, depression, and locomotor coordination (elevated plus maze, tail suspension and rotarod, respectively). Next, we isolated microglial cells from the brains of 6-month-old mice and made a detailed characterization by flow cytometry. Our findings revealed that deletion of CB_2 receptors leads to impaired microglia phagocytic activity and to a proinflammatory phenotype. Also, lysosomal activity was analyzed staining acidic organelles with LysoTracker Red. In addition, subtle differences were observed in neuritic plaque growth and structural features, including plaque volume, protein density, and plaque entropy.

These findings may suggest that CB_2 receptors exert a concerted action modulating microglia activation, phagocytosis and Aβ plaque formation in the context of amyloid-induced damage.

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SYSTEMIC CHANGES IN ENDOCANNABINOIDS AND ENDOCANNABINOID-LIKE MOLECULES IN RESPONSE TO PARTIAL NEPHRECTOMY-INDUCED ISCHEMIA IN HUMANS

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Introduction: Renal ischemia–reperfusion (IR), a routine feature of partial nephrectomy (PN), can contribute to the development of acute kidney injury (AKI). Rodent studies show that the endocannabinoid system (ECS) is a major regulator of renal hemodynamics and IR injury; however, its clinical relevance remains to be established. Here, we assessed the clinical changes in systemic endocannabinoid (eCB) levels induced by surgical renal IR.

Methods: Sixteen patients undergoing on-clamp PN were included, with blood samples taken before renal ischemia, after 10 minutes of ischemia time, and 10 minutes following blood reperfusion. Kidney function parameters [serum creatinine (sCr), blood urea nitrogen (BUN), serum glucose] and eCB levels were measured. Baseline levels and individual changes in response to IR were analyzed.

Results: The baseline levels of eCB 2-arachidonoylglycerol (2-AG) were positively correlated with kidney dysfunction biomarkers. Unilateral renal ischemia increased BUN, sCr, and glucose, which remained elevated following renal reperfusion. Renal ischemia did not induce changes in eCB levels for all patients pooled together. Nevertheless, stratifying patients according to their body mass index (BMI) revealed a significant increase in *N*-acylethanolamines (anandamide, AEA; *N*-oleoylethanolamine, OEA; and *N*-palmitoylethanolamine, PEA) in the non-obese patients. No significant changes were found in obese patients who had higher *N*-acylethanolamine baseline levels, positively correlated with BMI, and more cases of post-surgery AKI.

Conclusions: Unilateral renal ischemia induces an immediate elevation of kidney dysfunction biomarkers and *N*-acylethanolamines in non-obese patients. Obese patients, with higher eCB 'tone', showed no response. With the inefficiency of 'traditional' IR-injury 'preventive drugs', our data support future research on the role of the ECS and its manipulation in renal IR.

DEVELOPMENT AND APPLICATION OF GENETICALLY ENCODED SENSORS FOR ENDOCANNABINOIDS

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Introduction: Endocannabinoids (eCBs) are retrograde neuromodulators that play important roles in a wide range of biological processes. They include anandamide (AEA) and 2-arachidonoylglycerol (2-AG), whose release and *in vivo* dynamics remain largely unknown due to a lack of suitable probes for detecting eCBs with sufficient spatiotemporal resolution.

Results: We developed a genetically encoded eCB sensor $GRAB_{eCB2.0}$ by inserting a cpEGFP into the ICL3 of the human CB1 receptor. This sensor has proper cell membrane trafficking and a robust fluorescence change at physiological eCB concentrations. Using this sensor, we monitored eCB dynamics in several biological conditions in vitro and *in vivo*. Furthermore, we developed $GRAB_{AEA1.2}$ and $GRAB_{2\text{-}AG1.2}$ by structure-guided protein engineering based on $GRAB_{eCB2.0}$. These sensors showed specific responses to AEA and 2-AG respectively in HEK293T cells and cultured neurons. They could robustly report electrical stimulation evoked endogenous AEA or 2-AG release in cultured neurons and acute brain slices. Moreover, we improved the performances of these eCB sensors by point mutation screening to optimize fluorescent response amplitude and apparent affinity. We developed the next generation eCB sensors, including $GRAB_{eCB3.0}$ and $GRAB_{2\text{-}AG1.5}$, which showed improved fluorescent signals and maintained the pharmacological properties. We also developed the new AEA sensor, $GRAB_{AEA1.5}$, which showed improved response and apparent affinity to AEA, while still showing no significant signals for 2-AG at physiological concentrations.

Conclusion: $GRAB_{eCB}$ sensors are robust probes for measuring the dynamics of eCBs under both physiological and pathophysiological conditions, which could provide more details about endocannabinoid dynamics in various biological processes.

GENERATING CONDITIONAL MUTANT ALLELES IN *FAAH2* ORTHOLOGS IN ZEBRAFISH

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Introduction: In humans, the genes for the two fatty acid amide hydrolases, *FAAH* and *FAAH2* exhibit unique expression patterns and cellular localization, and evidence suggests these genes have distinct clinical implications. *FAAH2* mutations have been associated with neurodevelopmental delay, seizures and an autism phenotype. Currently, functional research on this gene is limited given that most placental mammals lack a *FAAH2* ortholog. However, zebrafish possess two *FAAH2* orthologs, *faah2a* and *faah2b,* in addition to a *FAAH* ortholog, *faah*. Given that zebrafish are genetically tractable, routinely used in compound screens and exhibit complex behaviors, they provide a great opportunity to study the role of anandamide metabolism on behavior and to study the impact of FAAH-modulating compounds on an organism with multiple FAAH enzymes.

Methods: Here, we are using the GeneWeld targeted integration approach to generate conditional mutant alleles in the zebrafish genes *faah*, *faah2a*, and *faah2b*. We designed and screened guide RNAs (gRNAs) targeting each gene and developed Uflip vector-based plasmids to reversibly silence these genes. We injected zebrafish embryos at the 1-cell stage to generate F0 mosaic mutant zebrafish, which we are raising to reproductive maturity to assess germline transmission. We conducted a pilot larval behavioral assay to screen endocannabinoid (eCB) modulating compounds.

Results: We found efficient gRNAs targeting each gene and are developing conditional mutant alleles in *faah*, *faah2a* and *faah2b* using the Uflip plasmid. We characterized the dosing and effects of FAAH inhibitor URB-597, MAGL inhibitor JZL-184 and dual FAAH/MAGL inhibitor JZL-195 on wildtype larval zebrafish behavior to serve as a basis for mutant screens and subsequent novel compound behavioral testing.

Conclusions: We are developing a repertoire of zebrafish with conditional mutations in each of the faah genes in tandem with a behavioral drug screen paradigm. This will allow us to distinguish the contributions of *faah*, *faah2a* and *faah2b* in zebrafish behavior as well as test eCB modulating compounds in an animal model with a similar eCB metabolism to humans.

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PERFORMANCE OF A NOVEL LC-MS/MS PANEL TO MEASURE PHYTOCANNABINOIDS AND ENDOCANNABINOIDS IN CANNABIS USERS

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Introduction: Phytocannabinoids represent a broad class of bioactive compounds originally identified in Cannabis sativa, and serve as exogenous ligands within the endocannabinoid system. The endocannabinoid system (ECS) is involved in influencing a wide range of physiological processes including nociception, inflammation, appetite, mood, memory, immune function, and locomotion. As such, dysregulation of the ECS has been implicated in the etiology of, and potential treatment for, a number of diseases and conditions affecting those processes. The quantification of endocannabinoids, the signaling molecules in this pathway, has been challenging due to their low abundance and structural variability. The ability of phytocannabinoids to directly influence the endocannbinoid systems to elicit a very diverse set of physiological profiles that impact biological pathways regulating pain, inflammation, neurological disorders, and cancer is the primary driver for investigating their potential utility in the treatment of pain and disease.

Methods: A targeted metabolomics panel utilizing UPLC-MS/MS has been developed that enables the simultaneous absolute quantification of eleven endocannabinoids, and fifteen phytocannabinoids and associated metabolites, from human plasma. The method utilizes protein precipitation and solid phase extraction of a 100 µL sample aliquot, followed by reverse-phase chromatographic separation on an Agilent UPLC with Sciex 5500 QTrap UPLC-MS system operated in MRM mode. The method is able to achieve lower limits of quantitation in the pg/mL range. Phytocannabinoids including tetrahydrocannabinols, cannabidiols, and cannabichromenes are quantified. Key endocannabinoid CB1/CB2 agonists such as anadamide/N-arachidonoylethanolamide (AEA) and 2-arachidonylglycerol and their biologically relevant conjugates are also measured.

Results: The method was analytically validated prior to use, with experiments performed to determine precision, accuracy, linearity, specificity, and stability. The analytes met all acceptance criteria for validation and achieved an average within-day and between day precision of less than 5 percent (%). All analytes were stable through three freeze-thaw cycles and 3 hours on ice during sample preparation. Endo- and phytocannabinoids were measured in a set of samples from consented young adult cannabis users and control subjects. Multiple phytocannabinoids, endocannabinoids, and related metabolites were quantified, and associated changes were seen between study groups.

Conclusions: A targeted metabolomics panel capable of measuring various phytocannabinoid and endocannabinoid metabolites has been developed. This panel may be used to simultaneously quantify phytocannabinoid and endocannabinoid metabolites in subjects using cannabis or cannabis-derived therapeutics and can help give insight into the complex regulation of the endocannabinoid system.

THE MONOACYLGLYCEROL LIPASE INHIBITOR ABX-1431 DOES NOT IMPROVE ALCOHOLIC HEPATIC STEATOSIS

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Introduction: Alcoholic liver disease (ALD) affects approximately fifteen million Americans and can be fatal. Alcoholic liver disease comprises a spectrum of conditions ranging from fatty liver (steatosis) to cirrhosis. Under normal conditions, hepatocytes store limited amounts of triglycerides (TG) in cytosolic lipid droplets and most TGs are oxidized and secreted into the bloodstream. Alcohol stimulates *de novo* lipogenesis and decreases lipid droplet autophagy and secretion. Triglycerides in hepatocyte lipid droplets are hydrolyzed by adipose triglyceride lipase, hormone-sensitive lipase and monoacylglycerol lipase (MAGL). Further, MAGL additionally plays a critical step in diminishing cannabinoid receptor 1/2 (CB1/2) signaling by catalyzing 2-arachidonoyl glycerol (2-AG) hydrolysis. Past studies in mice with global and myeloid-specific MAGL gene knockout showed reduced liver fibrosis and inflammation after liver-specific insults induced by various conditions but not alcohol. Whether MAGL inhibition can reduce ALD was the subject of our investigation.

Methods: We evaluated the efficacy of ABX-1431, a selective inhibitor of MAGL, in a mouse model of ALD wherein hepatic steatosis was induced by feeding animals the Lieber-DeCarli (LDC) alcohol-containing liquid diet. This clinically tested compound represents a pharmacologically relevant approach for MAGL inhibition and has excellent ADMET properties and selectivity. Mice were maintained either on a control diet or on LDC for 4 weeks. Animals on LDC were pair-fed and administered either vehicle (2% NMP/canola oil) or ABX-1431 at 0.3, 1 and 3 mg/kg once daily by oral gavage for an additional 31 days. At the end of the experiments, target tissues were collected for enzymatic measurements and histology.

Results: Treatment with ABX-1431 led to increased mortality in mice on LDC compared to vehicle. Further, enzymatic biomarkers did not significantly improve upon treatment with ABX-1431. The lack of efficacy was confirmed through liver histology – animals receiving ABX-1431 had similar liver injury compared to those receiving vehicle.

Conclusions: In summary, these studies indicate that inhibition of MAGL using ABX-1431 does not improve ALD in the mouse LDC model and that inhibition of MAGL is unlikely to be a viable therapeutic option for this condition.

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SELF-SELECTED FAVOURITE MUSIC INDUCES ANALGESIA IN HEALTHY PARTICIPANTS WITHOUT ALTERATIONS IN SERUM ENDOCANNABINOIDS

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Introduction: The endocannabinoid system is involved in a multitude of physiological processes, including pain modulation. A growing body of literature has investigated the analgesic effects of guided music listening (GML). Previous approaches exploring GML have used standardised music to assess the effect of music-induced analgesia (MIA) in acute experimental and chronic pain. The aim of this study was to investigate the effect of self-selected favourite music vs pink noise on a dynamic set of experimental heat pain intensity. A second aim was to determine if music analgesia is accompanied by alterations in peripheral endocannabinoid/*N*-acylethanolamine levels in male and female healthy human volunteers.

Methods: 39 healthy volunteers were recruited and consented to procedures approved by the University of Toronto Research Ethics Committee (19 female, 20 male; mean age ± SD: 27.5 ± 4.7 years). Participants were asked to fast for 10 hours before attending the research centre and provided a standardised snack upon arrival. All experiments were carried out in the morning. Questionnaire data and a baseline blood sample were obtained. Participants received a fluctuating tonic heat stimulus, with a baseline of 32°C and peaks ranging between 43 and 47°C, on their non-dominant volar forearm for the duration of the auditory stimuli. The order in which participants received the music or pink noise was counterbalanced and randomly assigned. Heat pain intensities were continuously recorded using an electronic visual analogue scale. The overall pain intensities for each condition were calculated as the area under the rating curve. A blood sample was obtained after each auditory stimulus session. Quantification of serum 2-arachidonoylglycerol (2-AG), anandamide (AEA), *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), was carried out by LC-MS/MS for 32 participants. Two-way repeated measures ANOVAs were performed, and $P<0.05$ was considered significant.

Results: There was a main effect of condition (pink noise vs music), whereby favourite music resulted in significantly lower pain intensity scores compared to pink noise (favourite music AUC: 2799.96 ± 2289.7, Pink Noise AUC: 3446.47 ± 2616, $p=0.037$). There were no changes in serum 2-AG, AEA, PEA and OEA levels.

Conclusions: This exploratory study supports the hypothesis that self-selected favourite music reduces subjective pain intensity ratings compared to pink noise. A potential role for the endocannabinoid system in MIA is yet to be determined.

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M3 MUSCARINIC RECEPTOR AGONIST PILOCARPINE DRIVES INCREASES IN ENDOCANNABINOIDS IN LACRIMAL GLANDS

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Introduction: There is a small but growing literature illustrating the relationship between muscarinic receptor activity and endogenous cannabinoid (eCB) system signaling (refs). Recently, we reported that the muscarinic receptor agonist, pilocarpine (PC), effectively drives an increase in tearing in male mice, as a positive control for examining levels of tearing to CB agonists and antagonists. The synthetic CB, CP, decreased tearing in male mice. However, CP had no effect on tearing in mice that were pretreated with PC suggesting an interaction of these signaling systems. Previous work by others suggests that M3 agonists are driving the production of eCBs in the in CNS. Here, we test the hypothesis that eCBs are regulated in the lacrimal glands by PC treatment.

Methods: Male mice were given a signal dose of pilocarpine (7mg/kg *s.c.*) and sacrificed 1 hour post injection and lacrimal glands collected. Lipids were extracted from lacrimal glands via methanolic extraction followed by centrifugation and then partial purification on C18 solid phase extraction columns. Lipids of interest were concentrated in the 100% methanol fraction and analyzed via HPLC/MS/MS.

Results: Levels of NAEs were increased in the PC treated lacrimal gland with levels of Anandamide and PEA being significantly higher. Importantly, levels of 2-AG were almost doubled in the PC treated lacrimal gland ($p \leq 0.02$), whereas all other 2-acyl glycerol species showed no changes.

Conclusion: The specific increases in 2-AG after the M3 receptor agonist treatment in an exocrine gland is further evidence that there is a signaling link between these endogenous signaling systems that is recapitulated throughout the body.

PLASMA LEVELS OF ENDOCANNABINOIDS AND RELATED LIPIDS BEFORE AND AFTER MTBI

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Introduction: Mild Traumatic Brain Injury (mTBI), or concussion, can be attributed to a majority of the head-injury related emergency room visits every year and are caused by a hard hit to the head, which can result in symptoms of cognitive impairment. Usually these symptoms will resolve within a matter of weeks with correct care, but can remain longer especially if multiple head hits occurred. Here we sought to elucidate some of the effects at a molecular level from one or multiple head hits by examining the levels of endogenous cannabinoids and related lipids in plasma post-injury. Specifically, we hypothesized that endocannabinoids, such as N-acylethanolamines (NAEs) and 2-acyl-sn-glycerols (2-AGs), as well as other major lipid families such as Free Fatty acids (FFAs) and N-acyl glycines would rapidly show differences in their concentration after at least one injury when compared to before.

Methods: Adult male Sprague Dawley rats underwent mTBI performed by a closed-head momentum exchange model to produce one (n=7), two (n=7) or three (n=6) mild head injuries (mTBI) in young adult male rats compared to non-injured, age and weight-matched controls (n=8). Plasma was collected prior to the first hit and 15 minutes after the 1st, 2nd, and 3rd hits. To extract lipids, samples were incubated in methanol + 5µL of 1µM internal standard (d8-AEA) for 2h in the dark on ice, then homogenized via sonication, centrifuged at 19,000G, 20˚ C for 20 min, and the lipid-containing supernatant decanted into water to make a ~25% organic solution. Lipids were partially purified from this solution using C18 solid phase extraction columns, eluting into 1-1.5mL of 65%, 75%, and 100% methanol. HPLC/MS/MS was then used to screen for ~87 lipids and calculate mol/gram concentrations for each tissue type. One-way ANOVAs with Fisher's LSD post-hoc tests were used to determine significant differences in lipid levels after one, two, and three head hits when compared to controls. A heatmap showing these changes was made.

Results: Results show that after one head hit, compounds in the NAE, FFA, and N-acyl glycine families increased, while compounds in the 2AG family both increased and decreased. Specifically, all of the compounds in the FFA family were significantly increased by at least three orders of magnitude after one head hit, while all but one compound in the N-acyl glycine family increased by at least two orders of magnitude. Interestingly, the NAE and 2AG families only had one of six and one of four compounds show an increase of two and four orders of magnitude, respectively. The only compound to show a significant decrease was in the 2AG family, which was 2-oleoyl-sn-glycerol by four orders of magnitude.

Conclusions: These findings provide a basis for investigating rapid plasma lipid level change post single heat hit injury in adult male mice. Future studies will investigate the effects of non-head hits (e.g. shoulder hits) and genetic sex.

THC EXPOSURE AND MITOCHONDRIAL DNA IN WHOLE BLOOD: A SECONDARY ANALYSIS FROM AN ACUTE AND RESIDUAL C ANNABIS EFFECTS DRIVING SIMULATOR STUDY

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Introduction: In recent years, research interest into the effects of cannabinoids on mitochondria has emerged. A few high-profile studies have demonstrated the presence of cannabinoid receptors on mitochondria in mice, which have been linked to various behavioural outcomes. Meanwhile, literature on the role of cannabinoids on mitochondria in humans is limited. In particular, there is a lack of studies looking at clinical biomarkers of mitochondrial health in humans who have been exposed to smoked cannabis. In the present study, we analyzed the levels of mitochondrial DNA across a number of timepoints in whole blood taken from individuals who smoked either placebo (THC removed) cannabis, or cannabis containing THC.

Methods: Blood samples were obtained for secondary analysis from a double-blind, placebo-controlled, parallel-group randomized clinical trial. In this study, cannabis users (1-4 days/week) aged 19-25 years were randomized with a 2:1 allocation ratio to receive active (12.5% THC) or placebo (0.009% THC) cannabis in a single 750 mg cigarette. Whole genomic DNA was extracted from blood samples taken at baseline (30 min prior to smoking) and at multiple timepoints following consumption of the cannabis cigarette. DNA was quantified using Quant-IT PicoGreen, and mt-ND1, mt-ND4 and β2M content was measured using SensiFAST SYBR No-ROX. Outliers were identified using ROUT, Q = 1.0%. Data was analyzed using repeated measures ANOVA, followed by Dunnett's multiple comparisons test.

Results: After measuring mitochondrial DNA copy numbers across all participants, we found that mitochondrial DNA in whole blood is reduced in participants who were exposed to the active cannabis cigarette up to the one-hour timepoint. In the placebo group, no significant changes to the levels of mtDNA were detected up to the one-hour timepoint.

Conclusions: THC in smoked cannabis cigarettes is able to reduce the levels of mtDNA in whole blood in healthy, young individuals who are habitual users of cannabis. The exact mechanism for alterations to mtDNA levels in these individuals remains unclear, however this study provides evidence for further work to identify the specific systems or cell types underlying this effect.

MATERNAL GESTATIONAL WEIGHT GAIN IS ASSOCIATED WITH HIGH LEVELS OF ENDOCANNABINOIDS IN BREAST MILK OF BRAZILIAN WOMEN

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Introduction: Anandamide (AEA) and 2-arachidonolylglycerol (2-AG) in human milk (HM) may promote early brain development and positive energy balance. We hypothesized that there is a physiological variation in the levels of AEA and 2-AG in HM between 2-119 postpartum days; that pregestational maternal overweight/obesity, excessive gestational weight gain (GWG), or maternal intake of n-6 polyunsaturated fatty acids during pregnancy are associated with the HM endocannabinoid concentration.

Methods: Women from Rio de Janeiro (n=92) were followed from the third trimester of pregnancy (baseline) to 119 days postpartum, and a total of 149 HM samples were analyzed between postpartum days 2-8 (T1), 28-47 (T2) and 88-119 (T3). HM AEA and 2-AG levels were assessed by high-performance liquid chromatography-mass spectrometry. The HM levels of AEA and 2-AG over time were investigated using Kruskal-Wallis with post-hoc Dunn's test. Multiple linear regression models were performed to test the hypotheses. *P<0.05.

Results: AEA HM levels were found higher in T2 (Md=0.57 ng/mL) compared with T1 (Md=0.13 ng/mL)* or T3 (Md=0.09 ng/mL)*, while 2-AG was higher in T2 (Md=1268.9 ng/mL) and T3 (Md= 916.6 ng/mL) compared with T1 (Md=187.8 ng/mL)*. Multiple linear regression models showed a significant direct association between excessive GWG and milk 2-AG (β=1629; 95%CI: 467-2792, P=0.008). No associations were observed between maternal overweight/obesity or n-6 intake and 2-AG and between maternal factors and AEA.

Conclusions: The precise physiological role of milk endocannabinoids remain to be determined. However, an unbalanced milk level of AEA and 2-AG may contribute to the developmental origins of metabolic diseases associated with maternal overweight or obesity.

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PHOSPHOLIPASE A AND ACYLTRANSFERASE (PLAAT) 1 DEFICIENCY AMERIOLATES OBESITY AND HEPATIC LIPID ACCUMULATION IN MICE

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Introduction: The phospholipase A and acyltransferase (PLAAT) family, consisting of five isoforms (PLAAT1-5), shows both phospholipase A_1/A_2 and *N*-acyltransferase activities. The latter activity of PLAATs produces *N*-acyl-phosphatidylethanolamines (NAPEs), the precursors of bioactive *N*-acylethanolamines (NAEs) including anandamide. PLAAT1 is mainly expressed in muscle, heart, and brain in mice and humans. However, the function of PLAAT1 *in vivo* has not been elucidated. In this study, we focused on the role of PLAAT1 in an obesity model of mice.

Methods: *Plaat1*$^{-/-}$ mice were generated by the CRISPR-Cas9 system. *Plaat1*$^{-/-}$ and wild-type (WT) mice were fed with high-fat diet (HFD) for 12 weeks. Total RNAs were isolated from the liver and were subjected to qPCR. Total lipids were extracted from the liver by the method of Bligh and Dyer, and were analyzed by liquid chromatography-tandem mass spectrometry.

Results: As a result of HFD feeding, WT mice showed obesity with hepatic lipid accumulation. On the other hand, *Plaat1*$^{-/-}$ mice exhibited slight gain of body weight and milder fatty liver. In the liver of *Plaat1*$^{-/-}$ mice, the mRNA levels of peroxisome proliferator-activated receptor-γ and its downstream lipogenic genes were suppressed. Lipid analysis revealed that glycerophospholipid levels tend to increase while lysophospholipid levels tend to decrease in *Plaat1*$^{-/-}$ liver. The levels of NAPEs and NAEs were, however, comparable between WT and *Plaat1*$^{-/-}$ mice.

Conclusion: These results demonstrated that the gene disruption of PLAAT1 improves HFD-induced overweight and hepatic lipid accumulation.

SEX DIFFERENCES IN CANNABINOID DISTRIBUTION, METABOLISM, AND LIPID MODULATION ACROSS THE BRAIN

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Introduction: Cannabinoids (CBs) have behavioral and physiological effects that are sex dependent. Our lab has previously shown that Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) modulate CNS endogenous lipids (endolipids). Here, we investigated how CBD, THC, or the combination of CBD and THC would modulate endolipids across the brain of both male and female mice. Specifically, we hypothesized that CBD, THC, and their metabolites would be present in the brain at different concentrations depending on the sex of the animal and brain region investigated. Additionally, we hypothesized that endolipids including *N*-acyl ethanolamines (NAEs) and 2-acyl-sn-glycerols would be modulated in a drug-, sex-, and region-dependent manner.

Methods: Male and female CD1 mice were injected *i.p.* with THC (10mg/kg, n_{male}=10, n_{female}=10), CBD (10mg/kg, n_{male}=11, n_{female}=9), THC+CBD (10mg/kg, n_{male}=11, n_{female}=11), or vehicle (, n_{male}=8, n_{female}=8). After 2hrs, animals were sacrificed, and their brains collected. Brain tissue was flash frozen on liquid nitrogen, dissected into 8 regions for lipid extraction (brainstem, hippocampus, hypothalamus, thalamus, midbrain, striatum, cortex, and cerebellum), and stored at -80˚ C until being processed. To extract lipids, samples were incubated in methanol + 5µL of 1µM internal standard (d8-AEA) for 2h in the dark on ice, then homogenized via sonication, centrifuged at 19,000G, 20˚ C for 20 min, and the lipid-containing supernatant decanted into water to make a ~25% organic solution. Lipids were partially purified from this solution using C18 solid phase extraction columns, eluting into 1-1.5mL of 65%, 75%, and 100% methanol. HPLC/MS/MS was then used to screen for ~87 lipids and calculate mol/gram concentrations for each tissue type. One-way ANOVAs with Fishers LSD post-hoc tests were used to determine significant differences in drug treatment groups within each sex, and independent t-tests were used to determine significant differences between sexes.

Results: Results showed that patterns in CB distribution were sex- and drug- dependent, though these patterns were markedly similar across the brain. Of note, in control animals, males had significantly higher levels of 2-sn-acyl glycerols in most brain regions, but NAE levels did not differ significantly from females. THC treatment primarily led to *decreases* in endolipids, though effects varied by brain region and sex. CBD had fewer effects on endolipids than THC or THC+CBD treatment. The effects of THC+ CBD were not wholly additive, with the combination treatment frequently causing a novel phenotype in lipid distribution.

Conclusion: These findings support the hypothesis that 10mg/kg CBD, THC, and THC+CBD affect endolipids in the brain 2hrs after *i.p.* administration. They also support that effects are sex- and brain region-dependent, as are concentrations of CBs and their metabolites.

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THE ACUTE VERSES REPEATED EFFECTS OF CANNABIDIOL ON WORRY AND ANXIETY: A DOUBLE-BLIND, RANDOMIZED PLACEBO CONTROLLED TRIAL

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Introduction: Millions of Americans suffer from anxiety symptoms (Terlizzi & Villarroel, 2020). Cannabidiol (CBD), a non-intoxicating molecule of the *Cannabis sativa* L. plant, displays therapeutic potential in the context of anxious arousal (Blessing et al., 2015); however, the effects of CBD on worry, a defining, cognitive feature of anxiety, remain untested. Commercially-available products suggest doses up to 50mg CBD; however, empirical data suggests acute and repeated administration of 300mg CBD alleviates anxious arousal (Crippa et al., 2021; Linares et al., 2019; Masataka, 2019). No study has 1) examined the effects of acute vs repeated CBD administration, nor 2) compared an empirically derived dose (300mg) vs a commercially-available dose (50mg).

Methods: The current study examined the acute and repeated effects of 300mg CBD, 50mg CBD, and placebo administered daily for 14 days on worry severity and anxiety symptoms. Participants (N = 63; M_{age} = 29.27; SD_{age} = 9.58) were elevated in trait worry, meeting a comprehensive list of eligibility criteria (e.g., no past month CBD or THC use). Participants were randomly assigned to condition (300mg CBD, 50mg CBD, placebo) and reported worry severity (Brief Measure of Worry Severity [BMWS]; Gladstone et al., 2005) and anxiety symptoms (Depression, Anxiety, and Stress Scales-21 anxiety subscale [DASS-A]; Antony et al., 1998) at baseline (prior to randomization), day 1 (acute test), and day 14 (repeated test).

Results: Mixed effects models revealed there was no significant time by condition interaction for BMWS scores when controlling for baseline. There was a significant time by condition interaction for 300mg vs 50mg (β = 1.97, t = 2.23, $p < .05$, η^2 = 0.15) and 300mg vs placebo (β = 2.61, t = 3.02, $p < .01$, η^2 = 0.15) for DASS-A when controlling for baseline. Simple slope tests revealed significant decreases in DASS-A scores from day 1 to day 14 within the 300mg (β = -3.54, CI[-4.79, -2.28]), and 50mg (β = -1.57, CI[-2.82, -0.32]) conditions, but not placebo (β = -0.92, CI[-2.12, 0.28]). Post hoc pairwise trend comparisons revealed significantly greater decreases in DASS-A scores within the 300mg condition vs placebo (β = -2.61, t = 3.02, $p <$ .05, d = 1.37), but no significant difference in slope vs the 50mg condition (β = -1.97, t = -2.23, p = .075, d = 1.03). There was no significant difference in slope within the 50mg condition vs placebo (β = -0.65, t = -0.75, p = .736, d = 0.34).

Conclusions: These findings suggest 300mg of oral CBD does not attenuate worry, following both acute and repeated administration. Some evidence for repeated administration of 300mg on anxiety symptoms was obtained. These findings fit with evidence suggesting CBD's anxiolytic effects may be specific to symptom domains (Kwee et al., 2022). The current study extends the literature with regard to the outcome studied, dosing level, and schedule. Future work should further explore the effects of CBD on various aspects of anxiety.

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CHRONIC CANNABIGEROL (CBG) PROVIDES 24/7 PAIN RELIEF FOR MALES AND FEMALES IN MOUSE MODEL OF CISPLATIN-INDUCED NEUROPATHY

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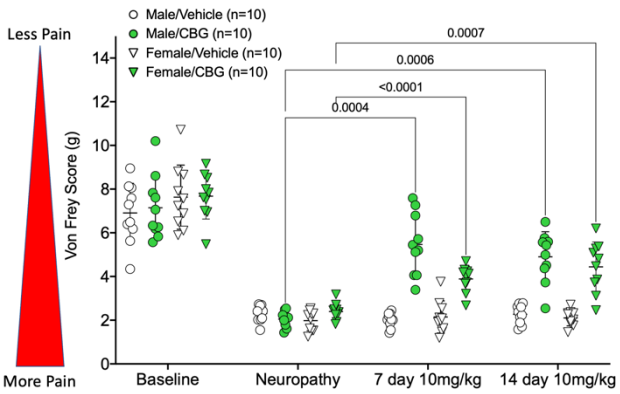
Introduction: Cannabigerol (CBG), is the precursor molecule to most cannabinoids including delta 9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Our group previously published the ability of acute CBG to reduce neuropathic pain for 1-6 hours. Here, we study the ability of daily CBG to modulate chronic, long-lasting neuropathy following chronic administration. We also study sex differences in response and estrous cycle contribution to pain. Finally, we aim to understand the mechanism through which CBG provides chronic pain relief by performing gene expression analysis of dorsal root ganglia (DRG).

Methods: Male and female C57BL/6J mice with cisplatin-induced neuropathy (4 weekly injections) received daily i.p. vehicle (1:1:18 DMSO: Tween80: saline) or 10 mg/kg CBG (n=10 per group) for 14 days. Mechanical hyperalgesia was measured by von Frey filament test before the experiment ("baseline"), after neuropathy induction, 7 days of treatment, and 14 days of treatment. Von Frey was performed ~24 hours after the previous drug injection to determine chronic response. Data were analyzed by two-way ANOVA with Tukey post-hoc test. Estrous cycle stage was identified through cytology of vaginal lavage 30min after von Frey. L4-L6 dorsal root ganglia (DRG) were dissected 24 hours after the last injection. RT-PCR was conducted on extracted tissue to quantify relative expression of genes and assessed using non-parametric t-test.

Results: Our model of cisplatin-induced neuropathy mimics patient experience with weekly chemotherapy injections and long-lasting mechanical pain sensitivity several months after stopping cisplatin. 7 and 14 days of chronic CBG provided 24/7 pain relief to 70-80% of pre-neuropathy levels **(p<.001)**, without any apparent tolerance. These findings had been replicated in a previous study of male mice. Chronic CBG did not affect weight (p>.05), estrous cycle trends, nor DRG gene expression (p>.05). Genes tested include: *CNR1, CNR2, GPR55, FAAH, MGLL, DAGL, NAPE-PLD, TRPV1, ADRA2A-C, DRD2,* and *OPRM1*.



Conclusions: These exciting and reproducible results encourage further study into CBG as a pain modulator. Our planned experiments delve deeper into the mechanism of CBG through pharmacokinetics and testing in a collaborative model of breast cancer treatment and recovery.

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THE IMPACT OF CANNABIDIOL (CBD) EXPECTANCY ON CORTISOL RESPONSIVITY IN THE CONTEXT OF ACUTE STRESS IN MALE AND FEMALE CANNABIS USERS

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Introduction: Cannabidiol (CBD), a non-psychoactive cannabinoid found in the cannabis plant, has gained interest for its' purported stress- and anxiety-reducing effects. However, the mechanisms underlying these effects remain unclear. Our group previously found that CBD expectancy alone resulted in lower state anxiety (vs. CBD-free expectancy) among those who strongly believed it was helpful for such purposes, in addition to influencing physiological measures (i.e., heart rate variability). Using data collected as part of this previously published larger study, we aimed to explore the extent to which CBD expectancy alone impacts cortisol in the context of a laboratory stressor.

Methods: A sample of 43 healthy adults (23 female) participated in one orientation and two experimental laboratory sessions. They received the same oil (CBD-free) both times but were told they received CBD oil in counterbalanced order in one of their sessions. Participants then engaged in a laboratory stressor (the Maastrict Acute Stress Test; MAST) and salivary cortisol samples were collected throughout. A linear mixed model was used to analyze the data.

Results: Findings indicated that a physiological stress response was elicited in the context of the MAST, which is consistent with what had been reported previously. Cortisol was also higher overall among males relative to females. Interestingly, while cortisol levels were significantly lower immediately following the MAST (likely representing the anticipatory stress period) in the CBD expectancy condition, this effect seems to be largely driven by males. Cortisol levels did not reliable vary across expectancy conditions at any other timepoint.

Conclusion: Findings indicate that CBD expectancy alone appears to blunt cortisol in anticipation of a stressor, particularly in males. These results suggest that it is important to consider the impact of drug related expectations when assessing CBD related effects on stress related processes.

THE CLINICAL TRANSLATION OF α-HUMULENE – A SCOPING REVIEW

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Introduction: **α**-humulene is a sesquiterpene, a subclass of terpenoid, and is present within the essential oils of many plant species, notably *Cannabis sativa*. There is growing interest in its biochemical properties and therapeutic potential. This scoping review aimed to summarise the evidence base on **α**-humulene to inform clinical translation and future research prioritisation.

Methods: A scoping review was conducted of EMBASE, MEDLINE and PubMed databases up to 12th February 2022. All studies describing original research on **α**-humulene cultivation, extraction, pre-clinical and clinical research were included for review.

Results: 336 full text articles were included. The reported yields of **α**-humulene varied from nil to 60.90% across various plant species. The antineoplastic effects of **α**-humulene were most consistently investigated. Cytotoxicity was demonstrated within *in vitro* models of adenocarcinoma of colorectal, pulmonary, breast (ductal), prostatic, lung and ovarian origin. However, other cell models showed varying response to **α**-humulene (malignant melanoma, hepatocellular carcinoma, and renal cell carcinoma). There was a paucity of reported mechanisms of action, however **α**-humulene (200 μM) was demonstrated to mediate mitochondrial dysfunction and reductions in intracellular glutathione, linked to increased generation of reactive oxidative species in a fibroblast cell line (L-929). **α**-humulene (50 mg/kg oral) was also investigated in rodent models of inflammation including phlogistic agent-induced paw oedema, and ovalbumin-induced airway allergic inflammation. This demonstrated reductions in interleukin-1β, tumour necrosis factor-**α**, nuclear factor kappa-light-chain-enhancer of activated B cells, prostaglandin E2, cyclooxygenase-2, and inducible nitric oxide synthase ($p<0.050$). **α**-humulene (50-200 mg/kg intraperitoneal) was also shown to have cannabimimetic properties mediated by cannabinoid 1 (CB1) and adenosine A2a receptors in a murine model. A single study demonstrated **α**-humulene to have dose dependent effects on the generation of phosphorylation of extracellular signal-regulated kinase in cells expressing CB1 receptors which was incompletely inhibited by rimonabant ($p<0.050$). Antimicrobial, antiparasitic, and larvicidal effects were also evaluated.

Conclusions: **α**-humulene demonstrated multiple pharmacological and toxicological properties with potential scope for therapeutic utilisation. The review also identified multiple species, including *Cannabis sativa* offering adequate yield of **α**-humulene for use in isolation or in combination with other active pharmaceutical ingredients extracted from plants. Whilst implications of its antineoplastic and anti-inflammatory effects are promising there is a need for further evaluation to delineate mechanism of action. Ultimately, pharmacokinetic studies in animals and humans will be necessary to determine optimum dosing strategies considering the poor bioavailability of most terpenes. This review, however, provides a basis for future evaluation of the promising therapeutic potential of **α**-humulene in clinical settings.

ANTINOCICEPTIVE EFFECTS OF CANNABICHROMENE IN ANIMAL PAIN MODELS

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Introduction: For centuries, cannabis has been utilized to address various medical conditions, particularly those that are associated with perceived pain. More recently, individual cannabinoid compounds have also been examined, including THC and CBD. However, the euphoric effects associated with some of these compounds have raised concerns about their therapeutic value, especially in light of the opioid epidemic. To address the need for non-euphoric pain treatments, we have focused our investigation on identifying non-psychoactive cannabinoids with anti-nociceptive properties. Cannabichromene (CBC) is a minor, non-psychoactive cannabinoid whose pharmacological profile suggests that it has the potential to reduce pain.

Methods: Therefore, we assessed the analgesic potential of CBC in: 1) a model of chemotherapeutic-induced peripheral neuropathy (CIPN); 2) hot and cold thermal assays; and 3) the formalin inflammatory pain assay.

Results: The results showed that CBC (at concentrations of 20 mg/kg, i.p.) reduced mechanical allodynia in a mouse model of CIPN. Additionally, we found that CBC increased the tail-flick latency in the hot thermal assay, while reducing the pain score in both phases of the formalin test (initial acute pain followed by delayed inflammatory pain). We found no CBC effect in the acetone cold thermal assay. Lastly, we did not observe any sex differences in CBC's analgesic properties, which is in contrast to the analgesic properties of other cannabinoids that have been previously examined.

Conclusions: Overall, our results suggest that CBC has antinociceptive properties that may be pain-type specific. CBC is reported to be a potent agonist of TRPA1 and also have activity at the CB2 receptor, activation of either or both of these receptors may be responsible for the antinociceptive activity of CBC and is currently under investigation.

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CHARACTERIZING THE EFFECT OF TERPENE BETA-CARYOPHYLLENE ON INTERACTIONS BETWEEN THE BLADDER UROTHELIUM AND UROPATHOGENIC *E. COLI*

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Introduction: Urinary tract infections (UTIs) are a common type of bacterial infection presenting frequently in urgent care. While typically manageable with standard antibiotic therapy, there is an urgent need for new therapeutic options to manage both the infection and painful symptoms associated with UTI. Beta-caryophyllene (beta-C) is a plant-derived terpene which can be found in cloves, black pepper and *Cannabis sativa*. It has local anesthetic and anti-inflammatory activity, which are partially mediated through cannabinoid receptor 2 (CB2R). Additionally, anti-bacterial activities have recently been described, but the mechanism of action remains unclear. We have previously demonstrated the utility of beta-C for the management of both urinary bacterial burden and symptoms of pain in a murine model of UTI. The goal of this project is to characterize the mechanism of activity responsible for the anti-bacterial effects of beta-C.

Methods: Gentamicin protection assays were used to evaluate changes in bacterial invasion into 5637 bladder urothelial cells subjected to beta-C treatment. In brief, 80% confluent cells were infected with uropathogenic *E. coli* at a multiplicity of infection of 100 and co-treated with 100μg/ml beta-C. After two hours, cells were treated with gentamicin and then lysed for bacterial enumeration. Western blotting was also employed to assess CB2R expression under the previously described conditions, with cell lysates collected following the two-hour infection period. Further experiments will use the CB2R antagonist AM630 to determine the involvement of CB2R in bacterial invasion. Imaging flow cytometry (Amnis ImageStream) will be used to shed further light on this pathway, providing additional detail on bacterial localization (i.e. adherent or internalized) and actin rearrangement within the cell.

Results: Experimental results from the gentamicin protection assay indicates that co-treatment with beta-C results in a significant reduction in bacterial invasion into urothelial cells. Western blot analysis also suggests an upregulation of the CB2 receptor post-infection, which is further increased by beta-C co-treatment. Preliminary ImageStream experiments also indicate beta-C treatment has no effect on bacterial adhesion, but results in decreased internalization.

Conclusion: Beta-C may exert anti-bacterial effects via modulation of the pathway to bacterial internalization, which may be associated with CB2-mediated changes in actin organization. This data lends support to the utility of beta-C as an adjunct therapy for UTI.

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CHARACTERIZATION OF HEXAHYDROCANNABINOL DIASTEREOMERS BY NMR, HPLC, GC-MS, AND TLC

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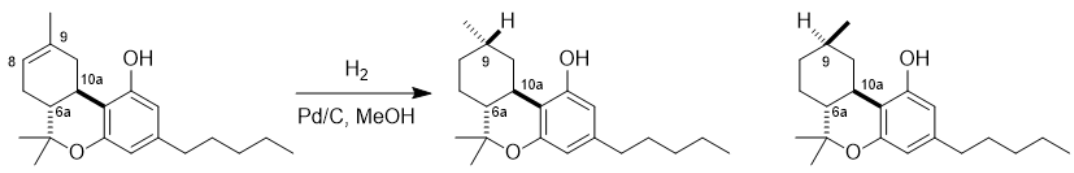
Introduction: Growth in hemp and cannabis-based products has exploded in recent years due in part to medical and recreational marijuana legalization at the state level and passage of the 2018 USDA Farm Bill. Initially, hemp-derived products were focused heavily on non-psychotropic substances like cannabidiol (CBD). However, hemp-derived CBD can be easily synthetically manipulated to psychotropic phytocannabinoids such as Δ^8-tetrahydrocannabinol (Δ^8-THC). Loopholes in the 2018 Farm Bill legislation have led to an increase of psychotropic THC products within the marketplace, ultimately leading to new legislative and regulatory responses. Hydrogenation of Δ^8- and/or Δ^9-THCs leads to formation of a mixture of hexahydrocannabinols (HHCs), comprised of the 9(*R*)- and 9(*S*)-HHC diastereomers. These compounds retain some psychoactivity but avoid classification as THCs and THC-related regulations. Separation of HHC diastereomers is possible by TLC, HPLC and GC-MS, but identity cannot be inferred without verified reference standards. Full NMR characterization of the two HHC diastereomers is necessary for confirmation and has not previously been described.

Methods: 1H and 13C NMR experiments were acquired on a JEOL ECZ-400S spectrometer, 2D experiments included COSY, HSQC and NOESY. All spectra were acquired in chloroform-d at ambient room temperature. HPLC was acquired on an Agilent 1260 Infinity II with a Gemini-C18 column with a 20:80:0.1 water/methanol/acetic acid mobile phase, 1 mL/min flow rate, 40°C column temperature, monitoring at 228 nm. GC-MS was acquired on an Agilent 8890 GC and 5977B MS Detector using a Restek Rtx-5 MS column, using a temperature program starting at 50°C for 1 minute, increasing at 30°C/minute to 300°C, with a 25-minute total run time. TLC developed using 10% MTBE/heptane eluent with F254 silica gel plates and visualized with CAM stain.

Results: HHC diastereomers, 9(*R*)-HHC and 9(*S*)-HHC, have been fully characterized by HPLC, GC-MS, and NMR. The associated analytical data presented aids in correct identification and differentiation of products resulting from Δ^8- and/or Δ^9-THC hydrogenation.

Conclusions: The information provided herein can aid in the correct identification and differentiation of the products resulting from hydrogenation of Δ^8- or Δ^9-THC. Prior to this study, misassignment of 9(*S*)- and 9(*R*)-HHC by an American cannabis analytical standards supplier led to confusion and many incorrect reported results, highlighting the need for high-level NMR experiments to confirm the stereochemistry.



Δ8-THC 9(*S*)-HHC 9(*R*)-HHC