29TH ANNUAL

SYMPOSIUM

OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

BETHESDA

MARYLAND, USA

JUNE 29 – JULY 4, 2019

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Registration: June 29th, 2019 (16.00 – 18.00)

Marriott North Conference Center
Bethesda, MD USA

Welcome Reception: 18.30 – 20.00

Day 1
Sunday, June 30th

7.30	**Breakfast**		
8.30	Welcome and Opening Remarks		
Oral Session 1. Cannabis Compounds, Genetics, chemovars and Extraction Methods Chairs: Jana Hajslova and John McPartland			
8.45	John M. McPartland* and Geoffrey W. Guy	RENAMING DINOSAURS: EXHUMING THE ANCESTORS OF "SATIVA" AND "INDICA"	1
9.00	Carrie Cuttler*, Alexander Spradlin and Rebecca Craft	SHORT- AND LONG-TERM ACUTE EFFECTS OF CANNABIS ON HEADACHE AND MIGRAINE: A NATURALISTIC STUDY OF MEDICAL CANNABIS USERS	2
9.15	Lipin Ji, Yingpeng Liu, Fei Tong, Marsha Eno, Shalley Kudalkar, Alex Straiker, Ai-ling Li, Othman Benchama, Chandrashekhar Honrao, Anisha Korde, Amey Dhopeshwarkar, Paula Morales, Shu Xu, Michaela Dvorakova, Dow Hurst, Simiao Wu, JodiAnne T. Wood, Nikolai Zvonok, Patricia Reggio, Ken Mackie, Lawrence Marnett, Andrea G. Hohmann, Alexandros Makriyannis and Spyros P. Nikas*	CHIRAL ENDOCANNABINOID LIGANDS	3

9.30	L. Cinnamon Bidwell*, Jarrod Ellingson, Sophie YorkWilliams, Hollis Karoly, Leah N. Hitchcock, Cristina Sempio, Jost Klawitter, Brian Tracy, Angela D. Bryan and Kent E. Hutchison	ACUTE SELF-ADMINISTRATION OF LEGAL MARKET FLOWER AND CONCENTRATED CANNABIS: CANNABINOID BLOOD LEVELS, SUBJECTIVE INTOXICATION, AND NEUROBEHAVIORAL OUTCOMES	4
9.45	Jana Hajslova*, Marie Fenclova, Frantisek Benes, Ethan Russo and Pavel Kubu	ASSESSING TRENDS OF CBD OILS QUALITY AT THE EU MARKET	5
10.00	**COFFEE BREAK**		
ORAL SESSION 2. CANNABIDIOL CHAIRS: MICHELLE GLASS AND SAOIRSE O'SULLIVAN			
10.30	Salahaden R. Sultan, Timothy J. England and Saoirse E. O`Sullivan*	ACUTE AND CHRONIC EFFECTS OF CANNABIDIOL ON HAEMODYNAMICS IN HEALTHY MALES	6
10.45	Staci A. Gruber*, Ashley M. Lambros, Rosemary T. Smith, M. Kathryn Dahlgren, Kelly A. Sagar, David P. Olson and Scott E. Lukas	HIGH ANXIETY? EXAMINING THE IMPACT OF FOUR WEEKS OF TREATMENT WITH A NOVEL HIGH CANNABIDIOL PRODUCT	7
11.00	Tory Spindle*, Edward Cone, John Mitchell, George Bigelow, Ron Flegel and Ryan Vandrey	ACUTE PHARMACOKINETICS AND PHARMACODYNAMICS OF ORAL AND VAPORIZED CANNABIDIOL IN HEALTHY ADULTS	8
11.15	Ewa Galaj*, Guo-Hua Bi and Zheng-Xiong Xi	CANNABIDIOL ATTENUATES COCAINE REWARD BY CB2, 5-HT_{1A} AND TRPV1 RECEPTOR MECHANISMS IN RATS	9

11.30 - 12.30	**PLENARY SPEAKER** US CANNABIS POLICY: IMPLICATIONS FOR PUBLIC HEALTH **SUSAN R. B. WEISS, PH.D.** Director Division of Extramural Research National Institute on Drug Abuse, Bethesda, MD, USA		
12.30	**LUNCH**		
12.30	NIDA STUDENT TRAINING SESSION OVERVIEW OF NIH/NIDA RESEARCH TRAINING AND GRANT OPPORTUNITIES IN CANNABINOID RESEARCH BETH BABECKI, WOODY LIN AND RAO RAPAKA		
13.30 - 14.30	**ICRS LIFETIME ACHIEVEMENT AWARD** CANNABINOID PHARMACOLOGY: MY FIRST HALF CENTURY **ROGER PERTWEE, M.A., D.PHIL, D.SC., HONFBPHS** Institute of Medical Sciences University of Aberdeen, Scotland		
ORAL SESSION 3. CB1 PHARMACOLOGY CHAIRS: RESAT CINAR AND YOSSI TAM			
14.30	Thuy Nguyen, Ann M. Decker, Thomas F. Gamage, Jun-Xu Li, Jenny L. Wiley, Brian F. Thomas, Terry P. Kenakin and Yanan Zhang*	ALLOSTERIC MODULATORS OF THE CANNABINOID CB1 RECEPTOR: DIARYL UREAS	10
14.45	Allyn C Howlett*, William T. Booth and W. Todd Lowther	STRUCTURAL ANALYSIS OF THE CB1 CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A)	11

15.00	Adi Drori*, Asaad Gamal, Shahar Azar, Liad Hinden, Rivka Hadar, Daniel Wesley, Alina Nemirovski, Maayan Salton, Boaz Tirosh and Joseph Tam	CANNABINOID-1 RECEPTOR REGULATES SOLUBLE LEPTIN RECEPTOR LEVELS VIA C/EBP HOMOLOGOUS PROTEIN (CHOP), CONTRIBUTING TO OBESITY-RELATED HEPATIC LEPTIN RESISTANCE	12
15.15	Resat Cinar*, Nathan J. Coffey, Steven P. Bodine, Joshua K. Park, Malliga R Iyer, Bernadette R. Gochuico, William A. Gahl, May Christine V. Malicdan and George Kunos	MRI-1867, A THIRD GENERATION CB1R ANTAGONIST, FOR EFFECTIVE THERAPY OF A RARE DISEASE, HERMANSKY-PUDLAK SYNDROME PULMONARY FIBROSIS	13
15.30	Michaela Dvorakova, Wesley Corey, Anaelle Zimmowitch, Alex Straiker* and Ken Mackie	A CRITICAL EVALUATION OF TERPENOID SIGNALING AT CANNABINOID CB1 RECEPTORS IN A NEURONAL MODEL	14
15.45	Tania Muller, Julia Leemput, Chloé Buch, Laurent Demizieux, Patricia Passilly-Degrace, Resat Cinar, Malliga R Iyer, George Kunos, Bruno Vergès, Pascal Degrace and Tony Jourdan*	HYBRID INHIBITOR OF PERIPHERAL CANNABINOID-1 RECEPTOR AND INDUCIBLE NITRIC OXIDE SYNTHASE MITIGATES THE DEVELOPMENT OF DYSLIPIDEMIA	15
16.00 – 18.00	**POSTER SESSION 1** RECEPTION		P1

Notes:

Presenting Author*

Day 2
Monday, July 1st

7.30	**Breakfast**		
8.30	Opening Remarks		
Oral Session 4. CB2 Pharmacology Chairs: Uwe Grether and Melanie Kelly			
8.45	U. Grether*, K. Atz, B. Brennecke, E. M. Carreira, C. Davies, J. Fingerle, T. Gazzi, J. Gertsch, W. Guba, A. Kimbara, C. Korn, M. Maccarrone, H. Mandhair, R. E. Martin, A. Mason, T. Miljus, M. Nazare, M. Nettekoven, S. Oddi, P. Pacher, A. Pavlovic, A. Pedrina-McCarthy, P. Pfaff, C. Raposo, M. Rogers-Evans, E. Roome, S. Röver, A. Rufer, R. Sarott, M. Soethoudt, C. Ullmer, M. van der Stelt, D. Sykes, Z. Varga, D. B. Veprintsev, M. Weise and M. Westphal	FLUORESCENTLY LABELED TYPE-2 CANNABINOID RECEPTOR (CB2R) LIGANDS: FROM INITIAL ATTEMPTS TOWARD A HIGHLY VERSATILE CHEMICAL TOOLBOX	16
9.00	M. Nazare*, T. Gazzi, B. Brennecke, M. Weise U. Grether, K. Atz, E. M. Carreira, J. Fingerle, J. Gertsch, W. Guba, C. Korn, M. Maccarrone, H. Mandhair, T. Miljus, S. Oddi, P. Pacher, A. Pavlovic, P. Pfaff, C. Raposo, A. Rufer, R. Sarott, C. Ullmer, M. van der Stelt, D. Sykes, Z. Varga, D. B. Veprintsev and M. Westphal	CB2 RECEPTOR DETECTION BY NEW FLUORESCENTLY LABELED SYNTHETIC LIGANDS	17

9.15	Zoltan V. Varga*, Katalin Erdelyi, Uwe Grether, Eszter Trojnar, Catarina Raposo, Jürgen Fingerle, Christoph Ullmer, Ozge Gunduz-Cinar, Michelle Glass, Bela Szabo, Miklos Palkovits, Andrew Holmes, Jürg Gertsch, Julian Romero, Cecilia Hillard and Pal Pacher	CANNABINOID 2 RECEPTOR (CB2) EXPRESSION REVISITED: DETECTION OF CB2 mRNA WITH NEW SENSITIVE TOOLS	18
9.30	Tomohiro Kimura, Alexei Yeliseev, Mihaela Mihailescu, Diane L. Lynch, Walter E. Teague Jr., Kirk G. Hines, Lioudmila Zoubak, Alan Grossfield, Patricia H. Reggio and Klaus Gawrisch*	2-AG LOCATION, STRUCTURE AND DYNAMICS IN MEMBRANES AND ITS INTERACTION WITH CB2 RECEPTORS	19
9.45	Jakub Mlost*, Marta Bryk, Magdalena Kostrzewa and Katarzyna Starowicz	CB2 BIASED AGONISTS AND THEIR THERAPEUTIC POTENTIAL IN OSTEOARTHRITIS	20
10.00	**COFFEE BREAK**		
ORAL SESSION 5. CANNABINOIDS AND REWARD CHAIRS: HEATHER BRADSHAW AND DAVID FINN			
10.30	Chloe J. Jordan*, Bree Humburg, Yi He, Xiao Han, Guo-hua Bi, Eliot Gardner, Xiang-qun Xie and Zheng-Xiong Xi	DISSECTING THE REWARDING VS. AVERSIVE EFFECTS OF CANNABINOIDS: FINDINGS FROM OPTOGENETIC BRAIN-STIMULATION REWARD MAINTAINED BY ACTIVATION OF VTA DOPAMINE NEURONS	21
10.45	Joseph F. Cheer*	ENDOGENOUS CANNABINOIDS AND REWARD PREDICTION	22

11.00	Matthew L. Eckard* and Steven G. Kinsey	GABAPENTIN ATTENUATES SOMATIC SIGNS OF Δ^9-THC WITHDRAWAL IN MICE	23
11.15	Withdrawn		24
11.30 - 12.30	**PLENARY SPEAKER** THE NEUROBIOLOGY OF DRUG ADDICTION: FROM DRUG REWARD TO HYPERKATIFEIA TO NEGATIVE REINFORCEMENT GEORGE KOOB, PH.D. Director National Institute on Alcohol Abuse and Alcoholism		
12.30	LUNCH		
13.30 - 14.00	**ICRS YOUNG INVESTIGATOR AWARD** ANANDAMIDE, FAAH, STRESS AND ANXIETY: A TRANSLATIONAL JOURNEY MATT HILL, PH.D. Associate Professor The Hotchkiss Brain Institute University of Calgary, Alberta, Canada		
ORAL SESSION 6. CLINICAL STUDIES CHAIRS: NALIN PAYAKACHAT AND ETHAN RUSSO			
14.00	Adi Zuloff-Shani*, Ephraim Brener and Ascher Shmulewitz	EFFICACY AND SAFETY OF THX-110, A PROPRIETARY THERAPEUTIC COMBINATION OF Δ^9-TETRAHYDROCANNABINOL AND PALMITOYLETHANOLAMIDE	25

14.15	Nalin Payakachat*, Ryan Vandrey, William E. Fantegrossi, Lauren Russell and Marcel O. Bonn-Miller	DOES CANNABIS HELP IMPROVE INSOMNIA AMONG PATIENTS WITH POSTTRAUMATIC STRESS DISORDER?	26
14.30	Carrie Cuttler*, Emily LaFrance and Aria Petrucci	ACUTE EFFECTS OF HIGH POTENCY CANNABIS ON EVERYDAY LIFE MEMORY	27
14.45	Bitya Raphael*, Natalya Kogan, Malka Attar-Namdar, Mukesh Chourasia, Maria G. Cascio, Avital Shurki, Joseph Tam, Moshe Neuman, Joseph Foldes, Roger G. Pertwee, Andreas Zimmer, Itai Bab and Yankel Gabet	HISTONE H4 ENCODES AN ENDOGENOUS PEPTIDE THAT SIGNALS VIA THE CB2 CANNABINOID RECEPTOR	28
15.00	Mark A. Ware*, Antonio Vigano, Pierre Beaulieu, Andrée Néron, Yola Moride, Michelle Canac-Marquis, Maja Kalaba, Marc O. Martel, Jordi Perez, Julie Desroches and William Barakett	THE QUEBEC CANNABIS REGISTRY, A DATABASE ON THE USE OF CANNABIS FOR MEDICAL PURPOSES: FINAL RESULTS	29
15.15	Marcel O. Bonn-Miller*, Megan Brunstetter , Alexandra Simonian, Hal Wortzel and Ryan Vandrey	A CONTROLLED PROSPECTIVE OBSERVATIONAL STUDY OF THE LONGITUDINAL ASSOCIATIONS BETWEEN CANNABIS USE AND PTSD SYMPTOMATOLOGY	30
15.30	Dylan Zylla*, Justin Eklund, Grace Gilmore, Alissa Gavenda, Gabriella VazquezBenitez, Pamala Pawloski, Tom Arneson, Angela Birnbaum, Stephen Dahmer, Matthew Tracy and Arkadiusz Dudek	A RANDOMIZED TRIAL OF MEDICAL CANNABIS IN PATIENTS WITH ADVANCED CANCERS TO ASSESS IMPACT ON OPIOID USE AND CANCER-RELATED SYMPTOMS: A PILOT AND FEASIBILITY STUDY	31
15.45	Joshua Rein*, Lindsay Texter, Mark Wurfel, Edward Siew, Amit Garg, Thida Tan, Paul Kimmel, James Kaufman, Vernon Chinchilli and Steven Coca	MARIJUANA USE AND KIDNEY OUTCOMES IN THE ASSESS-AKI COHORT	32

16.00 – 18.00	**Poster Session 2** Reception	P2
18.00	**Business Meeting**	

Notes:

Presenting Author*

Day 3
Tuesday, July 2nd

7.30	**Breakfast**		
8.30	Opening Remarks		
Oral Session 7. Development, Reproductive Function, Smell and Cancer Chairs: Anna Kalinovsky and Julian Romero			
8.45	Kylie Black, Shawyon Baygani, Ricardo Martinez, Tristen Mier, Alexandria Bell, Ken Mackie and Anna Kalinovsky*	CANNABINOID SIGNALING REGULATES CEREBELLAR DEVELOPMENT	33
9.00	Shahnaza Hamidullah*, Claudia D. Lutelmowskij and Jibran Y. Khokhar	BEHAVIOURAL AND COGNITIVE EFFECTS OF ADOLESCENT CANNABIS AND ALCOHOL CO-USE IN ADULTHOOD	34
9.15	Xiaofei Sun*, Yingju Li, Fenghua Bian and Sudhansu K. Dey	MICE MISSING CNR1 AND CNR2 SHOW IMPLANTATION DEFECTS	35
9.30	Ali Mokhtar Mahmoud*, Viviana Marolda, Magdalena Kostrzewa, Vincenzo Di Marzo, Roberto Ronca and Alessia Ligresti	NON-PSYCHOTROPIC CANNABINOIDS SUPPRESS TUMOR GROWTH BY ACTING ON METABOLIC REPROGRAMMING AND ONCOGENIC PATHWAYS IN HORMONE REFRACTORY PROSTATE CANCER	36

9.45	Huizhi Du, Maya Ploss, Alex Straiker and Thomas Heinbockel*	CANNABINOID RECEPTOR-MEDIATED MODULATION OF INTERNEURONS IN THE MAIN OLFACTORY BULB	37
10.00	**COFFEE BREAK**		
10.30	IN MEMORIAM		
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11.00	Madhusudhanan Narasimhan*, Henry Blanton, Jennifer Brelsfoard, Diana E. Sepulveda, Angela N. Henderson-Redmond, Daniel J. Morgan and Josée Guindon	CP55,940 ANTINOCICEPTIVE EFFECT, DEVELOPMENT OF TOLERANCE AND ACTIVATION OF JNK SIGNALING IN THE CISPLATIN-INDUCED NEUROPATHIC PAIN MODEL	39
11.15	Douglas E. Brenneman*, William A. Kinney and Sara Jane Ward	MOLECULAR AND PHARMACLOGICAL EVIDENCE FOR THE SODIUM-CALCIUM EXCHANGER-1 (mNCX-1) AS A MEDIATOR OF CBD- AND KLS-13019-APPLIED PROTECTION AGAINST PACLITAXEL-INDUCED TOXICITY IN DORSAL ROOT GANGLION CULTURES	40

11.30	Catharine A. Mielnik*, Chun Kit Li, Iain R. Greig, Mostafa H. Abdelrahman, Laurent A. Trembleau, WM Burnham, Ali Salahpour, Amy J. Ramsey and Ruth A. Ross	NOVEL NEGATIVE ALLOSTERIC MODULATOR (NAM) OF CANNABINOID RECEPTOR 1 (CB1) AMELIORATES SYMPTOMS DUE TO DOPAMINE DYSREGULATION IN PSYCHIATRIC DISORDERS	41
11.45	Giulia Donvito*, Ryan Mischel, Virginia McLane, Daisuke Ogasawara, Hamid Akbarali, Ku-Lung Hsu, Benjamin F Cravatt and Aron H Lichtman	THE DIFFERENTIAL ROLE OF DIACYLGLYCEROL LIPASES IN REVERSING MECHANICAL SENSITIVITY IN A MOUSE MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY (CIPN)	42
12.00 – 13.00	**KANG TSOU MEMORIAL LECTURE** VEGAS AND ULTRA-LSD: NEW CHEMICAL AND SYNTHETIC BIOLOGICAL TECHNOLOGIES **BRYAN ROTH, M.D., PH.D.** Director, NIMH Psychoactive Drug Screening Program Distinguished Professor, Department Of Pharmacology UNC Chapel Hill , NC		
13.00	**LUNCH**		
13.00	INDUSTRY BREAKOUTS		

14.00 – 16.00

NIDA Networking Session

CANNABINOID RESEARCH FINDINGS AND FUTURE RESEARCH DIRECTIONS

Chair: Steven Gust, Director, International Program

Division of Neuroscience and Behavior

BASIC RESEARCH OPPORTUNITIES AND PRIORITIES RELATED TO THE CANNABINOIDS
Rita J. Valentino, Division Director

BASIC RESEARCH ON CANNABINOIDS IN DNBR
Roger Sorensen, Chief, Integrative Neuroscience Branch

Division of Therapeutics and Medical Consequences

DEVELOPING CANNABIS-BASED MEDICATIONS, HOW CAN NIDA'S DIVISION OF THERAPEUTICS AND MEDICAL CONSEQUENCES HELP?
Robert Walsh, Chief, Regulatory Affairs Branch

SUPPORTING CANNABIS RESEARCH IN NIDA'S DIVISION OF THERAPEUTICS AND MEDICAL CONSEQUENCES
Aidan Hampson, Clinical Research Grants Branch

Division of Epidemiology, Services and Prevention Research

NIDA'S CANNABIS POLICY RESEARCH PORTFOLIO: CURRENT AND FUTURE DIRECTIONS
Marsha F. Lopez, Chief, Epidemiology Research Branch

NIDA'S RESEARCH INTERESTS IN THE PREVENTION OF CANNABIS MISUSE
Amy Goldstein, Chief, Prevention Research Branch

NIDA Intramural Research Program

CANNABIS-BASED MEDICATION DEVELOPMENT FOR THE TREATMENT OF SUBSTANCE USE DISORDERS
Zheng-Xiong Xi, Chief, Addiction Biology Unit

COCAINE-INDUCED ENDOCANNABINOID RELEASE MEDIATED BY EXTRACELLULAR VESICLES IN THE VENTRAL TEGMENTAL AREA
Carl Lupica, Chief, Electrophysiology Research Section

Day 4
Wednesday, July 3rd

7.30	**Breakfast**		
8.30	Opening Remarks		
Oral Session 9. Immune Function, Neurodegenerative and Cardiovascular Disorders Chairs: Irene Benito-Cuesta and Haley Vecchiarelli			
8.45	Ines Reynoso-Moreno*, Silvia Tiezt, Britta Engelhardt, Jürg Gertsch and Andrea Chicca	NEW STRATEGIES TARGETING THE ENDOCANNABINOID SYSTEM TO ATTENUATE DISEASE PROGRESSION IN A MOUSE MODEL OF MULTIPLE SCLEROSIS	43
9.00	Douglas J. Hermes*, Changqing Xu, Rick B. Meeker, Micah J. Niphakis, Benjamin F. Cravatt, Ken Mackie, Aron H. Lichtman, Bogna M. Ignatowska-Jankowska and Sylvia Fitting	GPR18 MEDIATES MICROGLIAL NEUROTOXICITY INDUCED BY HIV-1 TAT PROTEIN IN VITRO	44
9.15	Kevin S. Murnane*, Lindsey Phillips-Lindsey, Cedrick M. Daphney, Aboagyewaah Oppong-Damoah, Richard D. Khusial, Ayman Akil and Peter N. Uchakin	β-CARYOPHYLLENE IMPROVES MEMORY FUNCTION AND DECREASES KEY INFLAMMATORY CYTOKINES IN AGED MICE	45

9.30	Irene Benito-Cuesta*, Samuel Ruiz de Martín Esteban, Ana M. Martínez Relimpio, M. Asunción Barreda-Manso, Rosa M. Tolón, Cecilia J. Hillard, Julián Romero and M. Teresa Grande	THE ROLE OF MICROGLIAL CB2 RECEPTORS IN BETA AMYLOID PHAGOCYTOSIS: *IN VITRO* AND *IN VIVO* STUDIES	46
9.45	Janos Paloczi*, Csaba Matyas, Zoltan V. Varga, Resat Cinar, György Hasko, Thomas H. Schindler, George Kunos and Pal Pacher	ALCOHOL BINGE-INDUCED CARDIOVASCULAR DYSFUNCTION INVOLVES ENDOCANNABINOID-CB1-R SIGNALING	47
10.00	**COFFEE BREAK**		
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10.30	Ephraim Brener*, Adi Zuloff-Shani and Ascher Shmulewitz	PALMITOYLETHANOLAMIDE MODULATES CB1 AFFINITY TO THC	48
10.45	Sergiy Tyukhtenko*, Xiaoyu Ma, Kiran Vemuri, Spyros Nikas, Michael Malamas and Alexandros Makriyannis	MONOACYLGLYCEROL LIPASE INHIBITION: BIOPHYSICAL AND MECHANISTIC INSIGHTS	49
11.00	Emma Leishman*, Ken Mackie and Heather B Bradshaw	ELEVATED LEVELS OF ARACHIDONIC ACID-DERIVED LIPIDS, INCLUDING PROSTAGLANDINS AND ENDOCANNABINOIDS, ARE PRESENT THROUGHOUT ABHD12 KO BRAINS: NOVEL INSIGHTS INTO THE NEURODEGENERATIVE PHENOTYPE	50

11.15	Josephine Watson, Lauren Carnevale, William Arnold, Austin Weigle and Aditi Das*	METABOLISM OF ENDOCANNABINOIDS AND PHYTOCANNABINOIDS BY CYTOCHROME P450 TO PRODUCE NOVEL BIOACTIVE METABOLITES	51
11.30	Caroline Turcotte, Anne-Sophie Archambault, Élizabeth Dumais, Cyril Martin, Marie-Renée Blanchet, Elyse Bissonnette, Alain Veilleux, Michel Laviolette, Vincenzo Di Marzo and Nicolas Flamand*	ENDOCANNABINOID HYDROLYSIS INHIBITION UNRAVELS THAT UNSATURATED FATTY ACIDS INDUCE A ROBUST SYNTHESIS OF ENDOCANNABINOID-GLYCEROLS IN HUMAN MYELOID LEUKOCYTES	52
11.45	Linda A. Parker*, Gavin N. Petrie, Kiri L.Wills, Fabiana Piscitelli, Reem Smoum , Cheryl L. Limebeer, Erin M. Rock, Samantha Ayoub, Ashlyn Humphrey, Alexia Gene, Madeleine Sheppard-Perkins, Marieka DeVuono, Aron H. Lichtman, Vincenzo Di Marzo and Raphael Mechoulam	OLEOYL GLYCINE INTERFERES WITH ACUTE NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL, BUT NOT MORPHINE REWARD	53
12.00	**LUNCH**		
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ORAL SESSION 11. WOUND HEALING, MICROBIOTA AND OBESITY CHAIRS: ADI DRORI AND MITZI NAGARKATTI			
13.30	Natalia Murataeva*, Emma Leishman, Heather Bradshaw and Alex Straiker	A CENTRAL ROLE FOR 2-OLEOYLGLYCEROL IN CORNEAL WOUND HEALING	54

13.45	Sam R C Johnson*, James J Burston, Victoria J Tyrrell, Maceler Aldrovandi, Rossa Inglis, Robert Andrews, Jenna Cash, Paul Martin, Christopher P Thomas and Valerie B O'Donnell	12/15 LIPOXYGENASE ORCHESTRATES CELLULAR REMODELLING DURING WOUND REPAIR	55
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14.45	Amira Mohammed*, Hasan Alghetaa, Marcus Kaul, Prakash Nagarkatti and Mitzi Nagarkatti	THC TREATMENT IMPROVED NEUROTOXICITY BY ALTERING THE MICROBIOTA	59

15.00 – 16.00	**President's Lecture** THE STRUCTURES AND FUNCTIONS OF THE CANNABINOID RECEPTORS **Alexandros Makriyannis, Ph.D.** George D. Behrakis Chair of Pharmaceutical Biotechnology Director, Center for Drug Discovery (CDD) Northeastern University, Boston, MA, USA	
16.00 – 18.00	**Poster Session 3**	P3
18.30	**Reception**	
19.00	**ICRS BANQUET** AND **AWARDS CEREMONY**	

Departure: **Thursday, July 4th**

Notes:

Presenting Author*

POSTER SESSION P1

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Daniel Barrus*, Purvi Patel, Thuy Nguyen, Charlotte Farquhar, Tim Lefever, Yanan Zhang, Jenny Wiley, Thomas Gamage and Brian Thomas	PHARMACOLOGICAL CHARACTERIZATION OF THE SYNTHETIC CANNABINOID EG-018	P1-2
Paula Berman*, Liron Sulimani, Anat Gelfend, Keren Amsalem, Liran Baram, Gil Lewitus, Igal Louria-Hayon and David Meiri	CANNABINOIDOMICS – AN ANALYTICAL TOOL TO UNDERSTAND THE EFFECT OF MEDICAL CANNABIS TREATMENT	P1-3
Stefan Brand*, Hans-Jürgen Niemeyer, Carsten Röttger and Matthias Winkler	ENANTIOMERIC EXCESS DETERMINATION OF SYNTHETIC CANNABINOIDS	P1-4
Chris Breivogel*, Anicet Tresor Padjio Tchuisseu and Nshan Muradyan	CBD BLOCKS THE SEIZURE-INDUCING ACTIVITY OF CP55940	P1-5
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Marta Baranowska–Kuczko*, Hanna Kozłowska, Monika Kloza, Olga Sadowska and Barbara Malinowska	CHRONIC CANNABIDIOL TREATMENT IMPROVES VASCULAR FUNCTION OF HYPERTENSIVE DOCA-SALT RATS IN VASCULAR BED SPECIFIC MANNER	P1-7

Lukasz Ciesla*	CELLULAR MEMBRANE AFFINITY CHROMATOGRAPHY (CMAC) AS A TOOL TO IDENTIFY PHARMACOLOGICALLY ACTIVE COMPOUNDS INTERACTING WITH TRANSMEMBRANE PROTEINS	P1-8
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Luciana Leo*, Rufaida Al Zoubi, Pingwei Zhao, Daohai Yu, Eugen Brailoiu, Patricia H. Reggio and Mary E. Abood	MUTATIONAL ANALYSIS REVEALS BIASED SIGNALING AT THE CB1 CANNABINOID RECEPTOR	P1-32
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Thais Gazzi*, Marie Weise, Benjamin Brennecke, Claudia Korn, Wolfgang Guba, Chistoph Ullmer, Uwe Grether and Marc Nazare	GENERAL SYNTHETIC APPROACH TO SELECTIVE FLUORESCENT PROBES FOR THE CANNABINOID RECEPTORS CB1R AND CB2R	P1-61

Notes:

Presenting Author*

POSTER SESSION P2

MONDAY, JULY 1ST: 16:00 - 18:00

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Alexandria Bell*, Ricardo Martinez, Kylie Black, Jonah Wirt, Ken Mackie and Anna Kalinovsky	LOCALIZATION OF ENDOCANNABINOID SYNTHESIZING ENZYME NAPE-PLD DURING CEREBELLAR DEVELOPMENT	P2-6
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Nicole Bowles*, Saurabh Thosar, Maya Herzig, Noal Clemons, Garret Sauber, Alicia Stewart, Andrew McHill, Jonathan Emens, Cecilia Hillard and Steven Shea	BODY MASS INDEX BUT NOT SLEEP IMPACTS THE ENDOGENOUS CIRCADIAN RHTYHM OF THE ENDOCANNABINOID ANADAMIDE IN HUMANS	P2-9
Megan Sanctuary, Cinthia Wilkinson, Ashleigh Jones, Brittany Murphy, Edward Hoffenberg, Cecilia Hillard, Julian Romero and Colm Collins*	CELL-SPECIFIC CB2R DEFICIENCY ATTENUATES CHRONIC INTESTINAL INFLAMMATION	P2-10
Courtney Collins*, Heather Jackson, Nicolas J. Schlienz, Erin Martin, Ryan Scalsky, Marcel O. Bonn-Miller, Joel Munson and Ryan Vandrey	THE REALM OF CARING OBSERVATIONAL RESEARCH REGISTRY: EVALUATING THE HEALTH IMPACTS OF MEDICINAL CANNABIS USE	P2-11
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Presenting Author*

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Notes:

Presenting Author*

Plenary Speaker

Sunday, June 30, 2019
11:30 – 12:30

US CANNABIS POLICY: IMPLICATIONS FOR PUBLIC HEALTH

Susan R. B. Weiss, Ph.D.

Director
Division of Extramural Research
National Institute on Drug Abuse, Bethesda, MD, USA

Across the United States and the world, cannabis policies are changing rapidly, with public health impacts that cannot yet be foreseen based on the current state of our knowledge. Over the past decade, cannabis use has increased in young and older adults, and each year at least 4 million people in the U.S. meet diagnostic criteria for cannabis use disorder. The non-alignment of Federal and State laws creates obstacles for researchers, public health officials, and patients, as well as those in the cannabis industry. So does the fact that cannabis products vary widely in potency (i.e., tetrahydrocannabinol (THC) concentrations), constituents, and formulations. Edibles and concentrated THC products may pose particular health and safety risks; cannabidiol (CBD) products are now widely available to treat myriad conditions with little or no regulation.

This presentation will highlight the emerging public health and safety data from U.S. states that have implemented medical or adult use marijuana laws, with a focus on the challenges of implementing policies to minimize harm. It will also summarize what we currently know and what we still need to know about the adverse effects of cannabis and cannabinoids, as well as their potential therapeutic effects, so that we can better identify research needs and opportunities. More research on the long-term impact of cannabis use, particularly in adolescents, pregnant women (and their offspring), and persons with mental illness is greatly needed, as these groups are potentially the most at risk for adverse outcomes. Also, despite the widespread use of "medical marijuana" and CBD products, the potential therapeutic uses of cannabis and its constituent compounds require a great deal more study. To do so will require continuing to address long-standing barriers to research. As data accumulate, it will be important to remain unbiased in the assessment of both cannabis's harms and its benefits, so that the advancing science can most effectively inform policy and benefit the public health.

ICRS Lifetime Achievement Award

Sunday, June 30, 2019
13:30 – 14:30

CANNABINOID PHARMACOLOGY: MY FIRST HALF CENTURY

Roger Pertwee MA, DPhil, DSc, HonFBPhS

Institute of Medical Sciences
University of Aberdeen, Aberdeen, Scotland, UK

My cannabinoid research began at Oxford University, England, in the late 1960s, not long after the structural elucidation and first synthesis by Raphael Mechoulam *et al.* of two notable phytocannabinoids: Δ^9-tetrahydrocannabinol (THC) and cannabidiol.

The 1970s, 80s and 90s. In some of my initial research, I (1) contributed to the discovery in cannabis of the phytocannabinoid, tetrahydrocannabivarin, and to its initial pharmacological characterization, (2) developed the mouse ring test, an *in vivo* assay for measuring catalepsy induced, for example, by THC, and (3) discovered that THC lowers the thermoregulatory set point in mice, such that they still regulate their core temperatures, but at "sub-normal" levels.

After moving to Aberdeen University in 1974, I continued my cannabinoid research, for example by developing an "*ex vivo*" assay for "THC-like" drugs. This is performed with murine isolated vasa deferentia in which CB_1 agonists can produce concentration-related reductions in the size of electrically-evoked smooth muscle contractions, reductions now known to result from CB_1 receptor-mediated inhibition of the neuronal release of contractile transmitters. I used this assay in a project led by Raphael Mechoulam that began in the late 1980s, and was prompted by the then recent discovery of CB_1 receptors. This project provided the first evidence (1) that the endogenous compound, anandamide, is a CB_1 agonist and so an "endocannabinoid" and hence (2) for the existence of an "endocannabinoid system" of cannabinoid receptors and "endocannabinoids".

In the early 1990s I also helped found the ICRS, and later in that decade, interacted with the UK Government, with Medical organizations, and with UK and US multiple sclerosis patients who were self-medicating with cannabis. This I did in a manner that helped to encourage new medicalization of cannabis-derived cannabinoids.

The 21st century In this century, research in my laboratory has, for example, contributed to the discovery and/or development of (1) a water soluble synthetic analogue of THC, O-1057, (2) an allosteric site on the CB_1 receptor, and (3) novel synthetic compounds that behave as positive allosteric modulators (PAMs) of CB_1 or CB_2 receptors. These PAMs include two CB_1 PAMs (GAT211 and GAT229) that collaborators have shown to possess therapeutic potential for relieving neuropathic pain or ocular glaucoma, and a CB_2 PAM (EC-21a) that may, for example, have therapeutic potential for the treatment of blood cancer. My laboratory also recently helped to identify novel actions of the phytocannabinoids, cannabidiol, cannabidiolic acid, cannabigerol and tetrahydrocannabivarin, and of a stable synthetic analogue of cannabidiolic acid. These novel actions have revealed important potential therapeutic uses for most of these compounds.

In the more distant past, my laboratory also contributed to the pharmacological characterization of certain notable synthetic cannabinoids, including methanandamide, ACEA, ACPA, AM251, AM281, AM630 and HU-308, that had been designed and synthesized in the laboratories of some fantastic collaborators. Indeed, throughout the last half century, my research has involved productive and "synergistic" collaborations with many great scientists, based, for example, in Israel, the UK, the USA, Canada, Australia, Italy, Spain, Germany, Poland, Mexico or Russia.

Plenary Speaker

Monday, July 1, 2019
11:30 – 12:30

THE NEUROBIOLOGY OF DRUG ADDICTION: FROM DRUG REWARD TO HYPERKATIFEIA TO NEGATIVE REINFORCEMENT

George Koob, Ph.D.

Director
National Institute on Alcohol Abuse and Alcoholism
Rockville, MD, USA

Addiction is a chronically relapsing disorder characterized by compulsive drug seeking that is hypothesized to derive from multiple sources of motivational dysregulation. Positive reinforcement, incentive salience and pathological habits derive from super activation of reward neurotransmitter systems in the basal ganglia and set up negative reinforcement during withdrawal. The construct of negative reinforcement, defined as drug taking that alleviates a negative emotional state (hypohedonia, dysphoria, anxiety, hyperalgesia, irritability, and sleep disturbances- all comprising the term "hyperkatifeia"), is an addition source of motivation for compulsive drug seeking in addiction. The hyper negative emotional state associated with addiction has been termed hyperkatifeia from the Greek "katifeia" for "dejection or sadness" and is created by abstinence in the withdrawal/negative affect and protracted abstinence in the preoccupation/anticipation stages of the addiction cycle. In animal models, repeated extended access to drugs of abuse results in negative emotion-like states reflected in increased reward thresholds, decreased pain thresholds, anxiety-like and dysphoric-like responses. Such negative emotional states that drive negative reinforcement are hypothesized to derive not only from "within system" dysregulation of key neurochemical circuits that mediate incentive-salience/reward systems (dopamine, opioid peptides) in the ventral striatum but also from the" between system" recruitment of brain stress systems (corticotropin-releasing factor, dynorphin, norepinephrine, hypocretin, vasopressin, glucocorticoids and neuroimmune factors) in the extended amygdala. Excessive drug taking is also accompanied by deficits in executive function produced by neurocircuitry dysfunction in the medial prefrontal cortex that may facilitate the transition to compulsive-like responding and relapse. Thus, compelling evidence exists to argue that plasticity in the brain pain emotional systems is triggered by acute excessive drug intake, is sensitized during the development of compulsive drug taking with repeated withdrawal, persists into protracted abstinence, and contributes to the development and persistence of compulsive drug seeking.

Young Investigator Award Presentation

Monday, July 1, 2019
13:30 – 14:00

ANANDAMIDE, FAAH, STRESS AND ANXIETY: A TRANSLATIONAL JOURNEY

Matt Hill, Ph.D.

Associate Professor
The Hotchkiss Brain Institute
University of Calgary, Alberta, Canada

Endocannabinoid signalling has been well characterized as a modulator of the stress response, but divergent roles of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) have been identified. Work from our lab, and others, has found that in rodent models stress results in a rapid induction of FAAH activity, which results in an attenuation of apparent "tonic" signaling at the CB1 receptor. This loss of AEA signaling, particularly within the amygdala, appears to contribute to the generation of a stress response as inhibition of FAAH can reverse a multitude of neurobehavioral and endocrine responses to stress. The relevance of FAAH and AEA signaling in humans has also been examined by characterizing the impact of a gene variant in the FAAH gene (C385A) which results in a protein destabilization of FAAH and an elevation of AEA signaling. Consistent with the rodent data, carriers of the C385A allele of the FAAH gene exhibit reduced anxiety and fear, dampened responses to stress and blunted activation of the amygdala in response to threatening stimuli. More recently, we have begun to explore the impacts of pharmacological FAAH inhibition in humans to determine its impact on measures of fear and stress responsively as well. Together, these data provide a strong translational platform to indicate that AEA signaling may gate activation of the amygdala in response to stress and thus limit the generation of emotional states, such as fear and anxiety, and thus targeting FAAH may be a novel pharmacological approach to treating stress-related psychiatric disorders in humans.

Kang Tsou Memorial Speaker

Tuesday, July 2, 2019
12:00 – 13:00

VEGAS AND ULTRA-LSD: NEW CHEMICAL AND SYNTHETIC BIOLOGICAL TECHNOLOGIES

Bryan Roth, M.D., Ph.D.

Director
NIMH Psychoactive Drug Screening Program
Distinguished Professor, Department of Pharmacology
UNC Chapel Hill, NC, USA

In this talk I will highlight two new technologies. VEGAS is a synthetic biology technology which provides a platform for the directed evolution of proteins towards defined molecular objectives. I will show how this technology enables the creation of synthetic transcription factors, engineered receptors and allosteric state-dependent nanobodies. Ultra-LSD is an approach which provides a platform for the discovery of novel chemical matter from ultra-large virtual chemical libraries (Lyu, Wang et al, Nature 2019 for example). Recent examples will be provided which illustrate the potential utilities and power of these approaches.

President's Lecture

Wednesday, July 3, 2019
15:00 – 16:00

The Structures and Functions of the Cannabinoid Receptors

Alexandros Makriyannis, Ph.D.

George D. Behrakis Chair of Pharmaceutical Biotechnology
Director, Center for Drug Discovery (CDD)
Northeastern University, Boston, MA, USA

Although a number of cannabinoid analogs had been synthesized beginning in the 1960's, the discovery of the first cannabinoid receptor (CB1) took several decades. This happened through the joint efforts of a number of laboratories, and it involved the use of photoaffinity labelling of radioligands and imaging. The need for obtaining structural information on both CB1 and CB2 to serve as templates in target-based discovery led to computational efforts based on mutational studies for receptor modeling. More extended structural work involving the use of covalent ligands, targeted mutations and proteomic approaches in our laboratory, under the designation of Ligand Assisted Protein Structure (LAPS), provided more detailed experimental data and identified helix 6 in both receptors as being a key site involved in their activation/deactivation, validating some of the earlier computational efforts.

An intensive effort involving *Scripps*, *iHuman* in Shanghai, and our laboratory, the *Center for Drug Discovery* led to the crystal structure of the CB1 receptor, first in its inactive form using a suitably designed antagonist for crystallization and subsequently in its activated form, using two irreversible agonists. This work provided detailed information on the binding motif of CB1 ligands. It also validated the concept of a double-toggle (HX6 – Hx3) mechanism of activation. These first two successes were followed by the very recent crystallization of the CB2 receptor, which provided some initial evidence for the complementarity of the two receptors and introduced the activation/deactivation (ying-yang) receptor concept, which now serves as a basis for the development of CB2 agonist/CB1 antagonist therapeutic medications.

The availability of detailed structural information of a number of CB1 and CB2 ligand complexes, coupled with data on ligand-receptor dynamics, opens the door for the design of cannabinergic drugs with greater structural and functional specificity. The work also allows us, retrospectively, to examine the functional basis and potential improvement of pharmacologically useful ligands of potential therapeutic value. These include neutral antagonists, megagonists, functionally selective ligands, and CB1 irreversible chemical knock-out antagonists.

RENAMING DINOSAURS: EXHUMING THE ANCESTORS OF "SATIVA" AND "INDICA"

John M. McPartland* and Geoffrey W. Guy

GW Pharmaceuticals, Histon, Cambridge, CB24 9BZ, United Kingdom

INTRODUCTION: Everyone knows "Sativa" and "Indica" as colloquial names for distinct plants and plant products. Researchers desiring botanical nomenclature equate "Sativa" with *Cannabis sativa,* and "Indica" with *Cannabis indica*. This is erroneous.

C. sativa subspecies *sativa* refers to plants from Europe ("rope, not dope"), whose THC content ≤0.3% segregates them from Asian drug plants. "Sativa" and "Indica" are names for *two kinds of Asian drug plants*. They were distinguished by USA breeders in the 1970s. But they were known long before that, under different names. We exhumed old names from the literature, and linked them with herbarium specimens.

METHODS: We examined 1100 *Cannabis* specimens at 18 herbaria for morphological variation. Phytochemical and genetic data were obtained from published and unpublished sources. Old botanical names were assessed for legitimacy under protocols established by the *International Code of Nomenclature for Algae, Fungi, and Plants* (*ICN*).

RESULTS: Evidence traces "Sativa" to **South Asia** (India). It spread to the Middle East, Africa, and Southeast Asia by the 1200s. Europeans in India began assigning names in the 1500s, and preserving herbarium specimens in the 1600s. "Sativa" spread to America during the African slave trade, and spread to European medicine after O'Shaughnessy. They all share South Asian heritage. Europeans in **Central Asia** first described "Indica" (using other names) in the 1600s. By the 1800s British botanists collected Central Asian specimens from Afghanistan and Turkestan, and analyzed their phytochemistry. Afghani germplasm was smuggled into the USA in 1971, and cross-bred with "Sativa". Within 15 years, unhybridized plants of South Asian and Central Asian heritage had become difficult to obtain. Research on South Asian and Central Asian plants collected in the 1970s–1990s showed phytochemical and genetic differences. These differences began to disappear in 21st century studies of hybridized "Sativa" and "Indica".

DISCUSSION: The nomenclaturally legitimate variety names for the ancestors of "Sativa" and "Indica" are var. *indica* and var. *afghanica*, respectively. They are domesticated forms. Their wild-type progenitors are named var. *himalayensis* and var. *asperrima*, respectively. Widespread crossbreeding between *indica* and *afghanica* has obscured taxonomic differences. They face extinction through introgressive hybridization. Seen pessimistically, the varieties described here are becoming a lost world, an exercise in renaming dinosaurs. Optimistically, the formal recognition of Central Asian and South Asian varieties provides them with unambiguous names, and may help prevent their extinction. *Cannabis* biodiversity needs to be conserved—for future breeding efforts, at the very least. We will formalize the nomenclature in a botanical journal (this abstract does not conform to *ICN* protocols).

SHORT- AND LONG-TERM ACUTE EFFECTS OF CANNABIS ON HEADACHE AND MIGRAINE: A NATURALISTIC STUDY OF MEDICAL CANNABIS USERS

Carrie Cuttler*[1,2], Alexander Spradlin[1] and Rebecca Craft[1,2]

Department of Psychology[1], Translational Addiction Research Center[2],
Washington State University, Pullman, WA, USA

The use of cannabis to alleviate headache and migraine is relatively common in medical cannabis patients, yet research on its effectiveness remains sparse. To date there has only been one randomized, double-blind study of cannabinoid treatment for headache or migraine in humans; the results of which indicated that nabilone (a synthetic cannabinoid) was more effective than ibuprofen in reducing pain intensity. The present study expands on the limited scope of research in this area, providing a naturalistic account of patients' perceived changes in severity of headache and migraine as a function of inhaling different strains and doses of cannabis. Our objectives were to: 1) examine whether ratings of headache and migraine severity are significantly reduced after inhaling cannabis, 2) determine whether there are gender differences in these putative effects, 3) assess whether THC content, CBD content, and/or interactions between THC and CBD predict changes in severity ratings, 4) investigate differences in severity rating changes across various doses, 5) explore potential tolerance development across cannabis treatment sessions, and 6) assess changes in baseline (i.e., pre-cannabis use) ratings of headache and migraine severity across cannabis treatment sessions.

To achieve these objectives, we analyzed global data from the app Strainprint™. This app provides patients with a means of tracking changes in symptoms of a variety of medical conditions as a function of different doses and strains of cannabis. Specifically, patients indicate the symptom they are experiencing, rate its severity on a 0-10 scale, enter the strain they are about to use (THC and CBD content are obtained from Canadian licensed producers), enter the method of ingestion and dose (number of puffs) of cannabis used, and then re-rate their symptom severity 20 mins to 4 hrs after cannabis use. The sample used in the present study contained 1,587 individuals who used the app over 15,000 times to track changes in headache severity and 826 individuals who used the app over 10,000 times to track changes in migraine severity.

The results of a series of analyses using multilevel modeling revealed that patients reported a 48.5% reduction in headache severity and a 53.7% reduction in migraine severity following inhalation of cannabis. Men and women reported comparable reductions in headache severity but men reported significantly greater reductions in migraine severity following cannabis use than did women. No main effects or interactions between THC and CBD were detected, and all reported quantities used (1 to 10+ puffs) produced significant reductions in headache and migraine severity ratings. No change in baseline symptoms was detected across time/cannabis treatment sessions, and no tolerance was detected. Instead, larger reductions in migraine severity were reported across time/cannabis treatment sessions. The results indicate that inhaled cannabis reduces self-reported headache and migraine severity by approximately 50%, and repeated use of cannabis to treat headache and migraine does not appear to be associated with tolerance to its effects nor to medication overuse rebound effects.

CHIRAL ENDOCANNABINOID LIGANDS

Lipin Ji,[1+], Yingpeng Liu,[1+] Fei Tong,[1] Marsha Eno,[1] Shalley Kudalkar,[2] Alex Straiker,[3] Ai-ling Li,[3] Othman Benchama,[1] Chandrashekhar Honrao,[1] Anisha Korde,[1] Amey Dhopeshwarkar,[3] Paula Morales,[4] Shu Xu,[2] Michaela Dvorakova,[3] Dow Hurst,[4] Simiao Wu,[1] JodiAnne T. Wood,[1] Nikolai Zvonok,[1] Patricia Reggio,[4] Ken Mackie,[3] Lawrence Marnett,[2] Andrea G. Hohmann,[3] Alexandros Makriyannis[1]* and Spyros P. Nikas.[1]*
[+]equal contribution

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[4]Center for Drug Discovery, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27402, USA.

The chemistry and biology of the endocannabinoids anandamide (AEA) and 2-AG is rapidly expanding with the discoveries of new biological roles for these lipid mediators, pharmacological targets, and enzymes responsible for their synthesis, hydrolysis, transport, and oxidative metabolism. Structural modifications to enhance the biological activities and target specificities of these lipids while increasing their metabolic stabilities, is a challenge. In our approach we explore two design principles: a) the introduction of chiral methyl groups at judiciously chosen positions within the arachidonoyl chain of the prototype, and b) the inclusion of steric bulk and stereochemistry in the vicinity of the hydrolysable head group of the lipid. Here we report novel arachidonoyl ethanolamide (AEA) and 2-arachidonoyl glycerol (2-AG) analogs that combine distinct conformational properties with unique functional profiles at the cannabinoid CB1 and CB2 receptors, coupled with enhanced stabilities for their respective deactivating enzymes. Unlike AEA and 2-AG, the chiral endocannabinoid probes reported here do not generate arachidonic acid which exhibits non-CB-mediated biological actions and *in vitro* and *in vivo* toxicity. As such, these novel chiral endocannabinoid ligands along with the recent determination of the crystal structures of the CB1 and CB2 receptors, will inspire the design of improved analogs for *in vitro*, *in vivo*, and computational studies aimed at exploring the (patho)physiological roles of AEA and 2-AG.

Acknowledgements: Research supported by NIH/NIDA grants DA009158 and DA007215.

ACUTE SELF-ADMINISTRATION OF LEGAL MARKET FLOWER AND CONCENTRATED CANNABIS: CANNABINOID BLOOD LEVELS, SUBJECTIVE INTOXICATION, AND NEUROBEHAVIORAL OUTCOMES

L. Cinnamon Bidwell*[1], Jarrod Ellingson[1], Sophie YorkWilliams[1], Hollis Karoly[1], Leah N. Hitchcock[1], Cristina Sempio[2], Jost Klawitter[2], Brian Tracy[3], Angela D. Bryan[1] and Kent E. Hutchison[1]

[1]University of Colorado Boulder, Colorado, USA
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[3]Colorado State University, Fort Collins, Colorado, USA

Background: Our research program investigates the effects of commonly used cannabis strains and products, as they are used in everyday life, as opposed to the effects of U.S. government grown, lower potency cannabis in controlled laboratory experiments, which may underestimate effects of Δ9-tetrahydrocannabinol (THC) as available on the legal market. The use of novel cannabis research methods that are compatible with current federal regulations is imperative to study the effects of legal market cannabis. This study used novel observational research methodology to study higher strength THC flower and concentrated cannabis, which have been minimally researched and become increasingly available since legalization. Methods: Regular users of cannabis flower and concentrate were asked to use one of two legal market flower (16% vs 24% THC) or concentrated (70% vs 90% THC) cannabis products that they purchased from a local dispensary. Before, immediately after, and 1-hour after *ad libitum* use, flower (n=55) and concentrate (n=63) users were assessed in a mobile pharmacology lab. Measures included a blood draw to measure cannabinoid blood levels, self-reported subjective drug effects, and neurobehavioral tasks testing memory, inhibitory control, and standing balance/postural stability functions. Results: THC, and its metabolites, THC-COOH and 11-OH-THC, were each acutely elevated after use. The concentrate users had higher levels of THC and THC metabolites across all assessments, as well as a stronger peak acute effect for THC. Both flower and concentrate users reported increased subjective drug effects immediately after use; however, levels of subjective intoxication did not differ across flower and concentrate users or by potency within flower or concentrates. Thus, the results indicated that THC blood levels were higher for concentrate users overall and acutely, but that potency did not affect blood levels for either form of cannabis. In addition, despite these differences in blood levels, subjective levels of intoxication did not differ across flower and concentrate groups. Broadly, cognitive effects were minimal even for high potency forms of cannabis. However, acute cognitive impairment was found on verbal recall errors, but unexpectedly this impairment was greater for flower users acutely than for concentrate users. Postural stability was also acutely impaired immediately after cannabis use, and this acute effect on balance did not differ among flower and concentrate users. Conclusions: Findings suggest that users of high potency flower and concentrated cannabis products may demonstrate tolerance to both the subjective and cognitive effects of even very high potency cannabis. In addition, flower and concentrate users may titrate their use to subjective levels of intoxication regardless of product potency. Portably-performed quantitative measures of impaired postural stability appear to be responsive to acute use even in regular users. These studies provide the first data on the acute effects of legal market high potency THC products on important measures relevant to public health and cannabis harm-reduction.

ASSESSING TRENDS OF CBD OILS QUALITY AT THE EU MARKET

Jana Hajslova*[1], Marie Fenclova[1], Frantisek Benes[1], Ethan Russo[2] and Pavel Kubu[2]

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CBD oils represent a popular group of hemp-derived ´food supplements´, rich in the content of non-psychotropic phytocannabinoid cannabidiol (CBD). These products are prepared by enrichment of edible oils either by purified CBD or hemp extract. In the latter case, CBD oils may contain not only the health beneficial CBD but also various amount of psychotropic Δ^9-tetrahydrocannabinol (Δ^9-THC). Moreover, CBD oils may be a source of exposure to hazardous contaminants, such as polycyclic aromatic hydrocarbons (PAHs). It should be noted, that in spite of high demand for CBD oils, there is available a very limited information on the quality of these products being introduced at the markets. On this account, we decided to assess the quality of CBD oils available at the EU retail market focusing not only on CBD, but involving also Δ^9-THC and PAHs as quality indicators. In total, 70 CBD oil samples were collected within three sampling campaigns performed within the years 2016-2019.

The content of CBD and Δ^9-THC was determined by ISO 17025 accredited UHPLC-HRMSMS based method, the compliance of obtained data with the information provided by CBD oils producers on the label was evaluated. Further, the amount of Δ^9-THC in daily dose of tested oils recommended by producers was compared with the acute reference dose (ARfD) of 1 µg Δ^9-THC/kg b.w. set by EFSA. The content of PAHs was determined by HPLC-FLD and compared with the maximum limits of 2 µg/kg for benzo(a)pyrene (BaP) and 10 µg/kg for the sum of 4 carcinogenic PAHs benzo(a)pyrene, chrysene, benzo(a)anthracene and benzo(b)fluoranthene (Σ4 PAHs) according the EU Regulation 2015/1933. The results of chemical analyses showed significant issues in all of the monitored quality indicators. The lower than declared amount of CBD or its missing content information was observed in over 20 % of the tested samples. Despite the slight improvements over the testing years (CBD content even higher than declared), the situation could not be classified as satisfactory. The results of Δ^9-THC content were even worse. Although we detected this psychotropic compound in 89 % of the oils, in most cases, no information on Δ^9-THC presence was provided at the label. Considering the consumer with a body weight 70 kg, the ARfD was exceeded by almost 50 % of samples with the highest Δ^9-THC dose being even 4800 % of the ARfD. Such observation is alarming as even relatively low amounts of Δ^9-THC can influence normal function of cognition and impair comsumer's capacity to drive and make decisions in general. Raising significant personal as well professional life risks for consumer especially when not being aware of the controlled psychoactive substance intake at all. The continuously unsatisfactory situation was observed also in case of PAHs, which were detected in all of the tested CBD oils. The legislative limits for BaP and / or Σ4 PAHs were exceeded in 60 % of the samples with maximum concentrations being even 9- and 19-fold higher than maximum limits, respectively. As the PAHs contamination most probably results from improper drying procedures of hemp (contact with combustion gases), there is a strong need for improvement of respective processing practices.

ACUTE AND CHRONIC EFFECTS OF CANNABIDIOL ON HAEMODYNAMICS IN HEALTHY MALES

Salahaden R. Sultan[1, 2], Timothy J. England[1] and Saoirse E. O`Sullivan*[1]

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We previously showed in healthy males that acute oral administration of CBD (600 mg) causes a reduction in blood pressure at rest and in response to stress. The aim of this study was to see whether tolerance develops in this response to CBD with repeated dosing, and to establish whether other vascular endpoints are affected by CBD.

26 healthy males (26.3 ± 5.6, mean±SD) years were given 600 mg of CBD or placebo orally for seven days in a randomised, placebo-controlled, double-blind, parallel study (n=13 per group). Cardiovascular parameters were assessed after single (acute) and repeated (chronic) dosing at rest and in response to stress (isometric exercise) using a Finometer, Vicorder (measuring pulse wave velocity at the brachial site) and Duplex Ultrasound (measuring blood flow volume of the right internal carotid artery (ICA), and endothelial function of right brachial artery).

Compared to placebo, CBD significantly reduced resting mean arterial pressure (mean difference (MD) -2 mmHg, p=0.01), with a trend for a reduction in systolic blood pressure (MD -2.28 mmHg, p=0.08) and diastolic blood pressure (MD -1.76 mmHg, p=0.06) after the first but not the seventh dose of CBD. In response isometric exercise, volunteers who had taken CBD had lower systolic blood pressure after the first dose of CBD (MD -6 mmHg, p=0.007) and after the seventh dose of CBD (MD -5.7 mmHg, p=0.01). Chronic CBD dosing increased ICA diameter (ICA-D, MD +0.55 mm, p=0.01) and tended to increase ICA flow volume (ICA-FV, MD +0.12 l/min, p=0.07). Compared to day one, repeated dosing with CBD significantly increased ICA-D (MD +0.43 mm, p=0.05) and tended to increase ICA-FV (+0.08 l/min, p=0.09), reduce arterial stiffness (pulse wave velocity; MD -0.44 m/s, p=0.05, n=11 per group), and improve endothelial function (flow mediation dilatation MD +3%, p=0.05, n=6 per group).

These findings show that effects of CBD on resting BP develop tolerance, but that the BP lowering during stress seen with CBD persists. The reduction on arterial stiffness, and improvements in internal carotid artery blood flow and endothelial function after chronic CBD treatment indicate a positive effect on vascular function that warrants further investigation in relevant patient populations.

HIGH ANXIETY? EXAMINING THE IMPACT OF FOUR WEEKS OF TREATMENT WITH A NOVEL HIGH CANNABIDIOL PRODUCT

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Currently, 33 states and the District of Columbia have fully legalized medical cannabis (MC) programs and an additional 14 states offer limited access to MC products. Cannabis is comprised of over 100 phytocannabinoids, including delta-9 tetrahydrocannabinol (Δ9-THC), the primary psychoactive constituent of the plant, and cannabidiol (CBD), a primary non-intoxicating cannabinoid which may confer therapeutic benefits. Acute administration studies in both animals and humans have demonstrated that CBD may have anxiolytic properties; however, thus far, no clinical trials have assessed the impact of high CBD products in individuals who suffer from anxiety. Accordingly, we are conducting the first open-label to double-blind clinical trial assessing the impact of a novel, proprietary high-CBD, low-THC sublingual tincture in patients meeting a minimum threshold of "moderate" anxiety with no current use of any cannabinoid-based products. Study visits occurred at baseline (prior to beginning CBD treatment), and weekly over the course of the 4-week treatment period; at each visit, participants completed ratings of anxiety (Beck Anxiety Inventory [BAI], Overall Anxiety Severity and Impairment Scale [OASIS], Hamilton Anxiety Rating Scale [HAM-A]), mood (Beck Depression Inventory [BDI], and a number of other clinical and cognitive measures. Patients administered the custom-formulated, whole plant-derived tincture containing approximately 10mg/ml CBD three times per day.

Preliminary data from the open-label phase of the trial suggests significant improvement following 4 weeks of treatment when compared to baseline. Specifically, findings suggest that use of a custom-formulated, whole plant-derived high CBD sublingual tincture results in less severe anxiety (OASIS) and fewer anxiety-related symptoms (BAI) following 4 weeks of treatment relative to baseline. Patients also demonstrated significant reductions in depressive symptoms and improved mood. Further, we observed significant improvements in sleep and on several quality of life measures, which may be attributable to the improvements in clinical symptoms experienced by these patients. In addition, the study product appears to be well-tolerated with no discernible side effect profile. While these results are promising and reflect findings from our larger observational study noting clinical improvements in MC patients, results should be interpreted with caution as the open-label study is still ongoing. Furthermore, a definitive assessment of the impact of this novel treatment will be ascertained in the next phase of this trial, a double-blind, placebo-controlled phase of the study, which will provide empirically sound data regarding the efficacy of sublingual CBD for anxiety.

Acknowledgements**:** This project was funded by generous donations to the Marijuana Investigations for Neuroscientific Discovery (MIND) Program at McLean Hospital

ACUTE PHARMACOKINETICS AND PHARMACODYNAMICS OF ORAL AND VAPORIZED CANNABIDIOL IN HEALTHY ADULTS

Tory Spindle*[1], Edward Cone [2], John Mitchell[3], George Bigelow[1], Ron Flegel[4] and Ryan Vandrey[1]

[1]Behavioral Pharmacology Research Unit, Johns Hopkins University School of Medicine
[2] Pinney Associates, Inc.
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The use and availability of oral and inhalable cannabidiol (CBD) products has increased drastically in recent years. Given that limited preclinical evidence suggests CBD may convert to THC in the gut, controlled studies are needed to explore whether acute administration of CBD would produce positive results on a drug test. This study evaluated and compared the acute pharmacokinetics and pharmacodynamics of oral CBD, vaporized CBD, vaporized cannabis containing high CBD and low THC, and placebo. Six healthy adults completed four, double-blind, double dummy, acute CBD dosing sessions in a within-subjects cross-over study. A 100mg CBD dose was delivered in 3 formulations: encapsulated synthetic CBD powder, vaporized synthetic CBD powder, or vaporized cannabis containing 100mg CBD and 4mg THC. Placebo capsules were filled with cellulose, placebo cannabis was obtained from NIDA. Experimental sessions were separated by 1 week. Pharmacodynamic assessments (subjective drug effect ratings, Divided Attention Task, Paced Serial Addition Task, Digit Symbol Substitution Task) were evaluated at baseline and for 8 hours post-dosing. Whole blood, oral fluid, and urine samples were collected repeatedly over 5 days after each drug administration.

Vaporized CBD and high CBD cannabis increased ratings of Drug Effect and Pleasant Drug Effect compared with placebo. CBD did not increase subjective ratings for effects typically associated with acute cannabis effects and none of the active dose conditions significantly impaired cognitive performance. Following vaporization of pure CBD, delta-8 and delta-9 THC were measured in the oral fluid of all 6 participants; quantitative levels were below 2ng/mL within 4 hours of administration. No THC, 11-OH-THC, or THCCOOH were measured in blood, and trace amounts of THCCOOH were measured in urine after both oral and vaporized CBD administration. Overall, these results demonstrate that vaporization of pure CBD produces discriminable subjective drug effects and can result in the presence of THC in oral fluid, but does not produce "THC-like" subjective effects or cognitive/psychomotor impairment. These findings also suggest that a single acute exposure of 100mg CBD (oral or vaporized) would not result in a positive drug test using current federal testing standards beyond 1 hour after exposure. Additional research is needed to characterize the pharmacodynamic and pharmacokinetic effects of acute vaporized CBD at higher doses, both alone and in combination with varying vaporized THC doses, and also to explore the effects of chronic CBD use on drug testing outcomes.

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CANNABIDIOL ATTENUATES COCAINE REWARD BY CB2, 5-HT$_{1A}$ AND TRPV1 RECEPTOR MECHANISMS IN RATS

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Cocaine addiction continues to be a serious health problem in our society. Despite many years of intense research there is currently no FDA-approved medication to treat cocaine addiction. Current search for improved pharmacotherapeutics has been focused on agents targeting dopamine, GABA and glutamate systems, but limited success has been achieved at a clinical level. Cannabidiol (CBD) is a U.S. FDA-approved cannabinoid for the treatment of epilepsy and in recent years, CBD was reported to have other medical implications including the one for substance use and abuse. Here we evaluated the potential anti-cocaine therapeutic utility of cannabidiol and explored possible underlying receptor mechanisms by which CBD produces these effects. In a series of experiments, we demonstrated that systemic administration of CBD (3, 10, 20, 40 mg/kg, i.p.) dose-dependently reduced cocaine-enhanced electrical brain-stimulation reward, lowered a break-point for cocaine self-administration under a progressive-ratio schedule of reinforcement, and shifted cocaine self-administration dose-response curve downward. CBD had no effect on cocaine self-administration maintained by higher cocaine doses (0.5 or 1.0 mg/kg). Using *In vivo* microdialysis with HPLC, we showed that CBD alone significantly increased the extracellular DA level in the nucleus accumbens (NAc), whereas pretreatment with CBD dose-dependently blocked cocaine-induced increases in NAc DA. Regarding the receptor mechanisms, we found that pretreatment with AM251 (a CB1R antagonist), naloxone (an opioid MOR antagonist) or CID16020046 (a GPR55 antagonist) failed to block, while pretreatment with AM630 (a CB2R antagonist), WAY100135 (a 5-HT$_{1A}$ antagonist), or capsazepine (a TRPV1 channel antagonist) blocked CBD-induced reduction in cocaine self-administration and cocaine-enhanced brain-stimulation reward. These findings suggest that CBD may have certain therapeutic utility to blunt rewarding effects of cocaine, possibly by stimulation of CB2, 5-HT$_{1A}$, and TRPV1 receptors.

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ALLOSTERIC MODULATORS OF THE CANNABINOID CB1 RECEPTOR: DIARYL UREAS

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Blockade of the cannabinoid 1 receptor (CB1R) has shown great promise for the treatment of drug addiction and abuse, but the use of CB1R antagonists/inverse agonists in clinics has been limited by their psychiatric side effects. The development of CB1R allosteric modulators offers a much-needed alternative strategy to modulate CB1R signaling for therapeutic benefits.

Our group has developed and reported several series of CB1R allosteric modulators based on the diaryl urea scaffold. Continued effort has identified novel allosteric modulators including those bearing five membered aromatic ring systems such as a thiophene. The target compounds displayed nanomolar potencies in calcium mobilization and GTP-γ-S binding assays. Similar to PSNCBAM-1 and Org27569, these compounds exhibited a positive allosteric modulator-antagonist (PAM-Antagonist) profile, increasing the binding affinity of [^{3}H]CP55940 but decreasing agonist activity in functional assays. As expected with the allosteric mechanism, these compounds dose dependently reduced the E_{max} value of the orthosteric CB1R agonist CP55940. Most compounds possessed low nanomolar IC_{50} values at CB1R without any significant activities at the CB2 receptor. Several compounds showed good metabolic stability in rat liver microsomes. One such compound RTICBM-74 ($T_{1/2}$ > 300 min in RLM) displayed greater *in vivo* potency than PSNCBAM-1 ($T_{1/2}$ = 13 min) in attenuating the reinstatement of extinguished cocaine seeking behavior in rats. These results support the development of potent and selective CB1R allosteric modulators as potential medications for the treatment of drug addiction and abuse.

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STRUCTURAL ANALYSIS OF THE CB_1 CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A)

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The **c**annabinoid **r**eceptor **i**nteracting **p**rotein 1a (CRIP1a) modulates CB_1 receptor signaling via G proteins. CRIP1a appears to (1) attenuate agonist-mediated signaling by competing with certain Gi protein subtypes; and (2) control agonist-driven CB_1 receptor cell surface internalization by competing with β-arrestins. The molecular basis for these biological effects is not known. Previous studies have used a variety of computational approaches to generate models of CRIP1a and the related protein CRIP1b. The proposed CB_1 receptor binding interfaces, however, were not conserved and remain to be validated. In this study, we determined the first high-resolution structure of CRIP1a by X-ray crystallography. The DALI algorithm was able to identify several low-sequence identity homologs not related to those previously proposed. Interestingly, CRIP1a appears to belong to a unique class of proteins that are regulated by other protein partners. Experiments are underway to determine the identity and role of the CRIP1a and CRIP1a-like proteins and their association with and impact on CB_1 receptor signaling. Targeting the CRIP1a-CB_1 receptor interaction has tremendous therapeutic potential for pain management, epilepsy, neurotoxicity, motor disfunction and addiction.

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CANNABINOID-1 RECEPTOR REGULATES SOLUBLE LEPTIN RECEPTOR LEVELS VIA C/EBP HOMOLOGOUS PROTEIN (CHOP), CONTRIBUTING TO OBESITY-RELATED HEPATIC LEPTIN RESISTANCE

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Leptin plays a major role in regulating energy homeostasis by targeting hypothalamic leptin receptors to reduce food intake and increase energy expenditure. The soluble isoform of leptin receptor (sOb-R), secreted by the liver, binds free leptin and regulates its bioavailability and bioactivity. Diet-induced obesity (DIO) is associated with reduced sOb-R levels and hyperleptinemia, leading to leptin resistance. Obesity is also associated with increased activity of the endocannabinoid (eCB) system. In previous studies, we demonstrated that blockade of the cannabinoid-1 receptor (CB_1R) attenuates DIO-associated leptin resistance by reversing hyperletinemia via reducing leptin secretion from adipocytes, increasing its renal clearance, and restoring hypothalamic leptin signaling. Yet, a direct regulation of sOb-R production by the eCB system has not been reported.

In this work we determined the contribution of the hepatic eCB/CB_1R system to the expression and/or subsequent release of sOb-R as well as leptin sensitivity by using various pharmacological and genetic approaches in mice and cultured hepatocytes.

We found that both peripheral blockade and hepatic deletion of CB_1R reversed DIO-induced reduction in sOb-R gene expression and protein levels as well as hepatic leptin resistance. Hepatic over expression of CB_1R in global CB_1R null mice was sufficient to recapitulate the phenomenon documented in DIO wild-type animals. Interestingly, the peripherally restricted CB_1R inverse agonist, JD5037, failed to reverse DIO-induced reduction of sOb-R levels in mice that lack C/EBP homologous protein (CHOP). In addition, direct activation of CB_1R in hepatocytes reduced sOb-R levels in the culture media and cell lysates, in a CHOP-dependent manner. Moreover, CHOP stimulation increased sOb-R expression and release. Reporter assay and chromatin immuneprecipitation data suggest that CHOP is a positive regulator of the leptin receptor promoter.

These findings highlight a novel contribution of the hepatic eCB/CB_1R system to the development of leptin resistance by regulating sOb-R levels via CHOP.

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MRI-1867, A THIRD GENERATION CB_1R ANTAGONIST, FOR EFFECTIVE THERAPY OF A RARE DISEASE, HERMANSKY-PUDLAK SYNDROME PULMONARY FIBROSIS

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Hermansky-Pudlak syndrome (HPS) is a rare genetic disorder with 10 identified subtypes, each corresponding to mutation of a unique gene. Patients with HPS type 1, 2, or 4 develop pulmonary fibrosis (PF). HPS1 is the most common and severe form, leads to adult-onset fatal PF with no effective treatment. Considering the complex disease pathogenesis, targeting multiple pathways may improve therapeutic efficacy. Endocannabinoids acting via cannabinoid type 1 receptors (CB_1R) promote tissue fibrosis, including PF. The activity of inducible nitric oxide synthase (iNOS) is also increased in fibrosis.

First, we evaluated the role of the endocannabinoid/CB_1R and iNOS systems in bleomycin (Bleo)-induced PF in a mouse model of HPS1, *pale ear*, and in human HPS-PF using bronchoalveolar lavage fluid (BALF), plasma, and lung samples from patients with HPS and their controls. We found that CB_1R and iNOS were overexpressed in the lung of HPS-PF patients and in bleo-induced PF in *pale ear* mice. Anandamide (AEA) was significantly more abundant in BALF samples from HPS-PF patients and HPS1-PF mice compared to BALF from respective controls, and its level negatively correlated with pulmonary function results. Interestingly, plasma AEA levels from HPS and HPS-PF patients were also significantly elevated compared to normal volunteers, suggesting potential biomarker function of AEA. We then tested the therapeutic efficacy of combined inhibition of CB_1R and iNOS in Bleo-induced PF model of HPS. Tissue levels of endocannabinoids, CB_1R, iNOS and fibrogenic markers were increased 1-week post-Bleo infusion and remained elevated at 6 weeks along with progressive fibrosis. Bleo-challenged *pale ear* mice were treated daily between weeks 1 and 6 by oral gavage of vehicle or MRI-1867 (10 mg/kg/day), a peripherally restricted dual CB_1R/iNOS inhibitor. MRI-1867 treatment significantly attenuated PF progression and improved pulmonary function in *pale ear* mice.

In conclusion, we have identified CB_1R as a novel therapeutic target in HPS-PF. Furthermore, dual-targeting CB_1R and iNOS for inhibition is an effective anti-fibrotic therapeutic strategy for HPS-PF. MRI-1867 is being developed as a candidate drug to treat patients with HPS-PF.

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A CRITICAL EVALUATION OF TERPENOID SIGNALING AT CANNABINOID CB1 RECEPTORS IN A NEURONAL MODEL

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Cannabis has a long history of use by humans, stretching back thousands of years, and is experiencing a surge in use coincident with a shift in its legal status, having been formally legalized in Uruguay and Canada and several American states. Most attention has been paid to the two phytocannabinoids that are present in the greatest quantity in cannabis– tetrahydrocannabinol and cannabidiol. However, there is also a strong interest in the other phytocannabinoids and terpenoids present in cannabis. Cannabinoid-related terpenes are structurally unrelated to phytocannabinoids, but are aromatic, sometimes pleasingly so, and have been promoted commercially as having defined physiological effects, particularly in relation to cannabis. To take an example, myrcene is proposed to enhance the cannabis high, leading to the claim that consumption of a mango before smoking cannabis prolongs the high. Other claimed effects run the gamut from analgesia and sedation to suppressing inflammation. By and large, however there has been little or no systematic study of terpenoid interaction with the endocannabinoid signaling system.

We tested several of the more widely promoted terpenoids for their interaction with endogenous cannabinoid signaling in a well-characterized neuronal model. Autaptic hippocampal neurons express CB1 receptors as well as the cellular machinery to synthesize and metabolize the endocannabinoid 2-AG as well as several forms of CB1-mediated neuronal plasticity. This system affords the opportunity to test direct activation of this system as well as evaluating synergistic activity with the endogenous cannabinoids. We find that of myrcene, nerolidol, limonene, linalool and a-pinene, most compounds had little if any effect on cannabinoid signaling. Nerolidol however was found to have dual opposing effects: Nerolidol reduced DSE post-synaptically by inhibiting eCB synthesis, with significant effects even at 100nM. However, nerolidol also enhanced maximal CB1 inhibition of neurotransmission by 2-AG.

In summary, we have tested five terpenoids that have been proposed to have an assortment of cannabinoid-enhancing or modulating effects, finding that most have little or no effect on cannabinoid signaling in a neuronal model. However, nerolidol appears to have two opposing sites of action: inhibiting eCB production while enhancing CB1 receptor signaling.

HYBRID INHIBITOR OF PERIPHERAL CANNABINOID-1 RECEPTOR AND INDUCIBLE NITRIC OXIDE SYNTHASE MITIGATES THE DEVELOPMENT OF DYSLIPIDEMIA

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Diabetic dyslipidemia (DD) is characterized by increased plasma triglycerides (TGs) and decreased high-density lipoprotein cholesterol (HDL-C) levels. It represents one of the major factors contributing to Non-Alcoholic Steatohepatitis (NASH) and cardiovascular risk observed in type-2 diabetes (T2D), with no specific treatment currently available. A major contributor to elevated plasma TGs in subjects with insulin resistance/T2D is increased VLDL production, which could result from increased de novo lipogenesis (DNL), increased microsomal triglyceride transfer protein (MTP) activity and/or a reduction in ApoB degradation in hepatocytes. DD is multifactorial and the overactivity of the endocannabinoid - cannabinoid-1 receptor system (ECS/CB1R) is amongst the potential factors involved in its pathophysiology. Indeed, CB1R activation increases DNL and decreases fatty acid oxidation in the liver whereas CB1R inactivation or deletion improves liver lipid metabolism. Additionally, nitric oxide (NO) produced by inducible NO Synthase (iNOS) in the liver is associated with diet-induced steatohepatitis, insulin resistance and impaired hepatic lipid metabolism. Consequently, we hypothesized that simultaneous inhibition of ECS and iNOS activity could improve metabolic deregulations associated with DD.

Here we used MRI-1867, a peripherally restricted hybrid inhibitor of CB1R and iNOS, in mice with diet-induced obesity (DIO) to document its therapeutic efficacy in DD. MRI-1867 treated DIO mice displayed lower body weight and improved glucose homeostasis as evidenced by a lower blood glucose, improved glucose tolerance and insulin sensitivity, as compared to vehicle-treated DIO mice. Hepatic steatosis was decreased by > 60% and the plasma HDL-C/LDL-C ratio was improved by treatment with MRI-1867 compared to vehicle. MRI-1867 also reduced the secretion of TGs and VLDL from the liver, which could be partly attributed to a down-regulation of the MTP activity through a FoxO1-dependent mechanism leading to reduced VLDL assembly. Furthermore, MRI-1867 treatment resulted in a strong upregulation of the LDL receptor protein expression that could have been induced by the marked decrease in circulating levels of the proprotein convertase subtilisin/kexin type 9 (PCSK9) observed in these mice. Indeed, PCSK9 is able to bind to the LDL receptor and targets it for lysosomal degradation. Thus, reduced plasma PCSK9 levels and increased LDR receptor could lead to the lower LDL-C. PCSK9 is mainly regulated through an SREBP-2- and *E2f1*- dependent mechanism, the expression of both of which was strongly inhibited by MRI-1867. Additionally, MRI-1867 treatment also affected GLP-1 production, PPAR and FXR activity, all of which being potential therapeutic targets in DD.

In conclusion, combined blockade of peripheral CB1R and iNOS could improve DD via multiple mechanisms, thus representing a multi-target monotherapy.

FLUORESCENTLY LABELED TYPE-2 CANNABINOID RECEPTOR (CB_2R) LIGANDS: FROM INITIAL ATTEMPTS TOWARD A HIGHLY VERSATILE CHEMICAL TOOLBOX

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Activation of CB_2R holds great promise for the treatment of tissue injury and inflammatory diseases. Drug discovery efforts resulted in the identification of highly selective CB_2R agonists which show robust efficacy in various animal models of chronic and inflammatory pain, diabetic neuro- and nephropathy, liver cirrhosis, and ischemic-reperfusion injury. For the successful development of new drugs profound knowledge of their underlying molecular and cellular mechanism(s) of action is required. In addition, it's key to understand the cellular expression profile of the target protein in humans. Antibodies can be used to detect surface expression of GPCRs, but specific antibodies against CB_2R are currently lacking. Another important aspect in drug development is to verify that the drug candidate fully engages with its intended target *in vivo*. Fluorescent and biotinylated chemical probes are able to address these questions.[1]

On the way toward such probes several highly potent and selective CB_2R ligands, such as 2,5,6-trisubstituted pyridines/pyrazines,[2] triazolopyrimidines,[3] amidosulfones[4] and novel cannabinoid derived ligands[5] have been exploited. Their iterative optimization in cycles of design, synthesis, *in vitro* pharmacology profiling (human and mouse CB_2R binding and functional activity and selectivity) as well as in generating early absorption, distribution, metabolism and excretion data (*e.g.* solubility, permeation, lipophilicity and protein binding) toward a highly versatile chemical toolbox, will be the subject of this communication. Application data of most beneficial novel CB_2R fluorescent probes, including equilibrium competition binding experiments, binding kinetics studies, fluorescence activated cell sorting experiments, as well as detection of CB_2R in over- and endogenously expressing human and rodent systems, will be disclosed.

[1] M. Soethoudt et al., *J Am Chem Soc.* **2018**, *140*, 6067.
[2] R. Slavik et al., *J. Med. Chem.* **2015**, *58*, 4266.
[3] M. Nettekoven et al., *ChemMedChem* **2016**, *11*, 179.
[4] D. Riether et al., *WO 2011109324 A1* **2011**.
[5] M. Soethoudt et al., *Nat Commun.* **2017**, *8*, 13958.

CB2 RECEPTOR DETECTION BY NEW FLUORESCENTLY LABELED SYNTHETIC LIGANDS

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The type 2 cannabinoid receptor (CB2R) plays an important role in a number of pathophysiological processes like inflammatory pain, diabetic neuro- and nephropathy, liver cirrhosis, and ischemic-reperfusion injury. Still there are several persisting questions on target occupancy, expression and activation levels in tissues that remain to be clarified. In this context, fluorescently labeled selective chemical probes are potentially powerful tools to study CB2R in a spatiotemporal controlled manner in living native cells. The tremendous drug discovery efforts that have been devoted to the development of selective CB2R agonists have delivered a rich foundation of synthetic chemical structures of agonist ligands. For the generation of fluorescently labeled probes these available optimized drug candidate structures are particular promising synthetic starting points and may in principle bequest their favorable selectivity, physicochemical properties and low toxicity. In this presentation, we will illustrate successful strategies to convert a drug-like lead candidate into a set of potent, selective and efficacious fluorescent probes having appropriate physicochemical properties. Moreover, the identified construct proved to be largely insensitive towards the nature of the fluorescent dye. We will report results on CB2R and CB1R binding and functional activity as well as the structural considerations, which guided the probe optimization. We will disclose first applications and data obtained with these probes illustrating their suitability to characterize endogenous CB2R in cells.

CANNABINOID 2 RECEPTOR (CB2) EXPRESSION REVISITED: DETECTION OF CB2 mRNA WITH NEW SENSITIVE TOOLS

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G-protein coupled receptors, including cannabinoid receptors usually have low level of tissue expression and have multiple splice variants, that make difficult to study their presence with conventional biochemical tools. Accordingly, there is a consensus in the literature, that commercially available anti-CB2 antibodies lack specificity, and sensitivity. Therefore, reliable detection of CB2 mRNA is of high importance to elucidate tissue and cell-type specific expression of this drug target in various disease settings.

We developed and optimized new sensitive tools including droplet digital PCR, and multiplex fluorescent in situ hybridization (RNAScope combined with tyramide signal amplification), to detect low copy numbers of CB2 mRNA in various animal as well as human tissues and cells. Using these tools, we will show CB2 and CB1 mRNA expression levels in numerous human and mouse brain areas, as well as in mouse disease models with differing neuroinflammatory involvement. We will also correlate CB2 expression levels with multiple cellular and subcellular markers.

Our results will challenge some concepts and will help to better understand tissue specific as well as cell-type specific CB2 mRNA expression that may facilitate the ongoing disease-oriented drug development efforts.

2-AG LOCATION, STRUCTURE AND DYNAMICS IN MEMBRANES AND ITS INTERACTION WITH CB_2 RECEPTORS

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The endogenous cannabinoid ligand, 2-arachidonoylglycerol (2-AG), is a lipid-derived signaling molecule that activates cannabinoid receptors in brain and peripheral tissues. Because of high lipophilicity, 2-AG readily partitions into lipid bilayers where it can be stored at high concentrations. We studied location, structure and dynamics including lateral diffusion of 2-AG in membranes by solid-state nuclear magnetic resonance (NMR), neutron diffraction, and molecular dynamics (MD) simulations. Binding of 2-AG to purified cannabinoid type II (CB_2) receptor functionally reconstituted into liposomes was probed by NMR and MD.

In membranes, the 2-AG molecules locate with their glycerol group in the lipid water interface while the arachidonic acid chain inserts into the hydrophobic core of the lipid bilayer. The arachidonoyl chain of 2-AG is highly flexible with fast structural transitions on the timescale from pico- to nanoseconds between ±skew rotamers of =CH-**CH_2**-CH= bonds sandwiched by the cis-locked double bonds. The 2-AG molecules in membranes show liquid crystalline behavior with rapid lateral diffusion such that they easily reach membrane-imbedded receptors on the timescale of milliseconds. Uptake of specifically deuterated 2-AG by CB_2 as well as its release from the receptor by more strongly binding agonists and inverse agonists was followed 2H NMR with magic angle spinning for increased sensitivity. The data suggest a relatively rapid exchange of 2-AG between membrane- and CB_2-bound states on the millisecond timescale. At the physiological temperature of 37°C, the 2-AG molecules in bilayers undergo acyl chain migration to 1-AG with an exponential-decay constant of 43 minutes which greatly reduces affinity of interactions with the binding pocket of cannabinoid receptors.

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CB2 BIASED AGONISTS AND THEIR THERAPEUTIC POTENTIAL IN OSTEOARTHRITIS

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Purpose: A number of studies evaluated the therapeutic applications of cannabinoids in treatment of chronic pain. Because of broad anti-inflammatory properties of cannabinoids, endocannabinoid system (ECS) became a promising target to control both osteoarthritis (OA) symptoms and disease progression. However, still little is known about efficient treatment strategy with the use of cannabinoids. Recent data implies significant role of functional selectivity upon signal transduction pathways that may increase beneficial properties while reducing side effects. The aim of the present study was to evaluate therapeutic potential of two functionally biased CB2 agonists in different treatment regimens in order to propose the best pharmacological approach for OA management.

Methods: JWH133 (as cAMP biased agonist) and GW833972A (as β-arrestin biased agonist) were selected for the present study, based on the publication by Dhopeshwarkar and Mackie, 2016. Optimal doses were established by dose-response experiments; 1mg/kg for JWH133 and 5mg/kg for GW833972A. Drugs were dissolved in vehicle composed of 5% DMSO, 5% Kolliphor® EL, 5% ethanol and 85% saline. OA was induced by i.a. injection of 1mg (in 50μL of saline) monoiodoacetate (MIA) into rear right paw of Wistar rats. Drugs were administered i.p. in three treatment regimens: single or repeated injections - either each second day from day 10 to 28 (10 doses) or each second day from day 20 to 28 (5 doses) post MIA injection. Behavioural tests were carried out 1 hour after i.p. treatment with CB2 agonist. Pressure Application Measurement (PAM, UgoBasile) was used for joint pain assessment, whereas Kinetic Weight Bearing instrument (KWB, Bioseb) was used for measuring gait characteristics of freely moving animal. Among 20 available parameters measured by KWB - peak force, peak surface, swing duration and ratio of swing to laid duration phase of rear paws were selected for further analysis.

Results: Single injection with both JWH133 and GW833972A at doses of 1mg/kg and 5mg/kg, respectively, elicited significant increase in paw withdrawal force in PAM and restored all gait disturbances in KWB at day 21 post MIA injection. Repeated administrations of JWH133 from day 10 caused significant increase in paw withdrawal force in PAM throughout the course of the experiment, whereas repeated administrations of JWH133 from day 20 elicited increase in paw withdrawal force only at day 26 and 28. Repeated injections from day 10 with GW833972A caused significant increase in paw withdrawal force in PAM only at day 20 and then steadily declined, whereas repeated administrations of GW833972A from day 20 elicited increase in paw withdrawal force throughout the course of the experiment. In KWB, at day 21 we observed full restoration of gait parameters following JWH133 treatment from day 10 but no effects of following JWH133 treatment at day 20. On the other hand, GW833972A treatment from day 10 had no effects on gait parameters at day 21, whereas GW833972A given at day 20 was able to restore gait parameters at day 21. At day 28, we observed no effects of GW833972A on gait parameters in any treatment regimens. JWH133 (1mg/kg) treatment from day 20 did not affect gait parameters at day 28, whereas JWH133 treatment from day 10 was able to rebalance peak surface of rear paws.

Conclusions: The results show that functional selectivity towards CB2 transduction pathways have a significant impact upon their pharmacological properties *in vivo.* Both CB2 agonists exerted anti-nociceptive effects following acute administration, whereas in chronic treatment regimen, we observed tolerance following GW833972A treatment as evidenced by decline in its anti-nociceptive effects in both PAM and KWB. This implies functional selectivity as not only, a key factor in predicting clinical usefulness of drugs but also as a significant confounding variable in basic research.

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References: Dhopeshwarkar, Amey, and Ken Mackie. "Functional selectivity of CB2 cannabinoid receptor ligands at a canonical and noncanonical pathway." Journal of Pharmacology and Experimental Therapeutics 358.2 (2016): 342-351.

DISSECTING THE REWARDING VS. AVERSIVE EFFECTS OF CANNABINOIDS: FINDINGS FROM OPTOGENETIC BRAIN-STIMULATION REWARD MAINTAINED BY ACTIVATION OF VTA DOPAMINE NEURONS

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While some human cannabis users report subjective feelings of reward and euphoria, others report anxiety or dysphoria, suggesting that cannabinoids exert both hedonic and aversive effects. However, the neural mechanisms underlying these paradoxical effects are poorly understood. Cannabis reward is attributed to agonist activity at brain CB_1 receptors (CB_1R) on GABA inputs in the ventral tegmental area (VTA), which releases inhibition of VTA dopamine (DA) neurons. In contrast, we recently reported that Δ^9-tetrahydrocannabinol (Δ^9-THC) produces aversive effects by activation of CB_1R on VTA glutamatergic neurons (*Han et al., Sci. Rep., 2017*). In addition to acting on CB_1R, many cannabinoids exert activity at brain CB_2R on VTA DA neurons (*Jordan & Xi, NBR, 2018*). Therefore, we hypothesize that the net rewarding vs. aversive effects of cannabis depend upon the balance of activity at multiple cell type-specific receptor mechanisms.

To test this hypothesis, we used optogenetic and transgenic approaches to express light-sensitive channelrhodopsin (ChR2) in VTA DA neurons using DAT-cre mice. Optical stimulation of VTA DA neurons induced robust optical intracranial self-stimulation (oICSS) behavior in a frequency-dependent manner. Systemic administration of Δ^9-THC or WIN55,212-2, mixed CB_1R/CB_2R agonists, dose-dependently downward shifted frequency-rate oICSS curves, suggesting a reduction in brain reward function (i.e., aversion). In contrast, XLR-11, a synthetic cannabinoid with high $CB_1R > CB_2R$ selectivity (*Wang et al., NPP, 2019*), produced a dose-dependent upward shift in oICSS similar to cocaine or oxycodone, suggesting rewarding effects. Finally, Xie2-64 and Xie2-49, two new CB_2R agonists, produced downward shifts in oICSS curves, suggesting aversive effects. Together, these findings suggest that selective CB_1R agonists may exert rewarding effects, whereas CB_2R agonists exert primarily dysphoric effects. Mixed CB_1R/CB_2R agonists (Δ^9-THC or WIN55,212-2) may exert either rewarding or aversive effects, depending upon their CBR affinities and cellular distributions of CB_1R/CB_2R in different subjects (Fig. 1).

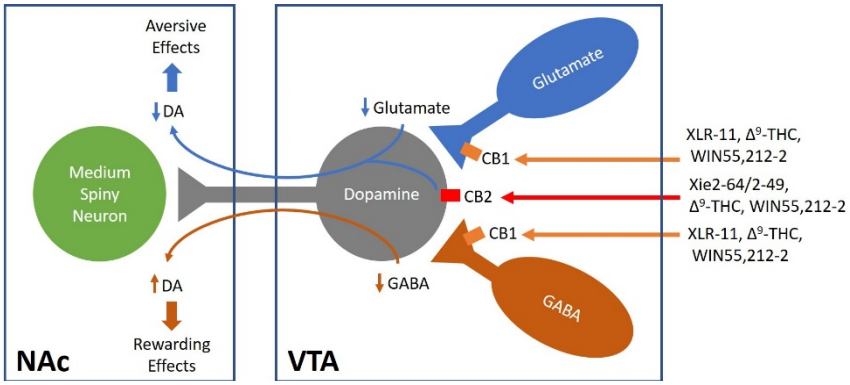

Fig. 1. Diagram of cannabinoid effects on the mesolimbic DA system.

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ENDOGENOUS CANNABINOIDS AND REWARD PREDICTION

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In the early stages of substance abuse, subjects receive a drug that is highly reinforcing and are thus likely to repeat the actions that led them to obtain it. This is termed positive reinforcement. However, in a minority of people who develop an addiction phenotype, negative reinforcement also causes a behavior to be repeated, but in this case, the action causes a bad feeling or situation to go away. The mesolimbic dopamine system, which is thought to generate a teaching signal, is involved in the selection of advantageous behavioral repertoires. This brain pathway is under control of endocannabinoid (eCBs), ubiquitous signaling molecules that bind to the same receptor targeted by marijuana (CB1) known to strengthen responses leading to the procurement of reward. Here, we investigate how eCBs modulate dopaminergic encoding of cues predicting either, appetitive stimuli, the avoidance of punishment or aversive outcomes. We find that disrupting eCB signaling by treating animals with a CB1 receptor antagonist dose-dependently decreased concentrations of dopamine release in the nucleus accumbens that were time-locked to a warning signal that predicts avoidance of punishment while simultaneously weakening shock avoidance behavior, effectively shifting the behavioral outcome from avoidance to escape. We further demonstrate, using directed mutagenesis approaches, that 2AG release from dopamine neurons in the midbrain is a canonical mechanism responsible for the pursuit of rewards. Together these data suggest that eCBs might modify distinct behavioral responses related to aversive stimuli by modulating conditioned mesolimbic dopamine release events. These findings suggest that therapies aimed at modifying tissue levels of eCBs may be used to prevent drug seeking driven by negative affective states.

GABAPENTIN ATTENUATES SOMATIC SIGNS OF Δ^9-THC WITHDRAWAL IN MICE

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Cannabis is the most widely used illicit drug, with a proportion of users developing withdrawal symptoms following cessation of use. However, no FDA-approved pharmaceutical treatments are available to treat cannabis dependence. Non-cannabinoid pharmacotherapies (e.g., anticonvulsant drugs; gabapentinoids) show partial efficacy in humans, but the findings are inconsistent. Gabapentin is an $\alpha_2\delta$-1 voltage-gated Ca^{2+} channel ligand that acts at presynaptic sites to modulate gamma butyric acid (GABA) activation, which is proposed to normalize the stress response observed during cannabis withdrawal. Yet, there is a lack of studies screening gabapentin in preclinical models of cannabinoid withdrawal. The current study was designed to test the hypothesis that gabapentin attenuates Δ^9-THC withdrawal in mice. Equal numbers of adult male and female C57/BL6J mice were treated with either Δ^9-THC (10 mg/kg, s.c.) or vehicle every 12 h for 6 days. Withdrawal was precipitated using the CB_1 inverse agonist rimonabant (3 mg/kg, i.p.). In line with previous reports, Δ^9-THC withdrawal increased head twitches and paw tremors (i.e., somatic signs), decreased marble burying, and increased time struggling in a tail suspension test. Pretreatment with gabapentin (≥10 mg/kg, i.p.) attenuated the increases in paw tremors and head twitches, but did not normalize marble burying or time struggling in the tail suspension tests. A separate cohort of male and female mice was used to test for gabapentin-induced locomotor effects. Gabapentin increased immobility only at the highest dose tested (50 mg/kg, i.p.). Thus, gabapentin blunting of somatic signs of Δ^9-THC withdrawal was not due to sedation. These data provide partial support for the use of gabapentin as a pharmacological tool to decrease cannabis dependence.

Withdrawn

EFFICACY AND SAFETY OF THX-110, A PROPRIETARY THERAPEUTIC COMBINATION OF Δ^9-TETRAHYDROCANNABINOL AND PALMITOYLETHANOLAMIDE

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THX-110 is a unique combination comprising two major components: Δ^9-tetrahydrocannabinol (THC), and Palmitoylethanolamide (PEA), a lipid-messenger that mimics many endocannabinoid activities although does not bind to CB1 receptors. The mechanism of action of THX-110 is based on the entourage effect hypothesis, in which it is presumed that PEA may stimulate the cannabinoid receptors by potentiating their affinity for their ligand or by inhibiting the metabolic degradation of endocannabinoids, and by doing so, may increase the uptake of cannabinoid compounds. As THC was found to ameliorate several diseases symptoms, the co-administration of PEA with THC is thus expected to (1) improve the beneficial effect of THC in said disorders, (2) reduce the required THC dosage and (3) minimize the THC associated adverse reactions and hence increase the safety of THC.

The efficacy, safety and tolerability of THX-110 in the treatment of Adults with Tourette Syndrome (TS) was studied at the TS/OCD Clinic at the Yale Child Study Center. The TS study was a single-arm, open-label trial, in which each subject both received one daily treatment of the drug via oral administration and was followed-up for a period of 12 weeks. Sixteen subjects participated in the study and received THX-110 at the Yale University Child Study Center at Yale University, USA. The primary endpoint of the study was to assess the performance of THX-110 in the treatment of adult patients suffering from symptoms of Tourette syndrome, as measured by the Yale Global Tic Severity Scale Total Tic Score (YGTSS-TTS), the gold-standard and customary index for assessing symptom severity. Treatment was given in a dose titration regimen with a maximum dose of THX-110 consisting of 10mg Dronabinol and 800mg PEA. The study showed that these 16 subjects with medication-refractory TS had a reduction of tic symptoms (paired t-test: YGTSS-TTS mean difference (mean +/- SD) =7.9+/-8.4, p=0.002) from baseline (YGTSS-TTS: 38.4 +/- 8.3) to endpoint (YGTSS-TTS: 30.5 +/- 10.9). This resulted in an average tic reduction of 21% across the entire sample.

Six of the 16 medication-refractory TS subjects experienced a response to treatment as defined by a reduction in YGTSS-TTS of greater than 25%. Improvement over time with treatment was also observed when generalized linear models were used to analyze repeated measures data on the YGTSS-TTS. The medication was generally well-tolerated by subjects with only two subjects stopping treatment early (one due to sedation and another due to lack of improvement in tic symptoms). Twelve of the 16 subjects elected to continue into a 24-week extension phase of the trial, which was also completed. Due to these encouraging results and after receiving all the required regulatory approvals, a randomized, double-blind, placebo-controlled trial is about to be initiated in Germany. We believe that THX-110 holds the potential to become the first-in-line, therapy of choice for treating moderate to severe TS patients.

DOES CANNABIS HELP IMPROVE INSOMNIA AMONG PATIENTS WITH POSTTRAUMATIC STRESS DISORDER?

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A growing body of preclinical literature and some early human clinical work has documented the potential benefits of cannabis for improving sleep quality (Babson et al., 2017). While a few studies have observed cannabis use specifically for the purpose of sleep improvement among individuals with posttraumatic stress disorder (PTSD; Bonn-Miller et al., 2014), such studies have not utilized a non-cannabis-using matched control group. With early clinical trial data suggesting therapeutic effects of THC on nightmares in this population (Loflin et al., 2017), better understanding "real-world" use patterns will only help to inform the development of future large-scale clinical trials.

The present study explored whether cannabis use was associated with improved sleep problems among individuals who self-reported PTSD within a cross-sectional, observational, online survey conducted in early 2018 (IRB#207440). Participants with PTSD were identified by a survey question asked whether they had PTSD (YES/NO), along with other approved conditions for medical cannabis. The online survey was distributed via email to people who participated in the Arkansas research registry. A study flyer including the survey link was posted on social media (e.g., Facebook) and in physical locations (e.g., paraphernalia shops, bus stops). Inclusion criteria for participation were age ≥18 years, residing in Arkansas, and being able to understand and complete the survey in English. Consent was obtained online from all participants. Propensity score matching (1:3) for age, gender, and race was conducted to match participants who reported having PTSD to those who did not. Mixed effects models were conducted in the matched sample to evaluate effects of cannabis use on sleep and wellbeing outcomes in participants with PTSD.

Of N=1,814, 260 adults (14.3%) reported having PTSD. A total of 253 individuals with PTSD were matched with 759 participants who did not, leading to N=1,012 for the analysis. Half of the participants with PTSD (n=120, 47.6%) reported using cannabis in the past month. Among cannabis users, the majority (n=221, 71.1%) reported using cannabis at least once a month on average. As a PTSD diagnosis validation check, scores on the Inventory of Depression and Anxiety Symptoms (IDAS) – Trauma Intrusion scale scores (score ranges from 4-20) were significantly higher among those with PTSD compared to those without (M=12.1 vs. M=6.9, $p<0.001$). Overall, individuals with PTSD reported greater sleep problems on the Insomnia Severity Index (ISI-score ranges from 0-28) (M = 15.5 vs. M = 12.2, $p<0.001$).

Mixed effects regression analysis, controlling for age, gender, race, and employment status, showed that individuals with PTSD who used cannabis in the past month (n=120) had significantly fewer sleep problems than non-users, as assessed by both ISI (-4.5 [95%CI: -2.4, -6.6], $p<0.001$) and IDAS-Insomnia scales (score ranges from 6-30; -3.1 [95%CI: -1.2, -5.1], p=0.002). Cannabis users with PTSD also reported greater well-being, as measured by the IDAS-Wellbeing scale (score ranges from 8-40; +3.9 [95%CI:1.8, 6.0], $p<0.001$).

The present study adds to the empirical evidence suggesting the benefits of cannabis for sleep difficulties among individuals with PTSD. Future studies would benefit from carefully investigating how individual cannabinoids and their combinations impact specific aspects of sleep in this population (e.g., nightmares, apnea, insomnia) in large, placebo-controlled, clinical trials.

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ACUTE EFFECTS OF HIGH POTENCY CANNABIS ON EVERYDAY LIFE MEMORY

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Trends toward legalizing recreational cannabis use in the United States (U.S.) create an urgent need to better understand its effects on humans. However, the current U.S. federal classification of cannabis as a Schedule I drug imposes numerous time and labor-intensive hurdles as well as legal restrictions that are stalling the progress of acute cannabis research. For instance, researchers providing cannabis to participants must obtain it from the National Institute on Drug Abuse (NIDA). However, at present, the NIDA drug supply is limited to products with relatively low levels of tetrahydrocannabinol (THC) that are not representative of the high-THC products currently dominating the recreational cannabis market. Consequently, our understanding of the acute effects of high potency cannabis on humans remains impoverished. Nevertheless, previous research indicates that acute cannabis intoxication impairs memory and that cannabinol (CBD) may offset some of these detrimental effects. However, this research has primarily utilized low THC products and has focused on a select few domains of memory (e.g., verbal, short-term, and working memory). As such, the acute effects of high-potency cannabis and cannabis concentrates on numerous aspects of everyday life memory remain unknown.

The present study was designed to examine the acute effects of various high potency cannabis products on four aspects of everyday life memory: prospective memory (the ability to remember to execute tasks in the future), source memory (the ability to remember the source of previously learned information), false memory (the recollection of items that were not previously presented), and temporal order memory (the ability to remember the order in which previous events occurred). Participants in this ongoing study are randomly assigned to one of four groups: i) a sober control group, ii) a high-THC (>20%)/no CBD (0.00%) flower group, iii) a high-THC (>20%)/with CBD (≥1%) flower group and iv) a cannabis concentrates (>60% THC, ≥1% CBD) group. After being randomly assigned to one of these groups, participants are provided with a list of products available at local recreational cannabis dispensaries which meet the specifications of the product they were randomly determined to use. Participants purchase the product using their own funds, and then engage in a video chat session with a researcher from their home environment. After providing informed consent and completing baseline tests, participants inhale their cannabis product while a researcher observes them over video chat, recording the duration and number of puffs they inhale before self-titrating. Participants then complete a series of everyday life memory tests.

Preliminary results indicate that relative to sober controls, participants in the high-THC/with CBD flower group performed significantly worse on the false memory test, those in the high-THC/no CBD flower group performed significantly worse on the source memory and temporal order memory tests, and those in the concentrate group performed significantly worse on the source memory, false memory, and temporal order memory tests. This is the first study to demonstrate effects of acute cannabis intoxication on these domains of memory.

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HISTONE H4 ENCODES AN ENDOGENOUS PEPTIDE THAT SIGNALS VIA THE CB2 CANNABINOID RECEPTOR

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The endocannabinoid (EC) system consists of the arachidonic acid derived ECs anandamide and 2-arachidonoylglycerol, their metabolizing enzymes, and CB1 and CB2 cannabinoid receptors. Studies of the skeletal EC system in mice and humans suggest the occurrence of a CB2 tone, which protects the skeleton against age-related bone loss. It is unlikely that fatty acid derived ECs generate such a tone, as they are short-lived neurotransmitter-like compounds synthesized 'on demand'. Although the existence of endogenous peptides that regulate the EC system has never been reported, we hypothesized that the CB2 tone is maintained by circulating peptide agonists. One such peptide candidate is H4(99-103), which is present in the circulation at significant concentrations. Studies on the skeletal EC system, showed a striking similarity between the biological activities of H4(99-103) and that of potent CB2 agonists, which target the same mitogenic signaling pathway and have similar skeletal effects. Docking simulation and competitive binding assays suggest that H4(99-103) binds CB2 at an allosteric site without altering the orthosteric binding affinity. The activity of H4(99-103) was tested *in vitro* and *in vivo* on wild type and CB2-deficient cells and mouse models, respectively, and in human derived osteoblasts. Our *ex vivo* results indicate that like a selective CB2 agonist, H4(99-103) triggers a proliferative dose-dependent response in osteoblasts, inhibitable by genetic or pharmacological ablation of CB2. Characteristic of CB2 agonists, H4(99-103) restrains *ex vivo* osteoclastogenesis dose-dependently in WT but not in CB2-deficient cells. A hallmark of CB2 activation is the attenuation of inflammation. Indeed, *in vivo*, H4(99-103) markedly decreases acute inflammation measured manually and histologically as ear swelling following topical application of xylene. This effect is completely absent in CB2 knockout mice. *In vitro*, a similar CB2-dependent inhibition of macrophage inflammatory response (RNA expression of TNFα and IL1β) was observed with H4(99-103). Our results demonstrate that H4(99-103) signals in bone and inflammatory cells via CB2. Consistent with our hypothesis that age-related bone loss is due to declining H4(99-103) serum levels with age, we found that the circulating levels of the peptide were significantly lower during the 5th decade of age relative to younger age in women. We also found that maintaining high levels of H4(99-103) by exogenous administration completely prevents the age-related bone loss in mice. These findings are the first demonstration of an endogenous peptide that signals via CB2 in health and disease. Furthermore, they suggest a role for H4(99-103) in maintaining the CB2 tone in the skeletal system and attenuating age-related bone loss.

THE QUEBEC CANNABIS REGISTRY, A DATABASE ON THE USE OF CANNABIS FOR MEDICAL PURPOSES: FINAL RESULTS

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Since 2014, access to cannabis for medical purposes in Canada has been available under an evolving range of federal regulatory frameworks. The Quebec Cannabis Registry (QCR) was launched in May 2015 as a pharmacovigilance initiative to allow adult patients in Quebec to access cannabis for medical purposes as part of a research framework. The main objectives of the QCR were to: (1) systematically collect data on indications, dosages, benefits and adverse events of the products used; (2) stimulate future research; and (3) support the ongoing development of a drug monitoring program. A descriptive analysis of patients included in the QCR and cannabis treatments was undertaken.

In October 2018, the QCR ended recruitment with 3,004 patients from 71 physician-collaborators across 11 regions in Quebec. The average age of patients was 49.8 years (range 18 to 91 years), evenly balanced by gender (51.5% male) with a mean of 3 follow-up visits. Primary diagnoses were classified into pain disorders (60.7%), mood disorders (15.6%) and oncology (8.8%) among others. Less than half (41.8%) of patients reported additional secondary conditions. Patients reported a mean duration of pain of 10.4 years. A primary analysis was conducted on 1,861 patients recruited before May 31st 2018 and who had at least one follow-up visit. A detailed analysis of outcomes is planned for July 1st 2019 on all patients recruited.

There was a significant difference in daily cannabis dose from initial (1.6 ± 1.6 g) to 15-month visit (1.8 ± 1.8 g) ($p < 0.01$), and from initial to 18-month visit (1.9 ± 1.6 g) ($p < 0.05$). The two most frequently authorized THC:CBD ratios at initial visit were 1:1 (24.1%) and 1:20 (21.1%). Eighty-two adverse events (AEs) were recorded (event rate 3.9 adverse events per patient-year) with (67%) associated with oil administration. Majority of AEs were non-serious (97.6%), and only (2.4%) were serious. Non-serious adverse events (NSAEs) were coded in 10 MedDRA System Organ Class, including Gastrointestinal disorders (25.6%), Nervous system disorders (23.2%), and Psychiatric disorders (15.9%). Of the pain-related concomittant medications used at baseline, anti-depressants (25%) and opioids (17%) were most common.

Prospectively collected data such as the Quebec Cannabis Registry provide important real-world evidence on safety and effectiveness of medical cannabis use. While conclusions about efficacy are limited by lack of control group(s) and blinding, data may generate important hypotheses for further research.

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A CONTROLLED PROSPECTIVE OBSERVATIONAL STUDY OF THE LONGITUDINAL ASSOCIATIONS BETWEEN CANNABIS USE AND PTSD SYMPTOMATOLOGY

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Cannabis is widely used by individuals with PTSD (VA PERC, 2015), but empirical research on the therapeutic effects and associated harms of cannabis use as a treatment for PTSD have been limited primarily to pre-clinical studies examining individual cannabinoids in acute fear extinction, anxiety, and sleep paradigms (Loflin et al., 2017). Human studies have almost exclusively been cross-sectional, limiting inference regarding directionality. Longitudinal data are needed to evaluate the impact of cannabis use on PTSD symptoms, compared with PTSD-diagnosed non-using controls, over time. The present study serves as the longest *a-priori* naturalistic prospective observational study of the relation between cannabis use and PTSD symptoms.

One-hundred-five patients with PTSD were enrolled in this observational study. Cannabis users (n=75) and non-users (n=75) were matched at study baseline on gender, primary trauma type, and number of comorbid psychiatric conditions. Participants were 73% male, 68% Caucasian, 43% married, and 81% military veterans. The majority of cannabis users reported using cannabis with high levels of THC/THC-A. Less than 20% of participants used cannabis with detectable levels of CBD or CBD-A. PTSD diagnosis and symptomatology were determined with the Clinician Administered PTSD Scale for DSM-5 (CAPS-5) and self-reported sleep dysfunction was assessed with the Insomnia Severity Index (ISI) and Pittsburgh Sleep Quality Index (PSQI). Measures were obtained at Baseline and again 3-, 6-, 9-, and 12-month follow-ups. Group differences were examined with t-tests at Baseline, and longitudinally with Generalized Linear Mixed Models (GLMM).

Both cannabis users and non-users had a decrease in PTSD symptom severity on the CAPS-5 over the course of 12 months, though the cannabis using group demonstrated a greater improvement relative to controls (Mean difference = 5.0; $p < .05$). Consistent with prior work (Bonn-Miller et al., 2011), changes in Negative Alterations in Cognitions and Mood (Cluster D) and Alterations in Arousal and Reactivity (Cluster E) predicted the observed group difference. No group differences in the experience of insomnia (i.e., ISI) were observed. However, cannabis users reported improved sleep quality (PSQI) relative to non-users, particularly during the first 6 months of follow-up.

Study data suggest that cannabis use may lead to clinically meaningful improvements in PTSD symptoms over the long-term, especially symptoms of negative mood and hyperarousal. Controlled clinical trials of defined cannabis products for the treatment of PTSD are warranted.

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A RANDOMIZED TRIAL OF MEDICAL CANNABIS IN PATIENTS WITH ADVANCED CANCERS TO ASSESS IMPACT ON OPIOID USE AND CANCER-RELATED SYMPTOMS: A PILOT AND FEASIBILITY STUDY

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Background: Cancer-related pain is common and negatively impacts quality of life. Opioids are the mainstay of pain management in patients with cancer. Higher pain and greater long-term opioid requirements have been associated with shorter survival in patients with cancer. Routine use of medical cannabis has been limited due to lack of rigorous scientific data, and concerns about side effects, legal ramifications, and high cost. We aimed to determine the effects of medical cannabis in patients with cancer.

Methods: 30 patients with stage IV cancer requiring opioid medications were randomized 1:1 to early cannabis (EC) (n=15) versus delayed cannabis (DC) (n=15). The EC group was provided with 3 months of medical cannabis at no charge, while the DC group received standard oncology care without cannabis for the first 3 months. Patients met with licensed pharmacists at one of two medical cannabis manufacturers approved in Minnesota, and pharmacists determined optimal cannabinoid dosing, formulation, and route during routine patient visits. Patients completed monthly pain and medication logs (including opioid and cannabis use), and validated Patient-Reported Symptom Monitoring surveys.

Results: As summarized in Table 1, a higher proportion of EC patients achieved a reduction in opioid use and improved pain control. On average over a 3 month window, EC patients did not require opioid dose escalation, showed a trend towards lower mean pain, and had similar quality of life compared to DC patients. Estimated mean daily THC and CBD use for EC patients at 3 months was 87 mg (range 8-149 mg) and 101 mg (range 0-516 mg), respectively. When asked about perceived benefit and negative impact of medical cannabis, mean benefit was 5.1 and mean negative impact was 2.7 (1 = no benefit/negative effects, 4 = some benefit/negative effects, 7 = a great deal of benefit/negative effects). Seven patients died during study (2 EC, 5 DC), and patient compliance with study logs limited analysis.

Conclusions: Conducting randomized studies of cannabis in the oncology setting is feasible, but rigorous data collection is challenging. The addition of cannabis to standard oncology care in patients with advanced cancers was well-tolerated and may lead to improved pain control and lower opioid requirements. Future prospective trials with cannabis focusing on specific cancer types/stages are needed.

	All Patients (n=20)[a]		**Early Cannabis (n = 12)[a]**		**Delayed Cannabis (n = 8)[a]**	
Mean age (range)	59 (38-77)		59 (38-76)		58 (47-77)	
Gender, % female	55		58		50	
Median days since stage IV diagnosis (range)	186 (12-3755)		136 (12-3755)		334 (43-1533)	
Mean pain, 0-10 scale	Bl 5.7	3M 5.3	Bl 5.3	3M 4.7	Bl 6.1	3M 6.0
Mean personalized pain goal	Bl 3.7	3M 3.4	Bl 3.4	3M 3.0	Bl 4.1	3M 3.8
% patients meeting personal pain goal	Bl 30	3M 20	Bl 25	3M 44	Bl 38	3M 13
Mean daily oral morphine equivalents (OME)	Bl 47	3M 60	Bl 55	3M 54	Bl 35	3M 67
% patients achieving at least 20% reduction in mean daily OME at 3 months	25		44		0	

Table 1. Demographics, pain, and opioid use among all patients, EC patients, and DC patients at baseline (Bl) and 3 months (3M). [a] Patient totals in each group may vary for certain measures, depending on available data.

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MARIJUANA USE AND KIDNEY OUTCOMES IN THE ASSESS-AKI COHORT

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Legal recreational and medicinal use of marijuana (MJ) is increasing worldwide. Animal kidney injury models show that activation of the cannabinoid receptor CB1 can exacerbate kidney disease while activation of CB2 may be protective. Whether these effects apply to whole plant MJ remains to be determined. We conducted a post-hoc analysis of MJ usage as a risk factor for kidney function decline and albuminuria in the ASSESS-AKI parallel matched cohort study that enrolled hospitalized adults with and without AKI from 4 US centers between 2009-2015, with a median of 4.1 years of follow-up. MJ usage was defined as responding yes to "have you used MJ since your last study visit?" at least once on any study visit questionnaire. Nonusers were defined as always responding no to this question. Kidney function decline was defined according to the parent study protocol. Association between MJ usage and the categorical and continuous outcomes were determined using multivariable Cox regression and linear mixed models, respectively.

MJ users represented 113 of 1599 (7%) participants, were younger (mean age 54 vs. 65 years), mostly white (78%), men (78%), and were more likely to be heavy tobacco users (≥20 cigarettes/day; 26% vs. 8%). Baseline eGFR was higher in users vs. non-users (87 +/- 30 mL/min/1.73 m^2 vs. 69 +/- 26 mL/min/1.73 m^2), while baseline UACR was similar (120 +/- 80 in users vs. 99 +/- 72 in nonusers). In those with baseline eGFR ≥60 mL/min/1.73 m^2, MJ use was not associated with incident CKD (adjusted HR 0.93; 95% CI, 0.5-1.8) or differences in eGFR slope over time (mean difference -0.12 mL/min/1.73 m^2/year, P=0.7). In contrast, in those with baseline eGFR <60 mL/min/1.73 m^2, MJ users had more rapid eGFR decline vs. nonusers (-3.2 vs -1.4 mL/min/1.73 m^2/year, P=0.002) and had a strong trend towards higher risk for CKD progression (adjusted HR 2.7; 95% CI, 0.83 to 8.5). MJ usage was not associated with the rate of change in UACR over time in those with (P=0.4) and without CKD (P=0.2). MJ usage was associated with more rapid eGFR decline in those with baseline CKD, but not in those without CKD, nor was it associated with changes in albuminuria over time in those with or without CKD. Reasons for the effect modification by CKD status regarding MJ and kidney function should be explored.

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CANNABINOID SIGNALING REGULATES CEREBELLAR DEVELOPMENT

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Brain complexity, which emerges gradually during development through an orchestrated program of neuronal proliferation and differentiation, is essential to support diverse computations in functionally distinct neural circuits. The cerebellum is a highly compartmentalized brain region distinguished by anatomical and cytological features such as a unique laminar structure, anterio-posterior folding pattern, and medio-lateral segregation into molecularly distinct stripes. This complex three-dimensional organization is remarkably conserved across Mammalia. Thus, insights gained from the study of cerebellar development in mice could illuminate the mechanisms of normal and pathological human development.

Endocannabinoid (eCB) signaling has been shown to play a key role in forebrain neuron proliferation, differentiation and migration; however, its role in cerebellar development has not yet been explored in detail. In this study we show that expression of endocannabinoid signaling machinery in the cerebellum is prominent and dynamic throughout development, and that eCB signaling through cannabinoid receptor 1 (CB1) plays a role in regulating the transition from proliferation to differentiation in GC. Disruption of eCB signaling through genetic ablation of CB1 causes reduction of cerebellar size and an altered pattern of folding, thus disrupting compartmentalization and possibly cerebellar circuits' wiring and function. Furthermore, we show that perinatal exposure to phytocannabinoid (−)-*trans*-Δ^9-tetrahydrocannabinol (THC) alters cerebellar developmental trajectory, leading to a decrease in size and an increase in foliation. Interestingly, we identified sex dependent differences in foliation patterns of the anterior cerebellar vermis, which are altered following perinatal exposure to THC.

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BEHAVIOURAL AND COGNITIVE EFFECTS OF ADOLESCENT CANNABIS AND ALCOHOL CO-USE IN ADULTHOOD

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Cannabis and alcohol co-use is prevalent in adolescence but its long-term effects on learning and cognition remain largely unexplored. Therefore, the objective of this study is to investigate the long-term effects of adolescent alcohol and Δ^9-tetrahydracannabinol (THC) co-exposure on learning and cognition. We hypothesize that co-exposure will produce more pronounced behavioral and cognitive deficits in adulthood compared to either drug exposure alone. Male Sprague Dawley rats received vapourized THC (10 mg/pad) or vehicle every other day and had continuous access to 10% ethanol in a two-bottle choice design during adolescence (post-natal day 28-42). Alcohol intake was measured during the exposure period to assess the acute effects of THC on alcohol consumption. In adulthood, a battery of behavioural tests (i.e., novel object preference, elevated plus maze, autoshaping and conditioned avoidance response) was performed.

Adolescent rats appeared to show higher alcohol preference on days in which they were not exposed to THC vapour. In adulthood, co-exposed animals trended towards better short-and-long-term memory retention on the novel object preference test compared to those that received either drug alone. Although no differences in acquisition were observed in the conditioned avoidance response, those exposed to THC alone exhibited higher resistance to extinction compared to co-exposed and alcohol exposed animals. Exposure to THC vapour appears to increase alcohol drinking in adolescent rats. In addition, co-exposure to THC and alcohol during adolescence can produce long-lasting behavioral effects in a variety of learning paradigms. These studies contrast our previous findings with injected THC exposure, suggesting that different routes of THC administration can produce varying short- and long-term consequences.

MICE MISSING *CNR1* AND *CNR2* SHOW IMPLANTATION DEFECTS

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Cannabinoid/endocannabinoid signaling is primarily mediated by CB1 (encoded by Cnr1) and/or CB2 (encoded by Cnr2). Here we show that *Cnr1*$^{-/-}$*Cnr2*$^{-/-}$ mice are subfertile due to compromised implantation. Upon implantation, the epithelium is smooth and adhered to the blastocyst trophectoderm within the implantation chamber (crypt) in wild type mice, whereas the epithelium in *Cnr1*$^{-/-}$*Cnr2*$^{-/-}$ mice is ruffled which compromises appropriate blastocyst-uterine interactions. The suboptimal implantation leads to higher incidence of pregnancy failure in *Cnr1*$^{-/-}$*Cnr2*$^{-/-}$ mice. Histological analysis revealed heightened edema around the implantation chamber in these deleted females. Using a reporter mouse line, we observed that CB2 is present on endothelial cells of uterine blood vessels and its absence leads to blood vessel leakage during implantation. These results suggest that appropriately regulated uterine edema is important to optimal implantation.

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NON-PSYCHOTROPIC CANNABINOIDS SUPPRESS TUMOR GROWTH BY ACTING ON METABOLIC REPROGRAMMING AND ONCOGENIC PATHWAYS IN HORMONE REFRACTORY PROSTATE CANCER

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Cancer cells follow a unique metabolic programming by preferring aerobic glycolysis as firstly observed by Otto Warburg in 1956. Cancerous phenotype is essentially backed by genetic mutations triggering several oncogenic signaling pathways that rewire the cellular metabolism to meet the highly bioenergetic and biomass requirements of proliferating cells. This crosstalk, although not completely understood yet, is a potential target for new promising interventions against cancer.

We previously demonstrated that CBD*, alone or in combination** with CBG, significantly reduced ($p<0.05$ and $p<0.001$, respectively) tumour progression in TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice, which uniformly and spontaneously develop multistage autochthonous (orthotopic) prostate tumours following the onset of puberty. After exposing TRAMP mice and TRAMP-C2 cells to a standard chemotherapy drug used for metastatic castration-resistant prostate cancer, enzalutamide (MDV3100), we set up *in vivo* and *in vitro* models of hormone refractory prostate cancer. Combined treatment with CBD and CBG (1:1)** significantly reduced tumour relapse ($p=0.0052$) in animals with hormone refractory status, and, *in vitro,* inhibited cell proliferation and induced apoptosis.

Here, we investigate how purified plant cannabinoids (CBD and CBG) affect the favourite metabolic system of malignant tumours. Extracellular flux analysis showed that CBD (1 and 3μM for 24h) up-regulates glycolytic capacity and inhibits oxidative phosphorylation in MDV3100-resistant cells and elevating mitochondrial ROS using mitoSox fluorescence probe. These metabolic changes lead also to notable shifts of specific oncogenic related signaling pathways in these cells (i.e. HIF-1a, BNIP3, PTEN and AMPK/ULK-1).

The effect on cancer energetics and its related oncogenic signalling proves the efficacy of phytocannabinoids as metabolic reprogramming agents, and sheds light on novel metabolic targets and interventions against highly malignant hormone refractory prostate cancer.

*(75mg/kg) **(37.5+37.5mg/kg), i.p. twice per week; 5-week treatments for early stage; 3-week treatments for advanced or hormone-refractory stages.

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CANNABINOID RECEPTOR-MEDIATED MODULATION OF INTERNEURONS IN THE MAIN OLFACTORY BULB

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In the main olfactory bulb, at least two populations of granule cells (GCs), GABAergic interneurons, can be distinguished based on their location in either the granule cell layer (GCL) or the mitral cell layer (MCL) where they are interspersed with principal neurons (mitral/tufted cells). Little is known about the properties of these two populations of interneurons. Using a combination of anatomical and functional approaches we have explored this question with respect to endocannabinoid signaling. Our understanding of the role of cannabinoid receptor type 1 (CB1R) in olfactory processing remains limited. Our previous work has demonstrated that endocannabinoid signaling is involved in the regulation of glomerular activity in the main olfactory bulb.

Antibody staining for diacylglycerol lipase DAGLα, which is responsible for the synthesis of 2-arachidonoylglycerol (2-AG), shows prominent expression in the MCL and GCL suggesting DAGL-dependent endocannabinoid signaling in these layers. Previously, whole-cell patch-clamp electrophysiology showed that Group I metabotropic glutamate receptor (mGluR) agonists potently depolarize GCs whereas mGluR antagonists reduce synaptic responsiveness. Differences in the physiology of the two GC populations were evident when we compared the responses from two mGluR k.o. mouse strains. MCL-GCs in slices from mGluR5 k.o. mice but not from mGluR1 k.o. mice responded to mGluR agonists, whereas GCL-GCs in slices from mGluR1 but not from mGluR5 k.o. mice were responsive to mGluR agonists suggesting different mGluR expression patterns. A CB1R agonist hyperpolarized GCs and made them less responsive to synaptic input, while a CB1R antagonist strongly excited GCs of both populations. These data indicate that the mGluR and endocannabinoid system can have different cellular and network effects in the main olfactory bulb. Furthermore, the data show that endogenous release of endocannabinoids and glutamate prominently modulates the excitability and synaptic responsiveness of interneurons in the main olfactory bulb.

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ENDOCANNABINOID ROLES IN SLEEP STABILITY AND SLEEP DISRUPTION BY CANNABINOID DRUGS

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Cannabis preparations have long been known to have hypnogenic effects and have been recently touted as sleep aids. However, sleep disruption has also been reported in humans following chronic cannabis usage. Less is known about endocannabinoid (eCB) roles in sleep. We explored endocannabinoid roles in sleep and effects of acute and chronic cannabinoid drugs on sleep with EEG recordings and unbiased scoring of vigilance states using an automated algorithm that we developed. Effects of acute increases in eCBs with inhibitors of monoacylglycerol lipase (MGL) or fatty acid amide hydrolase (FAAH) were examined in mice, as were effects of acute CB1 receptor activation or blockade. Effects of single or chronic (7 day) administration of delta-9-tetrahydrocannabinol (THC) were also examined.

We observed a transient elevation of slow-wave sleep (SWS, rodent equivalent of non-rapid-eye-movement sleep) following inhibition of either MGL or FAAH. Similar effects were observed after acute administration of CB1 agonist or THC. The early increase in SWS was followed by a reduction at longer durations following a single dose MGL inhibitor or CB1 agonist. The CB1 antagonist/inverse agonist AM281 blocked the early effects of CB1 activation and produced fragmented SWS in a manner that resembled the later phase responses to MGL inhibition or CB1 activation. Blockade of CB1 also prevented the rebound in SWS following sleep deprivation. SWS is disrupted during early phases of abstinence after chronic THC exposure, and the hypnogenic effect of THC is also lost at this timepoint. Our findings indicate a role for eCB signaling in SWS stability. Increasing eCBs or activating CB1 can enhance SWS, but compensatory SWS disruption appears to develop even after a single acute drug exposure. We are currently exploring the neural mechanisms underlying these sleep changes. These effects on sleep should be taken into account when considering using cannabinoid drugs as sleep aids.

CP55,940 ANTINOCICEPTIVE EFFECT, DEVELOPMENT OF TOLERANCE AND ACTIVATION OF JNK SIGNALING IN THE CISPLATIN-INDUCED NEUROPATHIC PAIN MODEL

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Chemotherapy-induced neuropathic pain (CIPN) is a clinical challenge for cancer patients. The development of novel targeted therapies with long term efficacy in alleviating CIPN is an ongoing focus of preclinical research. The endocannabinoid system has shown great promise and efficacy in alleviating CIPN in both preclinical and clinical studies. Our group has previously demonstrated that delta-9-tetrahydrocannabinol (Δ^9-THC) exhibits efficacy in measures of acute and chronic pain and that development of tolerance is partially mediated by c-Jun N-terminal kinase (JNK)/GRK/βarrestin2 signaling network. But, so far, the mechanisms underlying tolerance to CP55,940 in a CIPN model (cisplatin-induced neuropathy) remains elusive. The objective of our current work is thus to assess whether CP55,940, a synthetic, high potency cannabinoid agonist develops tolerance in a CIPN model through GRK/βarrestin2 and JNK-mediated mechanisms as found in Δ^9-THC tolerance. Tolerance to the antinociceptive effect of CP55,940 (0.3 mg/kg i.p.) were assessed by mechanical allodynia testing (von Frey) with the development of tolerance appearing after 11 days of chronic administration in male wild type mice. Unlike Δ^9-THC, the antinociceptive effects of CP55,940 are not prolonged following its co-administration with JNK inhibitor SP600125 (3 mg/kg i.p.) since tolerance appeared after 10 days. Chronic administration of CP55,940 (0.3 mg/kg i.p.) resulted in an enhanced protein-protein interaction between JNK1 and β-arrestin2 in the whole brain of CIPN wild-type mice. We also found using immunoblotting that chronic treatment of CP55,940 causes a pronounced activation of JNK signaling in the whole brain of cisplatin-treated wild-type mice. These results demonstrate the complexity of JNK and β-arrestin2 association in the development of tolerance following chronic administration of CP55,940 in a CIPN model. Future studies using JNK1 knockouts are necessary to confirm the role of JNK1 in the development of tolerance. We also need to assess the role of sex differences and the involvement of other JNK isoforms and pathways in the development of cannabinoid tolerance.

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MOLECULAR AND PHARMACLOGICAL EVIDENCE FOR THE SODIUM-CALCIUM EXCHANGER-1 (mNCX-1) AS A MEDIATOR OF CBD- AND KLS-13019-APPLIED PROTECTION AGAINST PACLITAXEL-INDUCED TOXICITY IN DORSAL ROOT GANGLION CULTURES

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Chemotherapy-induced peripheral neuropathy (CIPN) is a painful adverse effect caused by several classes of widely used anticancer drugs. Currently, there is no one drug or drug class that is considered both effective and safe in the treatment of CIPN. In a mouse model of CIPN that focused on paclitaxel-induced mechanical and cold allodynia, CBD was observed previously to be effective in preventing the onset for this treatment consequence. Now a new CBD analogue (KLS-13019) has been discovered in our laboratory that has improved drug-like properties in comparison to CBD, while retaining neuroprotective properties and significant preventative actions on allodynia in a mouse model of CIPN.

To study the toxicity of paclitaxel in a relevant cellular target, rat dorsal root ganglion (DRG) cultures were used as a model system to confirm the protective properties of CBD and KLS-13019 and to further explore the mechanism of action. Previous pharmacological studies with CGP-37157 (mNCX-1 blocker) conducted in hippocampal cultures indicated that all neuroprotective activity produced by CBD and KLS-13019 against ethanol toxicity could be prevented at inhibitor concentrations that were specific for this exchanger target.

In the present work, the previous pharmacological studies were confirmed in DRG cultures and the mechanistic studies extended with the use of siRNA to mNCX-1. Treatment with siRNA produced a 50-55% decrease in the immunoreactive (IR) area for NCX-1 in neuronal cell bodies and a 72-80% decrease in neuritic IR area as determined with high content image analysis. After treatment with 100 nM KLS-13019 and siRNA, DRG cultures exhibited a 75 $\pm$ 5 % decrease in protection from paclitaxel-induced toxicity; whereas siRNA studies with 10 μM CBD produced a 74 $\pm$ 3% decrease in protection. Treatment with mNCX-1 siRNA alone did not produce toxicity. The protective actions of cannabidiol and KLS-13019 against paclitaxel-induced toxicity were significantly attenuated with depletion of mNCX-1 that was not attributable to toxicity. These data indicated that the decrease in neuritic mNCX-1 corresponded with decreased protection after siRNA treatment. Pharmacological blockade of mNCX-1 with CGP-37157 produced complete inhibition of protection from paclitaxel toxicity in DRG cultures, supporting the siRNA effects on mechanism described for this cannabinoid-mediated protection. Both CBD and KLS-13019 were effective in preventing paclitaxel-induced mechanical allodynia and both of the protective actions of these cannabinoids were attenuated by mNCX-1 target knockdown. These studies strongly suggest a shared mechanism and potential therapeutic use. With the improved drug-like properties of KLS-13019 in comparison to CBD, a therapeutic alterative in the treatment of CIPN may be emerging for this intractable neuropathic pain.

NOVEL NEGATIVE ALLOSTERIC MODULATOR (NAM) OF CANNABINOID RECEPTOR 1 (CB_1) AMELIORATES SYMPTOMS DUE TO DOPAMINE DYSREGULATION IN PSYCHIATRIC DISORDERS

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The prevalence of psychiatric disorders is common, with anxiety, mood disorders, and schizophrenia reporting prevalence rates of 28.3%, 9.5%, and ~1% respectively. However, patient outcomes remain not ideal. Therefore, it is imperative to find novel treatment approaches for these disorders. Dopamine controls cognitive, emotional and motor aspects of goal-directed behaviour, with perturbations in the system playing a role in a number of psychiatric disorders and their underlying symptoms. Relating to the dopamine system, the endocannabinoid system serves as an important filter of afferent inputs, helping shape how incoming information is conveyed onto dopamine neurons and to output targets. Therefore, we hypothesize that compounds negatively targeting the endocannabinoid system could be candidates in treating positive and affective symptoms in psychiatric illness. We tested the effect of ABM300, a novel negative allosteric modulator (NAM) of the CB_1 receptor (IC_{50} of ~20nM).

Adult (>P70) GluN1-knockdown (GluN1KD - F1: C57Bl/6J x 129S1/SvlmJ) and DAT-knockout (DATKO - C57Bl/6J), balanced for sex, were treated with either vehicle (1:1:18 – Tween80 : 95% ethanol : saline) or a novel CB_1 NAM, ABM300, at 10mg/kg, tested on behavioural assays, and compared to littermate controls. Locomotor, stereotypic movements and vertical activity were tested, along with anxiety/mania and sensorimotor gating behaviours. All data were analyzed with two- or three-way ANOVA, as appropriate, and corrected for multiple comparisons. Behaviour effects of ABM300 were compared to an antipsychotic (olanzapine; 1mg/kg), a CB1 orthosteric inverse agonist (rimonabant; 10mg/kg), and a cannabinoid that is traditionally believed to act as a CB1 NAM (cannabidiol; 60 and 120mg/kg).

GluN1KD and DATKO mice display hyperactivity, impaired habituation and sensorimotor gating, along with increased stereotypy and vertical activity, in a state of mania-like behaviour. Following acute treatment with ABM300, amelioration of these dysregulated behaviours was observed. GluN1KD mice saw a reduction in locomotor and vertical activity, along with an amelioration of repetitive stereotypic movements and mania-like behaviour. DATKO mice also saw the same amelioration of behaviours as that of the GluN1KD, with additional amelioration of sensorimotor deficits. Effects of ABM300 were similar to those seen with olanzapine, while cannabidiol had no effect, and rimonabant exacerbated behavioural abnormalities.

The data suggest that CB_1 NAMs represent a novel treatment for psychiatric symptoms as a result of dopamine dysregulation. ABM300 ameliorates dopamine dysregulation in both animal models of psychiatric illness. Furthermore, targeting the endocannabinoid system offers the opportunity to normalize deficits that arise from differing underlying dysfunctions that manifest as similar behavioural changes; both of which are mediated by dopamine dysregulation.

THE DIFFERENTIAL ROLE OF DIACYLGLYCEROL LIPASES IN REVERSING MECHANICAL SENSITIVITY IN A MOUSE MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY (CIPN)

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Inhibition of diacylglycerol lipase (DAGL)-α and -β, the main biosynthetic enzymes for the endogenous cannabinoid 2-arachidonoylglycerol (2-AG), reduce inflammatory responses to a pro-inflammatory insults. Thus, here we tested whether blockade of these enzymes would ameliorate nociceptive behavior in a mouse model of Chemotherapy-Induced Peripheral Neuropathy (CIPN), a condition in humans that is resistant to traditional analgesics. Intraperitoneal (i.p.) administration of either the DAGL-α/β inhibitor DO34 or the selective inhibitor DAGL-β inhibitor KT109 dose-dependently reversed paclitaxel-induced allodynia. As DAGL-α (-/-) mice or DAGL-β (-/-) mice showed similar magnitudes of basal and paclitaxel-induced nociceptive behavior as wild type control mice, these transgenic mice were then used to screen each inhibitor. Both KT109 (40 mg/kg, i.p.) and DO34 (30 mg/kg, i.p.) fully reversed paclitaxel-induced allodynia in wild-type mice and DAGL-α (-/-) mice, but did not elicit antinociceptive effects in DAGL- β (-/-) mice, suggesting that DAGL-β plays a necessary role in the observed antinociceptive effects. In addition, the observation that i.p. injection of KT109 reduced the levels of proinflammatory cytokines/chemokines (IL-1β, IL-2, IL-17, IFN-γ, GM-CSF, MCP-1) in the dorsal root ganglia isolated from paclitaxel-injected mice and that KT109 showed low brain penetration when compared to peripheral tissue, and did not significantly inhibit the DAGL-β activity within the brain suggests a possible peripheral mechanism of action. In order to test whether these antinociceptive effects undergo tolerance, mice were given a daily i.p. injection of KT109 (40 mg/kg, i.p.) for six days. Not only did KT109 maintain its anti-allodynic effects following repeated administration, but also its duration of action persisted for at least 24 h after the final injection of drug. In contrast, the antinociceptive effects of an acute injection of KT109 are resolved by 8 h. In the final set of experiments, we sought to elucidate potential neural mechanisms mediating these effects by examining the *ex vivo* consequences of deletion or prolonged pharmacological inhibition of DAGL-β on paclitaxel-induced hyperexcitability of primary afferent neurons isolated from dorsal root ganglia (DRG) innervating the lumbar level of the spinal cord. Strikingly, DRG harvested from either DAGL-β (-/-) mice or wild type mice administered repeated KT109 were resistent to paclitaxel-induced hyperexcitability. Overall, these findings suggest that DAGL-β inhibitors may reverse paclitaxel-induced allodynia by attenuating proinflammatory cytokine/chemokine release at the level of the DRG to dampen neuronal hyperexcitability. Collectively, these findings identify DAGL-β as a viable target to treat neuropathic pain associated with chemotherapy.

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NEW STRATEGIES TARGETING THE ENDOCANNABINOID SYSTEM TO ATTENUATE DISEASE PROGRESSION IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is characterized by neurodegeneration associated with demyelination and excitotoxicity. Cannabis has been used for decades to mitigate MS-associated symptoms and preclinical evidence support the role of the endocannabinoid system as a key target [1-2]. Endocannabinoid (EC) increase has shown positive results in mouse models of MS; however, they might have limitations in clinical settings for chronic use [3]. Here we analyzed the therapeutic potential of WOBE437, a selective EC reuptake inhibitor (SERI) which is orally bioavailable and it has shown anxiolytic, analgesic and anti-inflammatory effects in different animal models, including mitigation of chronic inflammation through a polypharmacological mechanism [4-5]. Chronic-progressive experimental autoimmune encephalomyelitis was induced in C57BL/6 mice as a model of MS; daily treatments (i.p.) started individually at the onset and lasted 20 days. Our results indicate that WOBE437 significantly reduced disease severity and favored an earlier recovery, mediated mainly through a CB2 receptor-dependent mechanism. CB1 receptor itself seemed to play an important role for disease severity, as its blockage was detrimental for the mice. WOBE437 treatment increased EC levels only in cerebellum during the peak of disease, while a less localized increase was seen after full treatment without inducing desensitization of brain CB receptors. Not change on the infiltration or activation of immune cells was observed during the disease peak and ongoing experiments aim to elucidate the role of microglia. Our results show that SERIs may represent a novel therapeutic strategy for slowing MS progression.

References:
[1] Chiurchiù, et al. Prog Neurobiol. 2018; 160: 82-100.
[2] Notcutt WG. Neurotherapeutics. 2015; 12(4): 769-77.
[3] Toczek, Malinowska. Life Sci. 2018; 204: 20-45.
[4] Chicca, et al. Proc Natl Acad Sci U S A 2017; 114: E5006-5015.
[5] Reynoso-Moreno, et al. Front Mol Neurosci. 2018; 11: 180.

GPR18 MEDIATES MICROGLIAL NEUROTOXICITY INDUCED BY HIV-1 TAT PROTEIN *IN VITRO*

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Human immunodeficiency virus type 1 (HIV-1) is known to provoke microglial immune responses which likely play a paramount role in the development of the chronic neuroinflammatory conditions and neuronal damage related to HIV-1 associated neurocognitive disorders (HAND). In particular, HIV-1 Tat protein is a proinflammatory neurotoxin which predisposes neurons to synaptodendritic injury. Drugs targeting the degradative enzymes of endogenous cannabinoids have shown promise in reducing inflammation with minimal side effects in rodent models. Our lab has demonstrated that the HIV-1 Tat protein can produce indirect neurotoxic effects through microglial activity. Considering that markers of neuroinflammation can predict the extent of neuronal injury in HAND patients, we evaluated the neurotoxic effect of HIV-1 Tat-exposed microglia following blockade of fatty acid amid hydrolyze (FAAH), a catabolic enzyme responsible for degradation of endocannabinoids (e.g. anandamide). We have shown before that FAAH inhibition blunts Tat-mediated toxicity in neuronal cultures through cannabinoid receptor mechanisms. In the present study, cultured murine microglia were incubated with Tat and/or FAAH inhibitor (PF3845). After 24 hours, cells were imaged for morphological analysis and microglial conditioned media (MCM) was collected for neuron exposure experiments. Neuron cultures (DIV 7-11) were then exposed to MCM, and neurotoxicity was assessed using calcium (Ca^{2+}) imaging. These experiments were also repeated using FAAH KO derived microglial cells for comparison. Interestingly, PF3845's strong attenuation of microglial responses to Tat was found to be independent of $CB_{1/2}$ receptor mechanisms. Past reports have described the ability of GPR18 to regulate microglial function, therefore we evaluated the involvement of this receptor in microglial-mediated dysregulation of neuronal $[Ca^{2+}]_i$. We found that a purported GPR18 antagonist, CID-85469571, blocked the neuroprotective effects of PF3845. Moreover, the GPR18 agonist, N-Arachidonyl glycine (NAGly), also attenuated Tat-induced increases in neuronal $[Ca^{2+}]_i$. This suggests that the observed effects are dependent on GPR18 activation via a ligand targeted by FAAH. It is important to note that NAGly agonism of GPR55 and inhibition of FAAH could also play a role in the observed effects. These findings not only further elucidate neuroprotective mechanisms of endocannabinoid catabolic enzyme inhibition and also highlight the potential therapeutic benefit of a non-classic cannabinoid receptor in treating HAND and other diseases involving neuroinflammatory pathologies.

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β-CARYOPHYLLENE IMPROVES MEMORY FUNCTION AND DECREASES KEY INFLAMMATORY CYTOKINES IN AGED MICE

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Age-related cognitive decline has been associated with increased levels of inflammatory cytokines, but the precise relationship between aging, cognitive decline, and cytokine load remains to be elucidated. β-caryophyllene (BCP) is a naturally occurring sesquiterpene cannabinoid receptor 2 (CB_2) agonist that has established anti-inflammatory and neuroprotective effects in several models of neurotoxicity or neurodegeneration. It is also a safe and tolerable FDA-approved food additive. It is also known to improve memory function in several behavioral tasks in rodents, and have recently been shown to increase lifespan in C. elegans. It therefore of interest to explore the potential of BCP to reduce age-related cognitive decline, and to relate improved cognitive function to objective blood-based biomarkers that may have translational relevance. In this study, we sought to assess changes in circulating cytokines across the life-span to discover which cytokines are most closely associated with increased age. Furthermore, we also sought to determine whether BCP can reverse cognitive deficits in aged mice and whether improved cognitive function is associated with decreased levels of aging-related cytokines.

A full profile of 12 cytokines was conducted in Swiss-Webster mice at 3, 12, and 18 months of age using multiplexed flow cytometry. Working memory was compared in young young (3 months) and aged (12 months) mice using the spontaneous alternations Y maze assay. A dose-response function (100-300 mg/kg) of memory improvements was then determined using subchronic dosing regimens of BCP in young and aged mice. Finally, the effects of subchronic dosing of BCP on cytokine levels was assessed in aged mice using the most effective dose of BCP (178 mg/kg) in the memory study. The circulating levels of several cytokines significantly increased with age. Multilinear regression analysis corrected for collinearity revealed that IL-12 family cytokines were most strongly associated with increased age. Aged mice (12 month) showed deficits in Y-maze performance compared to young mice (3 month). BCP treatment had no significant effect on working memory in young mice, yet significantly improved working memory in aged mice. Furthermore, BCP treatment significantly reduced circulating levels of IL-12 family cytokines. BCP appears to reverse age-associated cognitive decline in the working memory domain as well as age-associated increases in circulating IL-12 family cytokines. BCP may be a promising treatment for the deleterious effects of aging and IL-12 family cytokines may be a biomarker for anti-aging therapeutic responses.

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THE ROLE OF MICROGLIAL CB_2 RECEPTORS IN BETA A MYLOID PHAGOCYTOSIS: *IN VITRO* AND *IN VIVO* STUDIES

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Alzheimer's disease (AD), the most common age-related neurodegenerative disease, is characterized by the accumulation of amyloid-beta peptide (Aβ) in neuritic plaques within the brain leading to a strong inflammatory response. Previously, our group observed an increase in the expression of CB_2 receptors that was restricted to plaque-associated microglia. Additionally, the activation of CB_2 receptors by specific agonists in animal models of AD resulted in the reduction of the inflammation associated with the disease as well as in the Aβ processing. These data suggest a role of CB_2 in Aβ clearance by microglia.

In the present work, we wanted to assess the role of CB_2 receptors in Aβ phagocytosis by microglia both *in vitro* and *in vivo*. Microglial cells obtained from $CB_2^{EGFP/f/f}$, $CB_2^{-/-}$ and WT mice were treated with HiLyte™ Fluor 555 labeled-Aβ (1-42) for 4h, and internalized Aβ was measured by flow cytometry and immunofluorescence. Inhibition of phagocytosis with cytochalasin-D was employed as negative control. To determine ~~the~~ Aβ phagocytosis *in vivo*, 5xFAD/$CB_2^{EGFP/f/f}$ and 5xFAD/$CB_2^{-/-}$ mice were injected with methoxy-XO4, a fluorescent dye for Aβ oligomers. 24h later, brains were harvested to isolate microglia and measure the internalized methoxy-XO4 signal by flow cytometry.

Our data show that the deletion of CB_2 receptor leads to a lower efficiency of Aβ phagocytosis by microglia both *in vitro* and *in vivo*. These results demonstrate a key role of CB_2 receptor in the ability of microglia to remove the pathogenic deposits of Aβ peptide in AD, and support the therapeutic potential of cannabinoids through CB_2 signalling. We are currently performing additional experiments in order to determine the molecular mechanisms underlying this process and to analyse its impact in the disease.

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ALCOHOL BINGE-INDUCED CARDIOVASCULAR DYSFUNCTION INVOLVES ENDOCANNABINOID-CB1-R SIGNALING

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Heavy episodic alcohol drinking (also termed binge drinking) is a serious health problem. There is ample of evidence demonstrating that binge alcohol consumption can lead to life-threatening adverse cardiovascular events including atrial fibrillation, acute myocardial infarction, and congestive heart failure. Despite numerous studies investigating the effects of chronic moderate or excessive alcohol consumption on cardiovascular function and/or risk, relatively few reports have explored the cardiovascular effects of binge ethanol use. It is well documented that under pathological conditions overactivation of endocannabinoid- cannabinoid 1 receptor (CB1-R) signaling may exert important effects on cardiovascular function, including abnormalities in cardiac inotropy, chronotropy, conduction and vascular tone. Emerging clinical evidence also describes severe (often life-threatening) cardiovascular consequences of potent synthetic cannabinoids. In this study, by using multiple approaches (echocardiography, Pressure-Volume analysis, laser Doppler flow probes and laser speckle analysis) we aimed to characterize the detailed hemodynamic effects of acute alcohol intoxication *in vivo* in mice and to explore the potential role of the endocannabinoid-CB1-R signaling in mediating these effects.

Alcohol intoxication induced marked time-dependent elevation of cardiac anandamide levels which paralleled with profound cardiac dysfunction and impaired blood redistribution *in vivo*. Acute administration of CB1-R antagonist rimonabant was able to reverse the alcohol binge-induced cardiac dysfunction, which was also attenuated in CB1-R knockout mice. Thus, the anandamide-CB1-R signaling plays an important role in acute alcohol intoxication-induced hemodynamic adverse effects. Since there is an increasing prevalence of alcohol ingestion in combination with synthetic cannabinoids leading to adverse cardiovascular effects in adolescents, this emerging threat raises major health concerns. Therefore, repurposing CB1-R antagonists for acute treatment of combined alcohol and synthetic cannabinoid intoxication by reversing/preventing cardiovascular collapse may save lives.

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PALMITOYLETHANOLAMIDE MODULATES CB1 AFFINITY TO THC

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We have previously shown that in a murine model of acute pain, Palmitoylethanolamide (PEA) potentiates analgesic effects of sub-effective doses of THC, while also counteracting the AEs exerted by THC higher doses. The working theory behind this is an "entourage effect" phenomenon. There is, however, an unanswered question of how does PEA, which does not bind the THC target receptor, CB1 receptor (CB1R), do it. In order to examine the entourage mechanism of PEA on THC, we assessed the THC binding to CB1 receptor and the effect of PEA on the affinity of CB1 receptor to THC in an *in vitro* setting. We've utilized the GPCR β-Arrestin assay, which takes advantage of Enzyme Fragment Complementation technology. Activation of the GPCR stimulates binding of β-arrestin to the ProLink-tagged GPCR and forces complementation of the two enzyme fragments, resulting in the formation of an active β-galactosidase enzyme. This interaction leads to an increase in enzyme activity that can be measured using chemiluminescent detection reagents. The tests demonstrated the efficacy range of THC to be 1-100 μM, and the optimal therapeutic window to be 90 to 180 min. Following the initial test, the effect of increasing concentrations of PEA was examined on pre-determined concentrations of THC. The chosen duration of the receptor stimulation was 120 min. PEA markedly enhanced lower doses of THC binding to the CB1R. PEA did not affect the binding affinity of CB1R in high doses of THC, probably due to receptor saturation. The effective working ratio between THC:PEA concentration was observed at approximately 1:100. Furthermore, when downstream to CB1R activation signaling cascade, Ca^{2}+ flux and cAMP production, were examined with THC versus THC with PEA, similar findings were demonstrated. Ca^{2}+ flux was measured utilizing the Fluo-8 AM dye in human CB1-expressing cells and cAMP production was measured using phosphorylated protein Kinase A. PEA at concentrations of about 40-fold higher than THC, increased both THC-induced Ca^{2}+ flux and cAMP production.

These findings suggest that PEA directly affects THC binding affinity to CB1R, further impacting CB1R induced signaling cascade. These results shed light on intricate interactions between THC, CB1R and PEA, which constitute the basis of the "entourage effect", originally coined by Prof. Mechoulam.

MONOACYLGLYCEROL LIPASE INHIBITION: BIOPHYSICAL AND MECHANISTIC INSIGHTS

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Monoacylglycerol lipase (MGL) inhibition provides a potential approach towards treating diverse pathological conditions such as chronic pain, neurodegenerative diseases and cancer, through the regulation of 2-AG levels, and possibly via the indirect stimulation of CB1 receptors. Understanding the mechanism of action of compounds that inhibit the degradation of 2-AG can provide important insights into enzyme function and assist in the discovery of safer therapeutics with predictable and varying durations of action.

Based on biophysical studies, we herein provide a comparative analysis while exploring the mechanisms of action of different classes of MGL inhibitors comprising of both reversible and irreversible compounds. The study includes analyses of MGL interactions with known and new inhibitors containing the carbamate and urea moieties, as well as transition state analogues and cysteine-targeting compounds. Identification of MGL allosteric sites and potential allosteric inhibitors will be also discussed. In addition, we will address the phenomenon where potent irreversible MGL inhibitors that are commonly used in pharmacological studies may have relatively shorter biochemical τ values due to fast hydrolysis of the covalent MGL-inhibitor complex and subsequent regeneration of the enzyme. This unique pseudo-irreversible mode of MGL inhibition, now shown via experimentation using a diverse set of compounds, represents a powerful mechanism for abrogating enzyme activity, as it is considered the most pharmacologically tractable mechanism of drug action. Our strategy of elucidating "reversible covalent" mechanisms using NMR techniques offers the advantage of discovering and rapid "fine-tuning" of compounds that mimic the action of irreversible inhibitors, with predictable dissociation rates that can be translated *in vivo*.

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ELEVATED LEVELS OF ARACHIDONIC ACID-DERIVED LIPIDS, INCLUDING PROSTAGLANDINS AND ENDOCANNABINOIDS, ARE PRESENT THROUGHOUT ABHD12 KO BRAINS: NOVEL INSIGHTS INTO THE NEURODEGENERATIVE PHENOTYPE

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Derived from arachidonic acid (AA)-containing phospholipids, the endogenous cannabinoid (eCB) 2-arachidonoyl glycerol (2-AG) is a substrate for the enzyme αβ-hydrolase domain 12 (ABHD12). Loss-of-function mutations of ABHD12 are associated with the neurodegenerative disorder polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC). Currently, there is no cure for PHARC. Representing an animal model of PHARC, ABHD12 knockout (KO) mice show PHARC-like symptomology in older adulthood. Here, we test the hypothesis that ABHD12 deletion age-dependently regulates bioactive lipids in the mouse CNS. Methanolic lipid extracts from frozen brainstem, cerebellum, cortex, hippocampus, hypothalamus, midbrain, striatum, and thalamus of young (3-4 months) and older (7 months) adult male ABHD12 KO and age-matched wild-type (WT) mice were partially purified on C18 solid phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS. Over 80 lipids were screened in each brain area, including eCBs, lipoamines, 2-acyl glycerols, free fatty acids, and prostaglandins (PGs).

Aging and ABHD12 deletion drove widespread changes in the CNS lipidome; however, the effects of ABHD12 deletion were similar between old and young mice, meaning that most alterations in the lipidome of ABHD12 KO brains precede PHARC-like symptoms. AA-derived lipids were particularly sensitive to ABHD12 deletion. Relative to WT, 2-AG concentrations increased in the striatum, hippocampus, cerebellum, thalamus, midbrain, and brainstem, whereas the eCB *N*-arachidonoyl ethanolamine increased in all 8 brain regions, along with at least 2 PGs. Levels of free AA were also elevated in the ABHD12 KO brainstem, cerebellum, cortex, hippocampus, midbrain, and thalamus, suggesting that increased availability of AA may underlie elevations in AA-derived lipids. In addition, the AA-derived bioactive lipoamines, *N*-arachidonoyl alanine, *N*-arachidonoyl serine, and *N*-arachidonoyl tyrosine increased with ABHD12 deletion in every area screened except the hypothalamus. The other AA-derived lipoamines in the screening library were elevated in at least two brain areas of ABHD12 KO mice. Aging also had a widespread effect on the lipidome and more age-related changes in bioactive lipids were found in ABHD12 KO mice than WT, suggesting that ABHD12 deletion exacerbates the effects of age. The most robust effect of aging (independent of genotype) across the CNS were decreases in *N*-acyl GABAs and *N*-acyl glycines. In conclusion, levels of bioactive lipids are dynamic throughout adulthood and deleting ABHD12 disrupts the wider lipidome, modulating multiple AA-derived lipids with potential consequences for neuropathology.

*All data was collected and analyzed while the author, Emma Leishman, was a Ph.D. student with the Program in Neuroscience at Indiana University.

METABOLISM OF ENDOCANNABINOIDS AND PHYTOCANNABINOIDS BY CYTOCHROME P450 TO PRODUCE NOVEL BIOACTIVE METABOLITES

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Cannabinoids are found in marijuana and also are produced naturally in the body from ω-3 and ω-6 fatty acids. Exocannabinoids in marijuana, are known to be responsible for some of its euphoric effects, but they also exhibit anti-inflammatory benefits. Our study revealed a cascade of enzymatic reactions that convert ω-3 fatty acids into anti-inflammatory endocannabinoid epoxides that act through the same receptors in the body as marijuana (PNAS 2017). Endocannabinoids are ligands for cannabinoid receptor 1 and 2 (CB1 and CB2). CB1 receptor agonists exhibit psychotropic properties while CB2 receptor agonists have anti-inflammatory effects. Consequently, there is a strong interest in the discovery of CB2 selective agonists to mitigate inflammatory pathologies. The work details the discovery and characterization of naturally occurring ω-3–derived endocannabinoid epoxides that are formed via enzymatic oxidation of ω-3 endocannabinoids by cytochrome P450 epoxygenases. These dual functional ω-3 endocannabinoid epoxides exhibit preference towards binding to CB2 receptor and are anti-inflammatory and vasodilatory and reciprocally modulate platelet aggregation. Some of the other regioisomers of ω-3 endocannabinoid epoxides are partial agonists of CB1 and stop tumor cell metastasis (J. Med. Chem 2018). By virtue of their physiological properties, they are expected to play important roles in neuroinflammation and pain. This finding demonstrates how omega-3 fatty acids can produce some of the same medicinal qualities as marijuana, but without a psychotropic effect. Separately, we showed that phytocannabinoids are also metabolized by cytochrome P450 to produce novel class of oxy-cannabinoids. Overall, we will present that both omega-3 fatty acid endocannabinoids and phytocannabinoids are metabolized by cytochrome P450 to form novel class of oxy-cannabinoids that are bioactive.

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ENDOCANNABINOID HYDROLYSIS INHIBITION UNRAVELS THAT UNSATURATED FATTY ACIDS INDUCE A ROBUST SYNTHESIS OF ENDOCANNABINOID-GLYCEROLS IN HUMAN MYELOID LEUKOCYTES

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CONTEXT. Endocannabinoids (eCB) modulate immune responses, either by activating cannabinoid receptors (CB) or through their multiple metabolites, notably eicosanoids. As such, eCB hydrolysis inhibition decreases eicosanoid levels and increase eCB-glycerol levels in humans and mice, and diminish inflammation in mice. Thus, eCB-glycerol hydrolysis inhibitors might represent a potent anti-inflammatory strategy. eCB synthesis by human leukocytes was ill-defined and the documented syntheses are suboptimal, the measured levels being below the concentrations needed to activate the CB2 receptor. Our working hypothesis was that eCB hydrolysis inhibition would lead to significant eCB concentrations by leukocytes.

RESULTS. eCB-glycerol hydrolysis inhibition dramatically increased 2-AG half-life in neutrophils. Under such setting, neutrophils, eosinophils and monocytes synthesized significant levels of eCB-glycerols but not eCB-ethanolamides, in response to unsaturated fatty acids (UFA). Lymphocytes, alveolar macrophages, platelets and erythrocyte did not generate eCB in response to UFA. The obtained eCB-glycerol levels match the documented necessary concentrations to activate the CB2 receptor. The efficacy of UFA at inducing eCB-glycerol in leukocytes is ~1000-fold greater than those of GPCR or TLR agonists. Triascin C, an inhibitor of fatty acyl-CoA synthetases and thimerosal, an inhibitor of acyl-CoA transferases both inhibited the UFA-induced eCB-glycerol synthesis in neutrophils, implying that UFA remodeling is essential in this process. eCB-glycerol synthesis correlated with the synthesis of an LPA intermediate although we could not confirm a causal relationship between the two. While GPCR agonists modestly induced the synthesis of eCB-glycerol in neutrophils, they inhibited that induced by UFA- by ~50%. This suggests that eCB synthesis by leukocytes is decreased when leukocytes are surrounded by a pro-inflammatory entourage, as it is the case in chronic inflammatory diseases.

CONCLUSION. Our data support the concept that human leukocytes use UFA to synthesize biologically significant concentrations of eCB-glycerol and that hijacking the immune system with eCB-glycerol hydrolysis inhibitor might diminish inflammation in humans.

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OLEOYL GLYCINE INTERFERES WITH ACUTE NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL, BUT NOT MORPHINE REWARD

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Oleoyl Glycine (OlGly), a recently discovered fatty acid amide that is structurally similar to N-acylethanolamines, which include the endocannabinoid anandamide (AEA) as well as endogenous peroxisome proliferator-activated receptor alpha (PPARα) agonists oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), has been shown to interfere with nicotine reward and dependence in mice. Here we show that OlGly also interferes with the affectively aversive properties and somatic behavioral effects of acute naloxone-precipitated morphine withdrawal (MWD) in male Sprague Dawley rats. Synthetic OlGly (1, 5 or 30 mg/kg, ip) produced neither a place preference or aversion on its own; however, at doses of 1 and 5 mg/kg, ip, it blocked the aversive effects of MWD in a place aversion paradigm in rats. This effect was reversed by the cannabinoid 1 (CB1) receptor antagonist, AM251 (1 mg/kg, ip), but not the peroxisome proliferator-activated receptor alpha (PPARα) antagonist, MK886 (1 mg/kg, ip). As has been previously reported in mice, OlGly (5 or 30 mg/kg, ip) did not interfere with a morphine induced place preference in rats. As well, OlGly (1 or 5 mg/kg, ip) did not interfere with reinstatement of a morphine conditioned place preference by a morphine prime. *Ex vivo* analysis of tissue (nucleus accumbens, amygdala, prefrontal cortex and interoceptive insular cortex) collected from rats experiencing naloxone-precipitated MWD revealed that OlGly was selectively elevated in the nucleus accumbens. MWD did not modify levels of the endocannabinoids 2-AG and AEA, nor those of the PPAR□ ligands, OEA and PEA, in any region evaluated. We are currently evaluating the potential of intra-nucleus accumbens and intra-interoceptive insular cortex infusions of OlGly to interfere with the affectively aversive effects of naloxone-precipitated MWD in a place aversion paradigm. OlGly also interfered with the nausea and vomiting in rats and shrews, respectively. These results suggest that OlGly may be neuroprotective in reducing the impact of the aversive aspects of MWD and may possess efficacy in treating opiate addiction.

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A CENTRAL ROLE FOR 2-OLEOYLGLYCEROL IN CORNEAL WOUND HEALING

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Corneal healing in response to abrasions occurs rapidly, over the course of ~20 hours in an avascular environment, through a process that involves directed migration followed by proliferation. Our work has implicated several cannabinoid receptors – CB1, CB2, and GPR18 – in corneal wound healing. These receptors likely regulate cell migration through chemotaxis, providing chemo-attractive (CB1, GPR18) and -repulsive (CB2) cues to corneal epithelial cells during the highly choreographed course of wound healing, though GPR18 also upregulates proliferation of corneal epithelial cells.

Deletion or pharmacological block of any of these receptors impairs the course of healing, however it remains unclear what their precise role is at a given time in the course of healing. In particular we do not know the 'architecture' of cannabinoid circuitry during the healing process. Which endocannabinoids mediate chemotaxic signals and what cells produce them? The involvement of these three receptors suggests that there are at least two different endocannabinoids involved. Using lipidomic tools we examined the lipid profile in mice at several time points (0, 3, 9 hours) after corneal abrasion. Though we see several time-dependent differences in lipid levels after injury, the most striking finding is that at 3 and 9 hours we saw a substantial drop in levels of 2-oleoylglycerol (2-OG). 2-OG has been shown to activate GPR119, hence we tested knockout mice for this receptor, but found that wound healing was unaltered in either male or female GPR119 knockouts. We next tested the consequence of 2-OG treatment on the course of wound healing in an *in vivo* model of corneal abrasion, finding that healing was greatly delayed. Lastly, we have identified a novel transgenic mouse model for real-time tracking of corneal epithelial cell migration that allows for observation of individual fluorescent cells. Using this model, we find that cell migration is differentially affected by treatment with 2-OG. We hypothesize that the GPR119 signaling system may serve as a sort of brake on cellular migration and that levels of 2-OG are tightly regulated to allow cell migration and so wound closure.

12/15 LIPOXYGENASE ORCHESTRATES CELLULAR REMODELLING DURING WOUND REPAIR

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12/15 lipoxygenase (12/15-LOX) is the murine ortholog of the human 15-LOX1. This enzyme generates multiple free and esterified lipid mediators (and biotransformations of 2-AG), however it is still unclear to whether it promotes or inhibits inflammation. In this study, we characterised the role of 12/15-LOX in the co-ordination of wound healing in a mouse model. Healing of full thickness wounds was evaluated in 8- to 10-week-old C57BL/6 wildtype (WT) and 12/15-LOX knockout (KO) mice for up to 14 days, utilising immunohistochemistry, LC/MS/MS, RNA-seq and collagen extracellular matrix evaluation. 12-HETE significantly increases in Day 1 (D1) in wildtype (WT) wounds compared to day 0 (D0) controls: (WTD0 27 ± 4.8 vs. WTD1 193 ± 24.1 pg/mg, $p<0.0001$). 12/15-LOX deficient mice show a more pronounced inflammatory phenotype with respects to their wound healing physiology. Specifically, KO wounds showed increased IFN-γ expression, decreased M2 macrophage polarisation ($p=0.03$) and high levels of neutrophil extracellular traps (NETs). KO wounds also show greater TGF-β expression and fibroblast proliferation (WTD7 105 ± 2.7 vs. KOD7 13 ± 5.1 mean grey intensity, $p=0.0006$), coupled with an increase in Ki-67 (101 ± 10.4 vs WTD7 74 ± 4.7 mean grey intensity, $p=0.04$). Analysis of RNA-seq data showed an increase in IL-6 and alpha-2-macroglobulin gene expressions in KOD7 wounds compared to WTD7 wounds, along with increased collagen levels detected by Masson's Trichrome staining. Alpha-2-macroglobulin can inhibit multiple matrix metalloproteases (MMPs), and MMPs degrade collagen. Thus, reduced collagen degradation may contribute to the KO phenotype. Collagen levels may also be augmented by the elevation in TGF-β seen in KO wounds, as TGF-β leads to fibroblast expansion. In summary, we demonstrate that 12/15-LOX is a key regulatory enzyme in the temporal orchestration of wound healing whose absence leads to an over proliferative fibrotic phenotype that could result in scarring.

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ACTIVATION OF CANNABINOID-1 RECEPTORS (CB1R) IN ADIPOSE TISSUE CONTRIBUTES TO METABOLIC RISK BY INHIBITING FAT MOBILIZATION AND ALTERING INSULIN SENSITIVITY

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Endocannabinoid tone is positively correlated to fat mass and associated with metabolic risk both in mice and human. Strong evidence suggests that activation of cannabinoid-1 receptors (CB1R) by endocannabinoids exerts coordinated actions on several organs that ultimately promote energy storage and fat accumulation. Indeed, CB1R activation in adipocytes favors lipid accumulation by different complementary mechanisms related to anabolic pathways, such as enhancement of lipoprotein lipase activity, glucose uptake and adipogenesis. In contrast, the impact of endocannabinoid system (ECS) on fatty acid lipolysis in adipose tissue (AT) has been poorly studied. Yet a decrease in fat mobilization is also predicted to promote fat storage and therefore contribute to the metabolic deregulations associated with AT hypertrophy. On the other hand, high lipolysis rate is considered a primary event contributing to the onset of insulin resistance, increasing ectopic fat storage and lipotoxicity in other organs such as muscle, liver, heart, and pancreas. In line with this, we performed various *in vivo* and *in vitro* experiments to study the consequences of ECS activation on AT lipolysis paying attention to the important regulatory role of insulin on this metabolic pathway.

We first showed in fasted mice (*i.e.* with low plasma insulin levels) that the elevation of the ECS tone induced by a treatment with JZL195 (an inhibitor of endocannabinoid degrading enzymes) was associated with a significant decrease in plasma glycerol appearance suggesting a lower lipolytic activity. Similarly, treatment of rat AT explants with JZL195 or anandamide (AEA) induced a decrease in both basal and catecholamine-stimulated lipolysis. We further observed that AEA treatment decreased cAMP levels compared to vehicle while co-treatment with Rimonabant abrogated theses effects. Akt phosphorylation was also stimulated by AEA and associated with a decrease in hormone-sensitive lipase activation. Data suggest that CB1R signaling can modulate cAMP levels independently on beta-adrenergic receptors but rather through an inhibition of adenylyl cyclase and / or an activation of PDE-3B.

Our observations were fundamentally different when experiments were performed in the presence of insulin. Indeed, AEA and JZL195 treatments increased glycerol release in mice injected with glucose, but not in $CB1R^{-/-}$ nor in 24-h fasted mice suggesting that the effect was dependent on CB1R and plasma insulin levels. We concomitantly observed an alteration of the antilipolytic action of insulin as evidenced by a decrease in the Akt cascade activity. Similar results were obtained with AT explants exposed to anandamide and insulin, thus excluding the impact of an *in vivo* alteration of insulin secretion and supporting the role of AT-expressed CB1R. Interestingly, similar experiments performed on white AT explants collected from healthy subjects further suggest that our findings might also apply to human physiology.

Taken together, our data support the concept that activation of CB1R in AT may interfere with pathways regulating lipolysis and ultimately contributes to the development of metabolic complications linked to fat mass expansion. Finally, our work maintains the view that the development of strategies to reduce the activity of peripheral CB1R presents a major therapeutic interest.

ENDOCANNABINOID SIGNALING MEDIATES SUSCEPTIBILITY TO HIGH FAT DIET-INDUCED INTESTINAL DYSBIOSIS AND REGULATES METABOLIC HEALTH

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The endocannabinoid (eCB) system regulates a variety of obesity-related physiological processes including appetite, inflammation, and metabolism. Recent literature also suggests that the gut microbiota is tightly linked to obesity development. However, studies on eCB regulation of obesity-induced intestinal dysbiosis have not been extensively explored. In the current study, genetic ablation of cannabinoid receptors CB1 and CB2, as well as pharmacological intervention were used in a mouse model of diet-induced obesity to determine the roles of the eCB system in modulating inflammation, metabolism, and the gut microbiome. As expected, CB1-/- but not wild type, CB2-/-, or double knockout CB1-/-CB2-/- mice were resistant to high fat diet (HFD)-induced weight gain, metabolic dysfunction, and visceral adipose tissue M1 macrophage accumulation. Of note, obesity development in double knockout CB1-/-CB2-/- mice highlights an important role for CB2 in regulation of metabolic processes that merits further investigation. Additionally, treatment of obese mice with the CB1 antagonist AM251 led to weight loss, decreased adipose inflammation, and improvement in metabolic parameters. Interestingly, CB1 was found to regulate HFD-induced leukocyte infiltration in the cecal-colonic lamina propria. Decreased intestinal inflammation in CB1-/- mice indicated CB1-mediated alterations in the gut microbiome might contribute to a decreased obese phenotype. Microbiota profiling by 16S V3-V4 sequencing showed that CB1-/- mice were resistant to development of HFD-induced gut dysbiosis. Moreover, AM251 intervention in obese mice shifted their microbiota towards a lean phenotype. Functional predictions by PICRUSt analysis revealed decreased abundance of operational taxonomic units belonging to bacterial metabolism and membrane transport pathways. Furthermore, transcriptome analysis of intestinal epithelial cells revealed differential expression of various antimicrobial peptides including alpha-defensins. Together these data suggest that the eCB system regulates metabolism by shaping intestinal immunity and mediating susceptibility to dysbiosis.

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BIDIRECTIONAL INTERACTION BETWEEN THE GUT MICROBIOME AND THE ENDOCANNABINOIDOME

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The gut microbiome and the endocannabinoidome (eCBome) are seemingly disparate systems sensitive to environmental and dietary factors that are critical regulators of several aspects of physiology, including energy metabolism, immune response and behaviour. Gut microbiota and eCBome interactions first came to light when microbiota modification with prebiotics (foods that stimulate the growth of beneficial bacteria) in obese *ob*/*ob* mice decreased CB1 and AEA levels in adipose tissue, modifying adipogenesis and lipid metabolism[1]. Dysbiosis in diabetic *db*/*db* mice is also associated with increased AEA and decreased 2-AG in fat[2]. Live beneficial microorganisms known as probiotics also modulate zebrafish eCBome gene expression, indicating that eCBome sensitivity to gut microbes is evolutionarily conserved[3]. Adipose tissue-specific knockout of a major AEA and *N*-acylethanolamine anabolic enzyme, NAPE-PLD, reduced PEA and OEA levels, inhibited adipose tissue browning and increased weight gain, while altering gut microbiota in mice fed normal and high fat diets[4], indicating, in conjunction with the above, the existence of a bidirectional eCBome-microbiome interaction.

We have investigated the gut microbiome-eCBome axis determining how the latter is altered in germ-free mice and how the former is altered in *Mgll*$^{-/-}$ mice in order to gain a deeper insight into their metabolic phenotypes and the mechanisms underlying obesity and its complications. We profiled over 50 eCBome genes and over 40 mediators using a qPCR array and LC/MS-MS, respectively, within several tissues of germ free (GF) and conventionally reared male and female mice at juvenile and adult ages. Our results show that the absence of the microbiome is accompanied by modifications in eCBome gene expression and lipid levels in, among others, ileum, colon and BAT of male mice. These results are consistent with the phenotype of GF mice and the roles that the eCBome is known to play in the regulation of BAT activity, gut motility and intestinal inflammation. Reconstitution of the gut microbiota in germ free mice partially reverted the changes in the eCBome of germ-free mice. Further, we show that the gut microbiota of *Mgll*$^{-/-}$ and wild-type male and female mice show a clear separation of the two genotypes by 16S metagenomic sequencing, suggesting that the presence or absence of MAGL may interact with the gut microbiome of mice. In particular, the *Roseburia* and *Parabacteroides* genera, which have been associated with improved glucose homeostasis, were increased in *Mgll*$^{-/-}$ mice, which are resistant to the development of diet-induced insulin resistance[5].

Our studies provide us with unique information on how the microbiome and eCBome influence each other, providing the groundwork for elucidating how these two complex systems work together to influence health. Also, they provide an opportunity for identifying and characterizing commensal bacteria that are influenced by eCBome lipid members and the activity of their receptors, and which possibly mediate the phenotype of *Mgll*$^{-/-}$mice and play a role in metabolism and glucose homeostasis.

1. Muccioli, G. G. *et al. Mol. Syst. Biol.* **6**, (2010).
2. Geurts, L. *et al. Front. Microbiol.* **2**, (2011).
3. Falcinelli, S. *et al. Sci. Rep.* **5**, (2015).
4. Geurts, L. *et al. Nat. Commun.* **6**, (2015).
5. Taschler, U. *et al. J. Biol. Chem.* **286**, 17467–17477 (2011).

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THC TREATMENT IMPROVED NEUROTOXICITY BY ALTERING THE MICROBIOTA

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HIV infection causes neuroinflammation resulting in neurodegeneration accompanied by progressive motor and cognitive dysfunction. The HIV-1 viral envelope glycoprotein gp120 is shed from infected cells to uninfected brain cells. Studies suggest that gp120 induces neurotoxicity. Tetrahydrocannabinol (THC), the active ingredient in *Cannabis sativa* has psychotropic effects and is used to treat anorexia, nausea as well as an analgesic. In the current study, we investigated the therapeutic potential of THC on neurotoxic effects. To this end, 7-month old GFAP-gp120 transgenic mice were treated with vehicle or THC. Colon microbiota was collected and shotgun genomic sequencing was performed. The data demonstrated that the major phylum was *Proteobacteria*, family Helicobacteraceae, species Helicobacter hepaticus, Strain GCF000007905 in the colon of vehicle-treated group. Moreover, THC treatment led to elevated *Firmicutes* phylum due to significant increase in the beneficial genus, *Lactobacillus.* Furthermore, virus taxonomic units Family Retroviridae, species mouse-mammary-tumor-virus, strain PRJNA14435 decreased in THC treated group. Additionally, among the metabolic pathways, we found that peptidoglycan biosynthesis pathway was increased in vehicle group due to *Bacteroides vulgatus* which was elevated too. Together, our data suggest that THC altered the microbiota in the colon of gp120 Tg mice to prevent toxicity.

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DOES ADMINISTRATION OF CANNABIDIOL ENHANCE EXTINCTION OF FEAR IN HUMANS?

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Animal research has demonstrated that the phytocannabinoid cannabidiol (CBD) enhances fear extinction in rats. We present two studies in which we examine whether CBD (vs. placebo) could enhance fear extinction in humans. Fear extinction is thought to underlie the benefits of the main behavior treatment for anxiety disorders (exposure), and can be modeled experimentally in a laboratory fear conditioning and extinction paradigm. Because the majority of preclinical studies demonstrate enhanced extinction of context fear, we induced both cue and context conditioning and extinction in a virtual reality setting. Outcome was assessed with fear-potentiated startle (FPS) and subjective fear ratings. Study 1 included 48 healthy volunteers who inhaled synthetic CBD (40 mg) or placebo after fear acquisition but before continuing with the extinction phase on the same day. Study 2 consisted of oral administration of the same compound (300 mg) in 56 healthy volunteers prior to extinction on a separate day. In both studies, retention of fear was assessed after one week.

In study 1, FPS and subjective fear did not reveal effects of CBD during extinction proper (within-session extinction), but at retention context-FPS was reduced in the CBD as opposed to the placebo group. The absence of an effect on within-session extinction could be due to the unexpected aversive nature of the CBD inhalation that served as a stressor prior to extinction in the CBD, but not the placebo group. Also, stronger effects at retention may have been prohibited by enhanced consolidation of not only the extinction memory but also the acquisition memory. Study 2 was set up to circumvent these issues. Results of study 2 are currently being analyzed and will be presented at the meeting.

The results so far suggest that CBD perhaps does not enhance fear extinction during extinction learning, but it does have potential for the strengthening of fear extinction between sessions. The latter feature would make CBD of clinical interest for the augmentation of exposure therapy, which is currently relatively effective (about 60% remission) but still leaves many patients impaired.

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PHARMACOLOGICAL CHARACTERIZATION OF THE SYNTHETIC CANNABINOID EG-018

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Background: Synthetic cannabinoids, which typically exhibit high affinity and efficacy for cannabinoid receptors, remain popular as psychoactive alternatives to cannabis despite continued regulatory enforcement initiatives and reports of their potential toxicity and lethality. EG-018, a structurally unique tricyclic synthetic cannabinoid analog, has received relatively little attention after its initial reports as an adulterant in illicit herbal products and subsequent detection in human biological samples. Therefore, EG-018 was tested *in vivo* (mouse tetrad and drug discrimination) and *in vitro* ([^{3}H]CP55,940 binding, [^{35}S]GTPγS binding and cAMP) to establish its relative affinity, efficacy and potency in cannabinoid pharmacological assays.

Results: EG-018 did not produce the standard profile of cannabinoid behavioral effects (i.e., decreased body temperature and motor activity, catalepsy, and antinociception) in adult mice at doses up to 100 mg/kg (i.p.) and produced only catalepsy and reductions in body temperature following 56 mg/kg (i.v). Catalepsy was partially reversed with administration of SR141716A. EG-018 did not substitute for Δ^9-THC at any dose up to 100 mg/kg in adult mice. EG-018 completely displaced ~1 nM [^{3}H]CP55,940 at hCB_1 and hCB_2 in HEK293 cell membranes (hCB_1: pK_i = 7.59±0.06, 25.5 nM; hCB_2 = pK_i 8.06±0.07, 8.62 nM). EG-018 stimulated [^{35}S]GTPγS binding in hCB_1 HEK293 cell membranes (E_{max} = 67.8%±5.8, pEC_{50} = 7.55±0.23), but to a lesser extent than THC (118.4%±9.43, pEC_{50} = 7.0±0.17). However, stimulation by EG-018 was only observed when the drug was pre-equilibrated for 30 min prior to addition of [^{35}S]GTPγS. EG-018 also inhibited forskolin-stimulated cAMP production in hCB_1 HEK293 cells (E_{max} = 32.9%±2.27, pEC_{50} = 7.51±0.23), and its maximal response was less than CP55,940's (E_{max} = 44.2%±3.65, pEC_{50} = 9.29±0.12), but not THC's (E_{max} = 41.5%±2.30, pEC_{50} = 8.22±0.14).

Discussion: Despite exhibiting high affinity for cannabinoid receptors, EG-018 was initially observed to lack efficacy in [^{35}S]GTPγS binding. These data were supported by *in vivo* findings of modest route-dependent effects in the mouse tetrad and lack of substitution for THC in drug discrimination. [^{35}S]GTPγS stimulation was only observed when EG-018 was pre-equilibrated with membranes prior to [^{35}S]GTPγS addition, suggesting that pre-equilibration prior to [^{35}S]GTPγS binding may facilitate assessment of lipophilic partial agonists that produce little G-protein activation. Inhibition of forskolin-stimulated cAMP production in hCB_1 HEK293 cells by EG-018 further confirmed that it can act as a partial agonist at cannabinoid receptors, albeit with lower efficacy than CP55,940. EG-018's greater relative efficacy in the cAMP assay compared to [^{35}S]GTPγS binding may be due to differences in receptor reserve or signal amplification, however, other mechanisms cannot be ruled out. Nevertheless, EG-018's minimal cannabis-like effects in rodents are consistent with its lower signaling efficacy at cannabinoid receptors, which appears to further translate into a lack of adverse effects or sustained use in humans.
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CANNABINOIDOMICS – AN ANALYTICAL TOOL TO UNDERSTAND THE EFFECT OF MEDICAL CANNABIS TREATMENT

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Cannabis is a complex plant composed of several hundred compounds of various chemical classes and wide concentrations. Among these, phytocannabinoids, the natural cannabinoids found in *Cannabis*, are unique to this plant. Their biological mechanisms of action are attributable mainly to their interactions with the endocannabinoid system (ECS), either by activating/inhibiting cannabinoid and non-cannabinoid receptors, and/or metabolic (biosynthesizing or degrading) endocannabinoid enzymes. In order to study the pharmacological effects of whole *Cannabis* extracts on the endocannabinoid metabolome we have developed an accurate high-resolution liquid chromatography-mass spectrometry (HR-LC-MS/MS) tool for identification and quantification of endocannabinoids and other endogenous cannabimimetic lipids, phytocannabinoids, and their metabolites in various biological matrices.

In this study, identification of target compounds by HR-LC-MS/MS was performed according to the retention times and MS/MS fragmentation patterns of available analytical standards as a reference for the identification of additional compounds from all subclasses. Overall, we identified (a) over 90 different phytocannabinoids from all 10 different phytocannabinoid subclasses by screening various natural and decarboxylated *Cannabis* flowers; (b) over 100 endocannabinoids and cannabimimetic lipids from 20 different lipid families by screening mice serum and tissues (brain, liver and spleen); and (c) 20 (-)-Δ^9-trans-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) metabolites in mice serum and liver samples following treatment with *Cannabis* extracts. Extraction methods were then developed and validated for quantifying the identified compounds in various biological fluids, tissues, and cells. This cannabinoidomic tool can be used to accurately determine changes in the levels of endocannabinoid metabolites as a result of *Cannabis* treatment for diverse physiological and pathological conditions.

ENANTIOMERIC EXCESS DETERMINATION OF SYNTHETIC CANNABINOIDS

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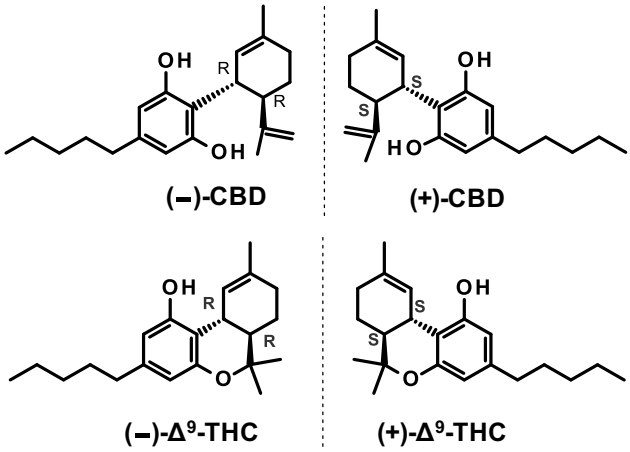

Synthetic chiral APIs require an exact enantiomeric purity determination according to the current regulatory guidelines to ensure product safety and to avoid patient harm, like the thalidomide tragedy in the late 1950s and early 1960s. The increasing interest in Cannabinoids (synthetic and from natural sources) for medical use requires sophisticated analytical methods. While stringent rules for the content and impurities are in place, enantiomeric purity has not received much attention. Whereas biological active compounds extracted from plant material are often implied without any analysis of its enantiomeric purity, synthetic chiral APIs require an exact enantiomeric purity determination according to the current regulatory guidelines to ensure product safety.

To approach this issue, (+)-and (−)-Cannabidiol as well as (+)-and (−)-Δ^9-Tetrahydrocannabinol were synthesized and tested with a selection of chiral chromatographic procedures to determine the best analytical process. The here demonstrated Ultra-High Performance Supercritical Fluid Chromatography UHPSFC is an advanced alternative to GC and HPLC in terms of analytical time and separation.

CBD BLOCKS THE SEIZURE-INDUCING ACTIVITY OF CP55940

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Cannabidiol (CBD) is a cannabinoid found in *Cannabis Sativa* that has recently been approved by the US FDA under the name Epidiolex® for the treatment of Dravet and Lennox-Gastaut Syndromes, two rare childhood seizure disorders. Synthetic cannabinoids sold under names like Spice or K2 have been reported to cause seizures in human users. CP55940 is a synthetic cannabinoid that was previously shown by our laboratory to produce seizures in mice. The hypothesis of these studies was that CBD would block seizures induced by CP55940. Using C57BL/6 male mice, CBD at 500 mg/kg or vehicle was injected i.p. ~40 minutes prior to 4 mg/kg CP55940 or vehicle. Body temperature and latency to tail withdrawal from a water bath set to 53 degrees were measured before each injection and 60 minutes after the second injection. Mice were video-recorded for 30 min after injection of CBD or vehicle and for 1 hour following injection of CP55940 or vehicle, and the recordings observed for visible convulsions. Change in body temperature, %Maximum Possible Effect (%MPE) in tail withdrawal and the number of episodes of convulsions for each mouse were determined.

CBD did not produce an effect in any of these measures. CP55940 produced a decrease in body temperature of 5.8 degrees, 66% MPE for tail withdrawal and an average of 8.5 convulsion events. Pre-treatment with CBD had no effect on the change in temperature or antinociceptive effects of CP55940, but decreased the number of convulsion events by over 80% from an average of 8.5 to 1.5 per mouse. Experiments in female mice, with other cannabinoids agonists and different doses of CBD are planned.

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LACK OF STANDARDIZATION IN CANNABIS VARIETY NAMES IN BOTH GREY MARKET AND LEGAL SUPPLY CHAINS IN CANADA: EVIDENCE FOR NECESSITY OF GENETIC VERIFICATION

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A review of *Cannabis* dried flower available for sale in selected markets in the United States and Canada revealed in excess of 1200 named varieties. While the nine most common variety names accounted for 10.3% of all material for sale, other variety names were observed at decreasing frequencies down to 845, single-occurrence variety names. Due to *Cannabis*'s history as an illicit substance the veracity of these self-applied variety names is unknown. It is conceivable for instance that in the past some growers may have arbitrarily named plant material to whatever variety names were expected to have highest market value; or conversely, growers may have taken common varieties and effectively rebranded them with novel names as a marketing strategy. Lacking generally accepted authenticated voucher specimens, approved variety appellations, and material pedigrees available in other high-value agricultural crops such as grapes or hops, the *Cannabis* industry faces challenges if it is to standardize product to consumers. Such standardization is desirable, particularly in the case of medical-use applications where someone may invest significant time and effort in determining suitability of a variety and its therapeutic dosing window. As many *Cannabis* varieties appear physically similar and have complex chemotypic makeups with nuanced differences, a reliable objective means for variety identification would be an essential first step in establishing verifiable variety identities.

To address this need, Segra has established and validated a 12-marker Variable Nucleotide Tandem Repeat (VNTR) genetic panel for variety typing in *Cannabis*. Data collected across several hundred samples to date has shown the method to be robust, reliable, scalable, and capable of resolution of even closely related accessions. Application of the method to Canadian grey market samples demonstrated multiple unequivocal examples of single clones bearing multiple names; multiple highly divergent samples sharing single names; and what appear to be legitimate variety name clusters with a single name covering limited diversity. Application of this VNTR variety typing panel to material in the tracked, legitimate federal and provincial supply chain demonstrated a similar picture; single variety names can occur on highly divergent material, and some individual clones are sold under different names. The need for consensus selection of variety voucher specimens and application of widespread variety typing by this or a similar genetic method will be essential as the *Cannabis* industry matures and standardization is demanded by customers.

CHRONIC CANNABIDIOL TREATMENT IMPROVES VASCULAR FUNCTION OF HYPERTENSIVE DOCA-SALT RATS IN VASCULAR BED SPECIFIC MANNER

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Cannabidiol (CBD), psychoactive inactive constituent of *Cannabis sativa*, has been suggested to play a beneficial role in the cardiovascular system (*Pisanti et al., Pharmacol Ther. 2017;175:133*). We demonstrated that CBD directly relaxed human and rat arteries and after 2h incubation augmented endothelial function in deoxycorticosterone acetate and high salt-diet treated (DOCA-salt) rats (*www.pa2online.org/abstract/abstract.jsp?abid=33343&period=66*). Thus, the aim of the study was to determine the influence of chronic CBD administration on the endothelium-dependent vasorelaxation and contractility in arteries of DOCA-salt hypertensive rats and their controls.

CBD 10 mg/kg or its vehicle was injected intraperitoneally once a day per 14 days. Twenty-four hours after the final does of CBD, experiments were performed in endothelium-intact thoracic aortae and small mesenteric arteries (sMAs) isolated from DOCA-salt hypertensive rats or their appropriate normotensive uninephrectomized (UNX) animals using organ bath and wire myography technique, respectively. Cumulative concentration-response curves were conducted (1) to phenylephrine, (2) a thromboxane A_2 analog U46619 and in phenylephrine pre-constricted arteries (3) to acetylcholine (4) sodium nitroprusside. In each individual preparation only one experimental curve was determined. Functional experiments were conducted according to Baranowska-Kuczko *et al.* (*Life Sci. 2016; 151:288-299).*

In comparison to CBD vehicle-treated DOCA-salt, but not to CBD vehicle-treated UNX, CBD administration augmented the potency of vasorelaxation induced by acetylcholine in aortae and sMAs but by the donor of NO, sodium nitroprusside only in sMAs. CDB treatment diminished the efficacy of U46619-induced vasoconstriction in CB_1-dependent manner (sensitive to AM251, 1 μM) only in sMAs both in DOCA-salt and UNX. CBD did not alter the phenylephrine-mediated vasoconstriction efficacy neither in sMAs nor aortae in either strain of rats compared to vehicle-treated controls.

Chronic treatment with CBD of DOCA-salt rats improves the endothelium-dependent vasorelaxation and/or reduced the U46619-induced vasoconstriction via activation AM251-sensitive mechanism in vascular bed-specific manner. More studies are required to explain CBD-mediated hypotensive mechanisms and its potential beneficial effect in the light of its vascular effects in systemic vessels in normo- and hypertension.

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CELLULAR MEMBRANE AFFINITY CHROMATOGRAPHY (CMAC) AS A TOOL TO IDENTIFY PHARMACOLOGICALLY ACTIVE COMPOUNDS INTERACTING WITH TRANSMEMBRANE PROTEINS

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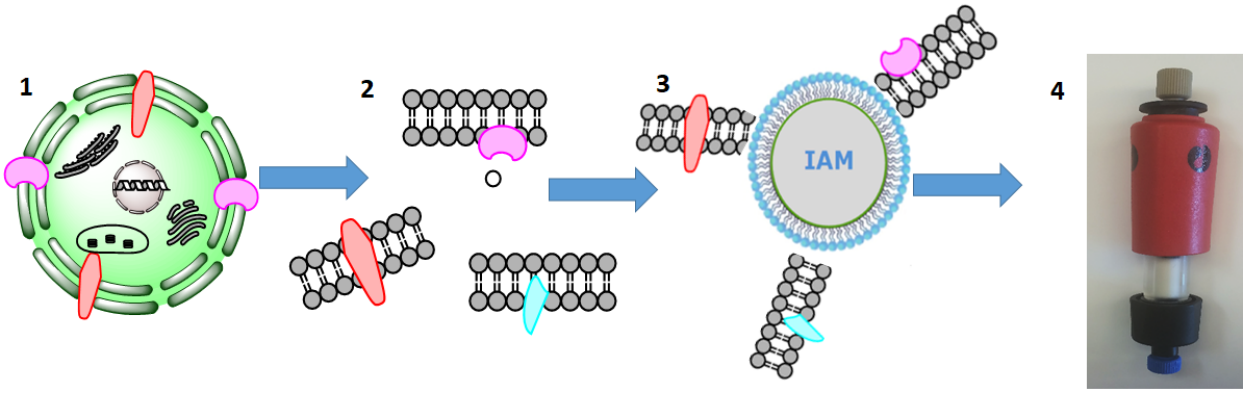
Identification of pharmacologically active compounds present in complex natural mixtures, e.g. plant, fungal or microbial extracts often requires high resource commitment. Despite the wealth of drug-like molecules produced by plants, the process of drug discovery from natural sources has diminished in the past two decades. The traditional approach involves isolation of individual compounds and subsequently testing them using *in vitro* cell-based assays or in different model organisms. This approach often leads to the isolation of known and most abundant secondary metabolites with well-defined pharmacological activity, but typically leaves out less abundant, although potentially important compounds. To overcome this barrier, novel tools allowing for targeted identification and isolation of natural compounds interacting with transmembrane receptors are needed.

This work describes cellular membrane affinity chromatography (CMAC) as a tool for rapid identification of pharmacologically active compounds from plant derived extracts. In CMAC, cell membrane fragments containing the targeted transmembrane protein are immobilized on the surface of a stationary phase coated with phosphatidylcholine. This allows quick identification of pharmacologically active metabolites, targeting transmembrane proteins without the need to isolate every single compound present in the extract. CMAC can be used to study the binding process between potential new ligands and the immobilized transmembrane protein target. Parameters that can be determined using CMAC include binding affinity (K_d), association rate constant (k_{on}), dissociation rate constant (k_{off}) and the equilibrium constant for complex formation (K). Examples, including compounds targeting the CB1 receptor will be presented.



Cell membrane fragment immobilization on the surface of IAM particles: (1) cells expressing targeted transmembrane proteins, (2) cell membrane fragments after cell lysis, (3) cell membrane fragments immobilized on the surface of IAM particles, (4) IAM particles packed in a glass column to yield CMAC column.

CANNABINOID CB_1R RECEPTOR IS OVERACTIVATED IN HERMANSKY-PUDLAK SYNDROME PULMONARY FIBROSIS

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Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder of lysosome-related organelle biogenesis that has 10 different genetic subtypes. Patients with HPS type 1, 2, or 4 develop pulmonary fibrosis (PF). Hermansky-Pudlak syndrome pulmonary fibrosis (HPS-PF) is a progressive life-threatening disease with poor prognosis. In the absence of an FDA-approved therapy, there is an urgent need to identify new therapeutic targets and treatments.

We evaluated the role of the endocannabinoid/CB_1R system in HPS-PF using human bronchoalveolar lavage fluid (BALF), plasma, and lung tissue samples from patients with HPS and controls without PF. We measured levels of the endocannabinoids anandamide (AEA) and 2-arachidonyl glycerol (2-AG) in BALF and plasma and correlated these with pulmonary function measurements performed in the same subjects. We also evaluated CB_1R protein levels by immunohistochemistry in explanted lung tissue samples from HPS-PF lung transplant recipients.

The endocannabinoid anandamide (AEA) was significantly higher in BALF samples from HPS1-PF patients (213 ± 18 fmol/ml) compared to BALF from non-fibrotic controls (146 ± 9 fmol/ml). Interestingly, plasma AEA levels from HPS1 carriers (3.4± 0.3 pmol/ml) and HPS1-PF patients (3.7± 0.2 pmol/ml) were also significantly elevated compared to plasma from healthy volunteers (2.3 ± 0.2 pmol/ml), whereas AEA levels in BALF and plasma from HPS3 carriers, who do not develop pulmonary fibrosis, were comparable to values in non-fibrotic controls, suggesting that plasma AEA is a potential biomarker in HPS-PF. Furthermore, AEA levels in BALF and plasma from HPS-PF patients negatively correlated with pulmonary function. We also found increased expression of CB_1R protein in explanted lung tissue from patients with HPS-PF compared to lung tissue from non-fibrotic controls.

In conclusion, we have found that the endocannabinoid/CB_1R system is overactivated in patients with HPS-PF. This suggests that CB_1R may be a therapeutic target for HPS-PF.

Acknowledgements: Funded by the intramural research programs of the NIAAA (to G. Kunos) and the NHGRI (W. Gahl), the American Thoracic Society Foundation Research Program and the Hermansky-Pudlak Syndrome Network (to R. Cinar).

APPLICATION OF OXFORD NANOPORE MINION PLATFORM IN CANNABIS GENOMICS AND METAGENOMICS: FIRST IMPRESSIONS

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The recent emergence of *Cannabis* cultivation as a legal mainstream activity has opened up opportunities for genomic studies to establish basic information on par with other high value industrial plant crops. In particular, next generation sequencing (NGS) technologies show promise in identification of single and multi-trait Mendelian and Quantitative Trait loci as markers for selective breeding programs; in the characterization of structural and copy number variations; and through metagenomics in the discovery, identification, and characterization of associated and possibly pathogenic microorganisms including bacteria, fungi, viruses, and viroids. In contrast to more mainstream NGS platforms with high equipment and per-run costs, the Oxford Nanopore MinIon technology has potential to democratize the application of NGS technologies by providing a relatively low entry cost platform with a scale well suited for application to *Cannabis.* We report here on our initial experiences of application of this platform to *Cannabis* genomics and metagenomics. These include preliminary development of an inexpensive and technically simple but effective dried plant material DNA isolation protocol enriched for longer fragments well suited to nanopore sequencing; observations on data yield and associated costs and times downstream of this preparation method, with discussion of scalability; a consideration of our experimentally observed read length distributions in the context of potential advantages of long read technologies over short read technologies in establishing genetic scaffolds in the presence of long repeat regions; and a summary of bioinformatics challenges, resources, and possible solutions for *Cannabis* laboratories considering gathering NGS data. Finally, we present examples of genomic and metagenomic data we have obtained with this method.

DISCOVERY OF STEROL CARRIER PROTEIN-2 INHIBITORS USING RATIONAL PROBE DESIGN

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Agents that modulate endocannabinoid (eCB) transport have therapeutic potential in disorders of stress, anxiety, and pain. A putative anandamide (AEA) uptake transporter that facilitates sequestration of extracellular AEA and transport within cells is considered to regulate eCB signaling. A number of AEA transport inhibitor probes have been reported, though the identity of the protein or proteins responsible for AEA transport remains a topic of much debate. We identified sterol carrier protein-2 (SCP-2) as a cytoplasmic binding and transport protein for eCBs (Mol. Neurobiol., 2014, 50, 149-158). More recently, we reported the results of binding studies using arachidonic acid derivatives, small molecules identified from high-throughput screening, and new agents discovered using computer-aided probe discovery (Methods in Enzymology, 2017, Ch. 5, Vol 593, 99-121). Here, we describe the results of a follow-up study aimed at more completely understanding the structure-activity relationships (SAR) of arachidonates and small molecules as SCP-2 inhibitors.

Compounds were selected from our in-house library of structurally diverse small molecules or purchased from commercial vendors. SCP-2 binding and efficacy was determined using an NBD-stearate (NBDS) displacement assay. *N*-arachidonoyl-L-serine was a more potent (Ki 1.69 ± 0.06 μM vs. 2.95 ± 0.12 μM) and efficacious (Emax 86.6 ± 0.3% vs. 69.2 ± 1.7%) inhibitor than its reduced analog *N*-arachidonoyl-L-serinol. Noladin ether was found to be approximately 15% more efficacious than 2-arachidonoylglycerol (2-AG) and 5-fold less potent. Replacement of the 4-*O*-(2-naphthyl ether) of csddd 9368 was detrimental to SCP-2 binding and inhibition. Of three new compounds discovered from virtual screening, two (csddd 4551 and csddd 9036) inhibited SCP-2 with Ki < 10 μM, though only the *N*-benzylindole csddd 9036 inhibited > 50% of NBDS fluorescence (Emax 58.0 ± 0.6). The structurally similar cyclooxygenase inhibitor indomethacin weakly inhibited NBDS fluorescence, suggesting that inhibition of SCP-2 is unlikely to contribute to the anti-inflammatory or analgesic effects of this medication.

Taken together, these results add to our understanding of the structural features required for binding and inhibiting SCP-2. SCP-2 substrates are generally amphiphilic, with the polar head group tolerant of charged and hydrogen bond-donating groups and lipophilic tail groups that can adopt an extended conformation.

NAUSEA PRODUCED BY HIGH DOSE THC: ASSESSMENT OF PHARMACOLOGICAL TREATMENTS

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Δ^9-tetrahydrocannabinol (THC), a partial agonist of the cannabinoid 1 (CB_1) receptor of the endocannabinoid (eCB) system, is known to effectively treat nausea and vomiting in humans undergoing chemotherapy treatment, and in animal models of toxin-induced nausea and vomiting. Paradoxically, findings in animals, and the characterization of "Cannabinoid Hyperemesis Syndrome" (CHS) in humans, suggest that high doses of THC can also *produce* nausea and vomiting. It has been shown that THC produces dose-dependent conditioned gaping in the taste reactivity paradigm, a rat model of nausea. High doses of THC (5 and 10 mg/kg), but not a low dose (0.5 mg/kg) produced nausea in rats via its action on the CB_1 receptor. A dose of 10 mg/kg of THC also produces upregulation of the degrading enzyme of the eCB, 2- arachidonoyl glycerol, (2-AG), monoacylglycerol lipase (MAGL), in the hypothalamus. To further investigate the mechanism of THC-induced nausea, we tested the ability of several pharmacological agents to interfere with THC-induced conditioned gaping in male rats assessed by the taste reactivity test.

An increase in MAGL expression by THC indicates low levels of 2-AG in the hypothalamus, which may contribute to an overactive stress response, and subsequent nausea. Pre-treatment with an MAGL inhibitor (MJN 110; 10 mg/kg i.p.) interfered with the establishment of conditioned gaping by 10 mg/kg THC. The effect of inhibiting fatty acid amide hydrolase (FAAH), the degrading enzyme of the eCB anandamide (AEA), with URB 597 (0.3 mg/kg, i.p.) is currently being assessed. To investigate the role of stress-induced nausea, the ability of the β-adrenoreceptor antagonist, propranolol (2.5 and 5 mg/kg, i.p), to interfere with THC-induced conditioned gaping was evaluated. A dose of 5 mg/kg propranolol significantly reduced the amount of conditioned gaping produced by 10 mg/kg THC. These results support the hypothesis that THC-induced nausea is a result of an overactive stress response due to eCB alterations caused by excessive agonism of the CB_1 receptor. Typical anti-emetic drugs, such as ondansetron, a 5-HT_3 antagonist, do not alleviate symptoms of CHS and THC-induced nausea in humans. In rats, ondansetron (0.1 and 0.01 mg/kg, i.p.) did not interfere with THC-induced conditioned gaping either.

Current experiments are evaluating the ability of Cannabidiol (CBD), another phytocannabinoid, to interfere with THC-induced nausea. CBD has known anxiolytic and anti-nausea properties and is a modulator of serotonin transmission as a 5-HT_{1A} receptor agonist. CBD is also an agonist of the transient receptor potential vanilloid 1 (TRPV1) receptor, and capsaicin, another TRPV1 agonist, may be effective at alleviating THC-induced nausea in humans.

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SELECTIVE INHIBITION OF THE CANNABINOID RECEPTOR CB1 FOR THE TREATMENT OF INFLAMMATION AND FIBROSIS

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Cannabinoid receptor CB1 inhibition has been proposed as a promising therapeutic approach to treat metabolic diseases, whereas CB2 receptor activation has been proposed as a promising therapeutic approach for the treatment of inflammation and fibrosis. Our current lead drug candidate lenabasum is a selective CB2 agonist that shows anti-inflammatory and anti-fibrotic effects in animal models and human subjects. Lenabasum is currently in phase 3 clinical trials for treatment of systemic sclerosis and dermatomyositis and phase 2 for treatment of cystic fibrosis and systemic lupus. However, it remained to be determined whether CB1 inhibition can ameliorate inflammation and fibrosis, an effect that can be useful for treating metabolic diseases that are accompanied by inflammation and fibrosis, such as non-alcoholic steatohepatitis (NASH). To answer this question, we selected a compound from a library of rationally designed peripherally restricted CB1 inverse agonists. This compound (CRB-4001) displayed over 1000-fold CB1/CB2 binding selectivity and was shown to improve metabolic parameters in diet induced diabetic mice. We first confirmed that CRB-4001 is highly potent in both CB1-mediated cAMP and βarrestin2 assays (low and sub-nanomolar, respectively). Then, we evaluated the effect of CRB-4001 on the inflammatory response of human PBMCs. We found that CRB-4001 reduced the LPS-induced pro-inflammatory cytokines MCP-1, IL-1β, TNFα, IL-6, IL-12, IL-17 and IL- 31 in a dose dependent manner (low μM potency) without affecting IL-8. In the BioMAP® phenotypic profiling platform that utilizes twelve human primary cell-based disease models, CRB-4001 reduced the levels of several tissue remodeling biomarkers in fibroblasts, including PAI-1, TIMP-1, type I and type III collagens and αSMA. These changes were accompanied by decreases in pro-inflammatory cytokines as well as inhibitory effects on cell proliferation in multiple BioMAP systems. The anti-inflammatory and anti-fibrotic activities together with a low brain/plasma exposure ratio indicated that CRB-4001 is a promising candidate for NASH.

CHARACTERISATION OF AMB-FUBINACA: A BIASED AGONIST AND TOXIC SYNTHETIC CANNABINOID OF ABUSE

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The abuse of new psychoactive substances is an unfolding crisis in international public health. Of these substances, synthetic cannabinoids (SCs) are the most numerous and rapidly proliferating – in New Zealand alone, more than 45 deaths have been attributed to SCs in the 12 months from June 2017, with ambulance services receiving in excess of 30 callouts per week to attend SC-related emergencies. In New Zealand, the most abundant abused SC to date is AMB-FUBINACA (e.g. (1))

Little is currently known about SC mechanisms of action or toxicity. Certainly, the psychoactive effects of SCs are mediated by the CB1 cannabinoid receptor, but activity in pathways downstream of receptor activation has not been systematically investigated. In the current study, we set out to characterise the activity of AMB-FUBINACA in common signalling pathways, to attempt to understand whether the toxicity is associated with biased activity (a "molecular fingerprint") relative to traditional cannabinoids such as THC. Pathways assayed include cAMP inhibition (method: real-time BRET/CAMYEL), pERK signalling (PerkinElmer AlphaLisa), receptor internalisation (live immunocytochemistry), and translocation of β-arrestins 1 and 2 (BRET).

While both THC and AMB-FUBINACA demonstrated largely equivalent efficacy in inhibiting cAMP, AMB-FUBINACA efficacy was greater than THC in both pERK and arrestin pathways, and potency was considerably greater in all pathways assayed. In contrast, while THC failed to elicit an arrestin translocation response, AMB-FUBINACA did so with high potency – suggesting that AMB-FUBINACA may be biased toward β-arrestin pathways. Further study is necessary to investigate both the roles of β-arrestins as CB1 effectors, and whether arrestin pathways have a role in the abuse and toxicity observed for AMB-FUBINACA and other SCs.

References:

(1) Stuff report: https://www.stuff.co.nz/national/health/96860356/the-ultrapotent-zombie-drug-ambfubinaca-has-come-to-town?rm=m, retrieved 23rd April 2019.

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COMPOUNDING OF CANNABIS PRODUCTS WITH SPECIFIC PLANT DERIVED ESSENTIAL OIL CONSTITUENTS FOR TARGETED THERAPIES

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Within today's cannabis industry a majority of formulated products are marketed and produced to be as true to the whole plant as possible while others take the plant derived extracts and reformulate them for different potencies and cannabinoid ratios. The ultimate goal of these varied potencies and ratios is for differing therapeutic effects and treatments. It is no secret that different cannabinoids hold different therapeutic benefits as well as the fact that many of these cannabinoids work in concert together, just like the therapeutic effects of other non-cannabis derived plant constituents. The addition of plant derived essential oils are to aid in the effect of the product. A cannabis product can be formulated with essential oils from lavender, for its calming effects, or citrus oils to improve attention or alertness. We at Constance Therapeutics have been working to build our formulation knowledge and expand our capabilities, to reach a broader range of ailments and increase efficacious results with formulations containing plant-derived essential oils and plant-derived cannabinoids.

One example of plant-derived essential oils is α-cedrene, commonly found in different types of cedarwood and previously shown to be cytotoxic to human leukemia HL60 cells with an IC50 of 22.20 μg/mL. Compounding cedarwood oil containing α-cedrene into a cannabis product along with other constituents and plant derived essential oils that show similar efficacious results can provide an improved cannabis-based therapy. By definition this falls under what is considered compound pharmaceuticals.

Safety is of utmost importance, especially when considering many previous formulation efforts have only utilized an isolated form of a constituent. Many plants that contain a beneficial constituent could also contain other harmful constituents, especially when not properly kept at or below certain safe quantities for use in ingestion or inhalation. The proper storage procedure and safety surrounding oxidation of certain constituents is also an important consideration. These factors are what lend to the formulating process being far more complicated due to the vast amount of variables associated with utilizing plant derived extracts and essential oils rather than working with isolated constituents.

APPLYING MACHINE LEARNING TO ENDOCANNABINOID TARGETS WITH ASSAY CENTRAL

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Collaborations Pharmaceuticals, Inc. aims to streamline the development of drugs for rare and neglected tropical diseases using our core technology, Assay Central. Assay Central is a collection of predictive Bayesian and Random Forest models in a self-contained executable for non-experts to evaluate the likelihood of activity at a target of interest. Using data available in the public domain such as ChEMBL and PubChem, we are able to expand our expertise to the endocannabinoid system.

Using machine learning models of relevant receptors (CB1/2, GPR55, GPR18, GPR119) we are able to predict target-specific lead compounds, as well as molecular properties, and potential *in vivo* liabilities. Deployment of models within a custom bundle to collaborators facilitates a seamless feedback loop and allows models to constantly be improved with new data. Visualization of active molecular features as well as insights into the training set data compliments human intuition to drive intelligent drug discovery.

Our business model is both scalable and replicable and applying it to the cannabinoid field is a natural step. This method aims to reduce superfluous synthesis, and by combining forces with many academic collaborators we have already leveraged a broad, collective expertise to identify treatments for parasites, bacteria, and viruses. We hope to develop new lead compounds for the treatment of rare pediatric diseases, such as West, Dravet and Lennox-Gastaut syndromes.

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STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF CB_1 PAM 2-PHENYLINDOLE SCAFFOLD

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Positive allosteric modulators (PAMs) of the cannabinoid type-1 (CB_1) receptor have shown promise in preclinical models of pain (e.g. ZCZ011, GAT211)[1,2] while producing minimal undesirable cannabinoid effects which typically occur with orthosteric ligands. Mechanisms through which CB_1 PAMs may be producing these effects through the CB_1 receptor include 1) enhancement of endogenous cannabinoid signaling via positive binding cooperativity (α), a bidirectional vector through which both ligands increase the affinity of the other for the receptor; 2) promotion of receptor conformations through which endogenous cannabinoids can exert enhanced efficacy (β); and/or 3) allosteric agonism in which the modulator activates the receptor in the absence of orthosteric ligand (τ_B). These can be observed when an allosteric modulator is co-applied in an agonist concentration response curve as 1) leftward shifts in the agonist pEC_{50}; 2) increases in E_{max}; and 3) increases in the bottom of the concentration-response curve (i.e. apparent increase in basal signaling)[3].

In order to examine how structure may impact these three allosteric properties, SAR studies were conducted using the 3-(2-nitro-1-phenylethyl)-2-phenylindole scaffold with a focus on substitutions of the nitro and phenyl groups on the 3-ethyl moiety. Concentration-response curves for the CB_1/CB_2 agonist CP55,940 were carried out in the absence or presence of 10 μM of each analogue in [^{35}S]GTPγS, [^{3}H]CP55,940 or [^{3}H]SR141716 binding assays.

Many of the compounds tested retained allosteric activity as determined by leftward shifts in CP55,940's pEC_{50}, increases in its $E_{max,}$ and/or increases in [^{3}H]CP55,940 binding. The percent increase in [^{3}H]CP55,940 binding by the compound predicted shifts in the bottom, [$p<0.001$; $r=0.90$ (0.81 – 0.95)], pEC_{50} [$p<0.001$; $r= 0.91$ (0.83 – 0.95)] and E_{max} [$p<0.001$; $r= 0.59$ (0.32 – 0.78)] parameters of CP55,940's concentration-response curve. An interesting pattern emerged from substitutions on the 1-phenylethyl group where ortho- substitutions enhanced alpha, meta-substitutions were mostly tolerated, but para- substitutions reduced alpha, suggesting that the conformation of the phenyl ring plays a significant role in how a ligand induces allostery and/or potentially interacts with the orthosteric site. Carbamate substitutions for the nitro group promoted NAM-like activity as CP55,940 pEC_{50} values were mostly right-shifted by these compounds, particularly with inclusion of para-substitutions on the 1-phenylethyl group.

These data provide insight into structural elements of the 2-phenylindole scaffold that could enhance allosteric parameters and/or affinity at the CB_1 receptor.

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1. Ignatowska et al. (2015). Neuropsychopharm, 40, 2948-2959.
2. Slivicki et al. (2018). Biol Psychiatry, 84(10):722-733
3. Kenakin, TP & Miller, LJ (2010). Pharmacol Rev, 62(2), 265-304.

PERIPHERAL CB_1 RECEPTOR BLOCKADE REDUCES VOLUNTARY ALCOHOL DRINKING BY INHIBITING THE FORMATION OF BIOLOGICALLY ACTIVE GHRELIN THROUGH A CB_1-DEPENDENT FATTY ACID OXIDATION IN THE STOMACH AND ITS SIGNALLING VIA GASTRIC VAGAL AFFERENTS IN MICE

Grzegorz Godlewski*[1], Resat Cinar[1], Nathan J. Coffey[1], Jie Liu[1], Tony Jourdan[1], Ozge Gunduz Cinar[2], Bani Mukhopadhyay[1], Lee Chedester[1], Ziyi Liu[1], Douglas Osei-Hyiaman[1], Malliga R. Iyer[1], Joshua K. Park[1], Roy G. Smith[3], Hiroshi Iwakura[4] and George Kunos[1]

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Endocannabinoids acting via CB_1 receptors (CB_1R) or the stomach-derived hormone ghrelin acting via ghrelin receptors ($GHS\text{-}R_{1A}$) are both known to promote food intake and alcohol seeking behaviors.

We report that the peripherally restricted CB_1R inverse agonist JD5037 reduces ethanol drinking when given orally, but not i.c.v. in a dose that elicits anxiogenic response in the elevated plus maze. Oral administration of JD5037 also inhibited the formation of biologically active octanoyl-ghrelin without affecting its inactive precursor desacyl-ghrelin. In stomach-derived ghrelin-producing MGN3-1 cells, JD5037 decreased the level of the substrate octanoyl-carnitine generated from palmitoyl-carnitine due to increased fatty acid β-oxidation of palmitic acid in control MGN3-1 cells but not in cells with shRNA-mediated knockdown of Cnr1. Using CRISPR-Cas9 we selectively deleted the membrane-bound O-acyltransferase domain containing 4 (Mboat4) gene in mice, which encodes ghrelin-O-acyl transferase (GOAT), the enzyme responsible for ghrelin acylation. Plasma levels of acyl-ghrelin were undetectable in $Mboat4^{-/-}$ mice whereas desacyl-ghrelin levels were similar in $Mboat4^{-/-}$ and its wild type counterparts, and JD5037 failed to affect voluntary alcohol intake in the mutant strain. Blocking gastric vagal afferents by capsaicin or subdiaphragmatic vagotomy also abrogated the ability of either CB_1R or $GHS\text{-}R_{1A}$ blockade to reduce ethanol drinking. We conclude that blocking CB_1R in ghrelin-producing cells reduces alcohol drinking by inhibiting the formation of biologically active ghrelin and its signaling via gastric vagal afferents. Thus, peripheral CB_1R blockade affecting the gut-brain axis may have therapeutic potential in alcoholism.

VARIABILITY AND PAUCITY OF MEDICALLY RELEVANT CANNABIS PRODUCTS IN STATE REGULATED CANNABIS RETAIL DISPENSARIES

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An accretion of evidence supports the inclusion of cannabidiol (CBD) in medical cannabis and cannabis-derived products. CBD has known analgesic, anti-inflammatory, anti-seizure, and anti-tumor properties, and has been shown to offset the negative psychotropic side effects of THC. Recent studies have demonstrated that hemp-derived CBD products purchased over the internet are frequently mislabeled, contaminated, or are outright fraudulent (contain no cannabinoids whatsoever). Therefore, patients are more likely to receive medical benefit from products that are routed through state-licensed cannabis markets, where laboratory testing for CBD content is required. This work aims to characterize the extent to which medically beneficial, CBD-containing products are available to consumers in states with medical cannabis laws. Data were collected by analyzing online menus for cannabis retail dispensaries throughout the United States. Despite the obvious medical benefits, the availability of CBD-containing products in state-licensed cannabis retail stores is highly variable and surprisingly sparse. For example, in one Pennsylvania dispensary, only 39 of 196 products (20%) contained CBD. These results highlight the need for expanded patient access to CBD products.

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TRANSCRIPTOME-LEVEL ANALYSIS OF CBD-MEDIATED NEUROPROTECTION IN A SEROTONERGIC CELL LINE

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Despite being the third leading cause of death in the United States, there are currently no FDA-approved drugs to promote neuroprotection following a stroke injury. Recent studies have identified potential neuroprotective effects of cannabidiol (CBD) in neonatal pig models of hypoxia. However, in multiple cell models of hypoxic injury on pure neuronal populations, CBD did not have robust neuroprotective effects. This suggests that CBD-mediated neuroprotection may be mediated by indirect interaction with neurons. In the current experiments, we implemented next-generation sequencing (NGS) to identify changes in gene expression in a neuronal cell line in response to CBD. Cells were exposed to CBD at 1uM and 10uM for 24hrs in the presence or absence of cobalt chloride, a chemical inducer of hypoxia-related gene expression. Additionally, to further elucidate the signaling mechanisms of CBD, gene expression was also measured in the presence of a selective 5HT-1A receptor antagonist. Data will demonstrate the effect of CBD on survival, as measured by MTT, and the differential expression profiles of neurons in response to two physiologically relevant concentrations of CBD.

EVALUATION OF THE RESPIRABLE FRACTION OF CANNABIDIOL METERED DOSE INHALERS

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According to the National Organisation for the Reform of Marijuana Laws (NORML) cannabis is currently legalised in 11 states in the US and legal for medical use in 34 (including the completely legal states) while medical cannabidiol (CBD) laws are present in 17 states (four of which have laws on medical cannabis). Besides smoking, dry powder inahalers (DPIs), nebulisers and pressurised metered dose inhalers (pMDIs) offer alternative ways to administer cannabinoids to the lungs. However, it is unknown if these devices are able to administer a medically effective dose to the lungs in a user-friendly way.

CBD has been reported to show anti-nausea, anti-inflammatory, anti-psychotic, anticonvulsive, sedative, hypnotic and anxiolytic effects. For seizure disorders (2400 mg) and psychosis (800 mg) daily doses have been established. Exact doses are, however, unknown for the other effects. In other indications 5 – 20 mg/day (orally, two to three doses) are sufficient to obtain benefits for many patients (MacCallum & Russo, Eur. J. Intern. Med. 49 (2018) 12-19). Due to the inhaled bioavailability of CBD being about twice as high as the oral bioavailability (31 % vs 16 % (Scuderi et al., Phytother. Res. 23 (2009) 597-602)) the corresponding daily dose would be 2.5 – 10 mg being divided into three doses (0.83 – 3.33 mg per single dose).

As pMDIs are already commercially available for recreational purposes (i.e. Mystabis® and Aeroinhaler®), the aim of this study is the evaluation of the fine particle fraction (FPF) of different pMDI formulations to evaluate their suitability for medical purposes. Aforementioned recreational pMDIs contain the following three ingredients: HFA 134a (propellant), ethanol (solvent/surfactant) and cannabis oil/extract. The Aeroinhaler® emits 100 µL of formulation per puff, containing approximately 5.0 mg of active ingredient (THC/CBD) (product information of the Aeroinhaler®). Revealing that the CBD concentration in the formulation is about 50 µg/µL. Three formulations were prepared based on the recreational preparations to evaluate their medical potential; though, pure CBD was used instead of cannabis oil or an extract. The CBD concentrations of the formulations were 10, 30 and 50 µg/µL. The aerodynamic performance of an aerosol formulation is heavily dependent on the orifice diameter of the device; therefore three different actuators (orifice diameters of 0.22 mm, 0.30 mm and 0.42 mm) were used to administer the aerosol to the fast screening impactor (FSI; Copley Scientific, Nottingham, UK).

Table 1: Mean percentage of respirable aerosol (< 5 µm), n = 3

	0.22 mm orifice	0.30 mm orifice	0.42 mm orifice
10 µg/µL	27.4 %	36.0 %	39.0 %
30 µg/µL	27.9 %	36.9 %	37.0 %
50 µg/µL	25.5 %	29.4 %	23.4 %

It can be seen that a larger orifice diameter is beneficial for the used dosing valves. Data shows that only a relatively small amount of the emitted CBD is actually reaching the lungs. The formulations emit following mean doses/shot (50 µL valve): 10 µg/µL: 528.3 µg; 30 µg/µL: 1543.9 µg; 50 µg/µL 2119.6 µg). Concluding it can be said, that pMDI formulations need further optimisation to make them suitable for CBD inhalation in a medical setting.

AN EPIGENETIC ANALYSIS OF THE EFFECTS OF A SYNTHETIC CANNABINOID

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Traumatic brain injury (TBI) has been shown to be one of the leading causes of death and disability worldwide. The main cause of post-TBI complications is neuroinflammation, the brain's immune response to pathological insults. This response is facilitated by cytokines, chemokines, prostaglandins, and other byproducts that participate in the inflammatory cascade. The early effects of inflammation have been shown to be beneficial, but prolonged inflammation leads to detrimental effects including neuronal cell death. Repeated TBI's throughout life can lead to the development of chronic traumatic encephalopathy (CTE), caused by long-term, constant neuroinflammation. A solution is needed to combat the short and long- term effects of TBIs and neuroinflammation. Cannabinoids are a chemical family that has been shown to be effective at lowering inflammation. Therefore, in this experiment we tested the chemical WIN 55,212-2, a synthetic cannabinoid, on HeLa cells in order to determine its potential anti-inflammatory effects.

We performed an epigenetic analysis, MeDIP Chip (Methylated DNA Immunoprecipation), in order to better understand the anti-inflammatory effects of WIN 55,212-2, mesylate at the gene expression level.

Several hundred genes were altered as a result of the treatment, and major gene groups affected are involved in inflammatory responses, and, surprisingly, epigenetic control. This latter result was unexpected and we are further analyzing the data. Overall, the hypothesis of the involvement of the synthetic cannabinoid in inflammation processes was validated, but raises new questions about the mechanism of action of WIN 55,212-2 mesylate, and possibly other cannabinoids.

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CB1 MUTATIONS TO TEST THE RELEVANCE OF TARANABANT CONTACT WITH N-TERMINAL RESIDUES F102 AND M103 IN CB1 CRYSTAL STRUCTURE

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Receptor regions in GPCR x-ray crystal structures, particularly N-termini, may have unexpected contacts with the ligand binding pocket that are not physiologically relevant, such as His54 in the mu-opioid active state with agonist BU72 (Huang et al., Nature, 2015). In the x-ray crystal structure of Taranabant bound to the inactive state of CB1, the N-terminus penetrates deeply into the orthosteric pocket, with residues F102/M103 directly contacting the co-crystallized ligand (Shao et al., Nature, 2016). We undertook a combined mutation and modeling study to test the importance of F102, M103 to the binding of Taranabant. Binding studies used [3H]-CP55,940. The M103G, M103A mutations had no effect on Taranabant binding, while F102A had a 5-fold effect. Modelling studies were performed using the crystal structure (PDB-ID:5U09) to create each of the mutants, energy minimize with Prime (Schrödinger), and predict per residue interaction energies with Taranbant (30 iterations/10K steps; target gradient 0.1kcal/mol; implicit membrane depth 31.6Å). The mutation from M103 to G103 had a 10.0kcal/mol loss of interaction energy with Taranabant, a large change that is not reflected in the binding data above. When 103 was changed to A103, the loss was 6.7kcal/mol, this significant loss of interaction energy is also not reflected in the binding data. The mutation from F102 to A102 only lost 0.1kcal/mol in interaction energy, too small a change to be consistent with the five-fold loss in the binding studies. These results suggest that residues F102, M103 are not in direct contact with Taranabant in the CB1 inactive state. [Support: NIDA DA003934 and School of Medical Sciences, University of Auckland]

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF POSITIVE ALLOSTERIC MODULATORS OF THE CANNABINOID CB_1 RECEPTOR

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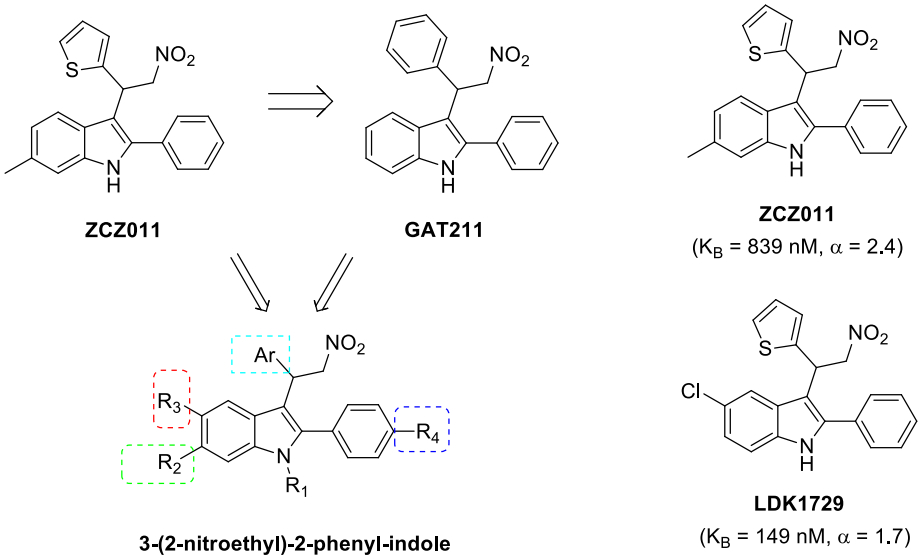
CB_1 allosteric modulators hold tremendous potential to improve drug discovery targeting the endocannabinoid system. To date, positive allosteric modulators (PAMs) as well as negative allosteric modulators (NAMs) of the CB_1 receptor have been disclosed. The CB_1 PAM 3-(2-nitroethyl)-2-phenyl-indole ZCZ011 potentiated CB_1 agonist binding and decreased binding of inverse agonists. ZCZ011 also reduces nociceptive behavior in mouse neuropathic and inflammatory pain models through a CB_1 receptor dependent mechanism, without eliciting psychoactive effects commonly produced by CB_1 orthosteric agonists. GAT 211, a homologous analog of ZCZ011, similarly suppresses nociceptive behavior in inflammatory and neuropathic pain models. These findings suggest that CB_1 PAMs derived from the 3-(2-nitroethyl)-2-phenyl-indole scaffold may offer an innovative template for developing new analgesic agents directed at putative allosteric sites of action. To optimize the 3-(2-nitroethyl)-2-phenyl-indole scaffold, we carried out an SAR study, which was driven by the *in vitro* binding parameters of the newly synthesized compounds (i.e. the equilibrium dissociation constant, K_B, and the binding cooperativity, α). Selected compounds were further assessed for their allosteric modulation of the CB_1 receptor in *in vivo* assays of cannabimimetic activity. Initial SAR studies indicate that the substitution on the indole ring critically impacts the pharmacological effects. Through this SAR study, we identified several analogs (e.g. LDK1729) that show promising allosteric modulation.



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GPR6: MODEL DEVELOPMENT, DRUG DESIGN AND SYNTHESIS

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G protein coupled receptor 6 (GPR6) is an orphan Class A GPCR that belongs to the MECA cluster which includes the CB1 and CB2 receptors. GPR6 is predominantly expressed in the central nervous system. One critical feature of GPR6 is that it exhibits quite high constitutive activation of adenylyl cyclase. Dr. Zhao-Hui Song's group has shown that phytocannabinoids such as cannabidiol and cannabinoid antagonists, including SR144528, have GPR6 activity. As demonstrated by several research groups, GPR6 represents a possible target for the treatment of neurodegenerative disorders such as Parkinson's disease, Alzheimer´s disease, and Huntington's Disease. Diminishing the constitutive signaling of GPR6 as a treatment for Parkinson's disease and other dyskinesia syndromes is currently being pursued by several pharmaceutical companies. Currently, GPR6 patents have focused on pyrazine derivatives.

At present, there have been no GPR6 X-ray, cryo-EM or NMR structures published. The goal of the present study was to develop a human GPR6 homology model to gain insights into unique structural features of GPR6. This should help us to elucidate the structural determinants governing ligand-receptor interactions at GPR6 and help us to design novel GPR6 chemotypes that can be synthesized and pharmacologically evaluated.

Our inactive state model of hGPR6 was developed using a suite of computational techniques with the X-ray structure of the sphingosine-1-phosphate receptor 1 (S1P1) used as a template. The most potent GPR6 pyridopyrazine inverse agonists from the patents were docked in the resultant GPR6 inactive state model, to test the model and identify the key residues in the binding crevice for ligand recognition. A subset of these compounds was used as starting points for the design of novel potent GPR6 inverse agonists using a Core Hopping approach (integrated in the Maestro software). In this process, we took into consideration the importance of retaining crucial interactions that keep the receptor in its inactive state, while building in additional interactions with the receptor. The candidate ligands were identified according to visual inspection, ranking of docking scores, synthesizability and promiscuity moiety filters. The best candidates were chosen to be synthesized following an efficient synthetic route that will enable derivatization in order to generate a rational SAR with new scaffolds. Cell based assays for cyclic AMP (cAMP) production, beta-arrestin-1 (βArr-1) and βArr-2 recruitment will be used to evaluate the synthesized compounds.

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INHIBITORY CONTROL DEFICITS ASSOCIATED WITH UPREGULATION OF CB_1R IN THE HIV-1 TAT TRANSGENIC MOUSE MODEL OF HAND

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In the era of combined antiretroviral therapy, HIV-1 infected individuals are living longer lives; however, longevity is met with an increasing number of HIV-1 associated neurocognitive disorders (HAND) diagnoses. The transactivator of transcription (Tat) is known to mediate the neurotoxic effects in HAND by acting directly on neurons and indirectly via its actions on glia. The Go/No-Go (GNG) task was used to examine HAND in the Tat-transgenic mouse model. The GNG task involves subjects discriminating between two stimuli sets in order to determine whether or not to inhibit a previously trained response. Data reveal inhibitory control deficits in female Tat(+) mice ($p = .048$) and upregulation of cannabinoid type 1 receptors (CB_1R) in the infralimbic (IL) cortex in the same female Tat(+) group ($p < .05$). A significant negative correlation was noted between inhibitory control and IL CB_1R expression ($r = -.543$, $p = .045$), with CB_1R expression predicting 30% of the variance of inhibitory control ($R^2 = .295$, $p = .045$). Furthermore, there was a significant increase in spontaneous excitatory postsynaptic current (sEPSC) frequencies in Tat(+) compared to Tat(-) mice ($p = .008$ across biological sex). The increase in sEPSC frequency was significantly attenuated ($p < .001$) by bath application of PF3845, a fatty acid amide hydrolase (FAAH) enzyme inhibitor. Overall, the GNG task is a viable measure to assess inhibitory control deficits in Tat-transgenic mice and results suggest a potential therapeutic treatment for the observed deficits with drugs which modulate endocannabinoid enzyme activity.

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FUNDAMENTAL PARTITIONING RELATIONSHIPS OF COMPOUNDS INDICATIVE OF CANNABIS PLANTS OR CANNABIS EXPOSURE

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Passive air samplers and breath collection devices in which an air (or breath) sample equilibrates with an adsorbent material (sorbent) require partition coefficients to quantify the original concentration of the compound(s) of interest. Cannabis plants grown for medicinal or adult recreational use can be distinguished from hops (a similar aromatic crop) and from cannabis plants grown for industrial/nutritional use (hemp) by three sesquiterpenes with the formula $C_{15}H_{24}$: alpha-santalene, valencene, and beta-bisabolene (Wiebelhaus et al. *Forensic Chemistry* **2** (2016) 1-8). Cannabis plants can also be distinguished by two cannabinoids with the formula $C_{21}H_{30}O_2$: tetrahydrocannabinol and cannabidiol. However, these larger compounds have extremely low vapor pressures (Lovestead et al. *Forensic Chemistry* **5** (2017) 79-85) and only small quantities will be captured at ambient temperatures. Capturing unique, more volatile compounds may enable reliable non-invasive detection of illegal possession or recent use. Our ongoing work focuses on characterizing sorbent materials for the capture and release of terpenes and cannabinoids. We present our research on polydimethylsiloxane, which can be coated onto glass fibers and has been used to capture analytes from breath, and activated carbon, which has a long history of use in personal exposure badges, in this poster.

We determined polydimethylsiloxane – air partition coefficients ($K_{PDMS/AIR}$) at temperatures from 60 °C to 200 °C by combining previously reported Kovats' retention indices with isothermal gas chromatography retention time measurements. Our long-term goal is to accurately predict $K_{PDMS/AIR}$ for compounds of interest (hydrocarbons and oxygen-containing hydrocarbons) without any experimental input. We developed a group contribution modelling approach that incorporates first-order chemical structure as described by 18 functional groups to predict $K_{PDMS/AIR}$ as a function of temperature. Our preliminary model was built from 275 compounds ranging in size from C_6 to C_{14}. $K_{PDMS/AIR}$ predictions correlated well with experimental data ($R^2 = 0.984$), but also revealed gaps in the training set. We will present an expanded model built from 350 chemicals ranging in size from C_6 to C_{16} and $K_{PDMS/AIR}$ predictions for terpenes and other compounds not included in the training set.

We have also developed a systematic approach to investigate the effect of mixtures on partitioning. To directly probe competitive adsorption, we created nuclear magnetic resonance spectroscopy samples in which an adsorbent (activated carbon strip) is added to the sample tube along with a flame sealed capillary containing water. The capillary acts as a standard intensity (or peak area), allowing us to observe the adsorption of multiple species simultaneously. We developed the method with a simple 50:50 mol% methane:propane mixture pressurized to 0.05 MPa. Preliminary experiments indicated that methane does not adsorb to the carbon strip in the presence of propane, at least under our experimental conditions. We are currently investigating pure, binary, and ternary mixtures of alpha-pinene, caryophyllene, limonene, and myrcene.

LASTING DECREASE OF CORTICOSTRIATAL COHERENCE IN RATS AFTER ACUTE EXPOSURE TO VAPOURIZED Δ^9-TETRAHYDROCANNABINOL

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Introduction: Over 14% of Canadians use cannabis, with nearly 60% of these individuals reporting daily or weekly use. Little is known about the differential effects of varying routes and frequency of cannabis use on brain and behaviour. In this study, we assessed changes in neural circuit dynamics in rats exposed to vapourized Δ9-tetrahydrocannabinol (THC). We hypothesized that THC would reduce coherence between cortical and striatal brain regions.

Methods: Sprague-Dawley rats were implanted with electrode arrays targeting the prefrontal cortex (PFC), orbitofrontal cortex (OFC), ventral hippocampus (vHIP), and dorsal striatum (dStr). Rats were administered THC using a Volcano® vapourizer and monitored in a plexiglass chamber.

Results: Decreased power was observed within the PFC, OFC, vHIP, and dStr in the gamma range (30-100 Hz) following vapourized THC administration. Specifically, a 20% to 35% decrease was observed in the low gamma frequency band, whereas a 40% to 50% decrease was observed in the high gamma frequency band. The changes in low gamma remained after 1 week, indicating that the effect of acutely administered, vapourized THC may be long-lasting. Comparing vHIP theta frequency signal to dStr gamma frequency signal revealed that there was a decrease in gamma power that preceded a peak in vHIP power, following THC administration.

Conclusion: Vapourized THC exposure led to acute neurophysiological changes consistent with some of the known effects of cannabis and symptomatic electrophysiological activity in patients with schizophrenia. Further cognitive and behavioural testing must be performed to determine whether this electrophysiological hypofunction contributes to the cognitive changes characteristic of cannabis use and schizophrenia.

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THE CB_1 POSITIVE ALLOSTERIC MODULATOR ZCZ011 BLOCKS Δ^9-THC WITHDRAWAL IN MICE

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Despite recent increases in attention to cannabinoid use disorders, there remains a need for effective pharmacological interventions to complement behavioral therapy. ZCZ011 is a CB_1 positive allosteric modulator that increases the effect of CB_1 agonists bound at the orthosteric site. CB_1 positive allosteric modulators avoid common effects of exogenous cannabinoid agonists, including sedation and cognitive deficits. We hypothesized that ZCZ011 significantly attenuates behavioral signs of cannabinoid withdrawal. Equal numbers of male C57BL/6J mice were administered Δ^9-THC (10 mg/kg, b.i.d., s.c.) or vehicle for six days, then withdrawal was precipitated using the CB_1 selective inverse agonist rimonabant (3 mg/kg, i.p.). As previously reported, rimonabant-precipitated Δ^9-THC withdrawal induced paw tremors and head twitches. Acute ZCZ011 (≥10 mg/kg, i.p.) significantly attenuated both paw tremors and head twitches. ZCZ011 (≥10 mg/kg, i.p.) also attenuated paw tremors and head twitches induced by spontaneous Δ^9-THC withdrawal (i.e., 36 hr of abstinence after 6 days of Δ^9-THC exposure). The locomotor effects of ZCZ011 were tested, and the observed reduction in somatic signs of withdrawal were not due to sedative effects. Because ZCZ011 is a racemic mix, the individual enantiomers were also probed. These data support the use of CB_1 positive allosteric modulation as a strategy to reduce the psychological effects of cannabinoid dependence.

CHEMICAL COMPOSITION-ACTIVITY ANALYSES OF 59 MEDICAL CANNABIS SAMPLES: A MEDICINAL CHEMISTRY APPROACH

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Medical cannabis is a natural product with a large variety of chemovars and/or genovars, essentially termed cultivars, and these contain a characteristic combination phytocannabinoids and other phytochemicals on their genetics. Medical cannabis users in Canada can obtain any variety of these medical cannabis products with a medical authorization from a physician and decide on a suitable product for themselves. Polypharmacy and polypharmacology of medical cannabis are complex concepts for an objective evaluation. We undertook the analyses of medical cannabis using a medicinal chemistry approach, with a slight twist: to understand any relationship between the chemical composition of these varieties/cultivars of medicinal cannabis and the corresponding receptor responses.

We collected 59 samples of medical cannabis which were extracted and were chemically analyzed to profile for 64 phytochemicals and followed by quantitation of the four major phytocannabinoids, Δ^9-THCA, CBDA, Δ^9-THC and CBD. These extracts were then evaluated in a cAMP functional assay for the agonist-like and antagonist-like responses against CB1 and CB2 receptors *in vitro*, obtaining four distinct biological activities (i.e. EC_{50} as an agonist and IC_{50} as an antagonist) for each medical cannabis extract. These biological activities were then correlated with the chemical composition of each medical cannabis extract and a partial-least squares (PLS) analysis was employed to derive correlation between the chemical compositions of the cannabis extracts and the resulting receptor responses. Chemical composition-receptor response relationship studies of medical cannabis will be presented with a prospective discussion on the predictability of biological activity using chemical composition.

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INTEGRATING CANNABIDIOL WITH MITOCHONDRIAL MODIFIERS IN THE TREATMENT OF PTSD AND PSYCHOSIS

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Recent clinical trials have indicated the efficacy of Cannabidiol (CBD) in the treatment of neuro-inflammation underlying both psychiatric and neurological Disorders. Maroon and Bost (2018) in a review of the neurological benefits of CBD state that "CBD appears to stimulate synaptic plasticity and facilitates neurogenesis that may explain its positive effects on attenuating psychotic, anxiety, and depressive behaviors." Separate research has elucidated the role that neuroinflammation, anti-oxidant stress and mitochondrial dysfunction plays in the etiology of schizophrenia and other psychiatric disorders.

To date, no one in the research community has suggested an integrated approach to the treatment of psychiatric disorders using an integrative model combining CBD with nutraceuticals to affect underlying conditions of neuroinflammation, oxidative stress, mitochondrial dysfunction and neurotransmitter deficits.

Two case studies from the author's practice will be used to elucidate the theoretical biochemical pathologies and the practical treatment approach to these psychiatric conditions. In both cases an integrated protocol was used to treat psychiatric conditions that had not responded well to conventional pharmaceutical therapy.

One patient with a history of psychosis was able to be weaned off her anti-psychotic medication which was producing deleterious side-effects using the integrative protocol. The second patient with PTSD and a diagnosis of a Bipolar/Borderline Personality Disorder and who had not responded well to either psychiatric medications or psychiatric institutionalization had a complete remission of her condition after 6 months of using the integrative protocol.

The integrative protocol consists of IV NAD+, CBD, oral liposomal glutathione, magnesium, SAM-E, phosphatidyl choline and other complementary mitochondrial nutrients.

MUTATIONAL ANALYSIS REVEALS BIASED SIGNALING AT THE CB1 CANNABINOID RECEPTOR

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The therapeutic potential of CB1 cannabinoid receptor agonists in various central nervous system disorders has long been hindered by negative side effects promoted by CB1 activation. Biased signaling may have the potential to refine cannabinoid therapeutics. Biased ligands could elicit therapeutic effects while inducing fewer adverse effects. The stabilization of the receptor in different conformational states can favor coupling to either G-proteins or β-arrestins. However, little is known about the CB1 receptor conformational state that promotes β-arrestin coupling. Molecular dynamics simulations of CB1 bound to a β-arrestin biased ligand suggests this conformational state involves an outward movement of the intracellular domain of transmembrane helix 7 and helix 8, accompanied by stabilization of Y7.53 Chi1 dihedral in the trans conformation. In order to elucidate the role of this conformation of Y.53 in β-arrestin coupling, we proposed amino acid mutations that are predicted to stabilize Y7.53 Chi1 dihedral in trans, increasing β-arrestin biased signaling: I2.43A, I2.43T and S7.57E. Wild-type (WT) and mutated human CB1 receptors were stably transfected into HEK293 cells, and signaling in response to 2-AG, an unbiased ligand, was analyzed.

Our first step was to evaluate ERK1/2 phosphorylation (pERK1/2), which can be induced by either G-proteins or β-arrestin. None of the mutations altered the potency or efficacy of 2-AG in pERK1/2. To evaluate $G\alpha_{i/o}$-independent signaling, we analyzed pERK1/2 in the absence or presence of pertussis toxin (PTx). All three mutations increased $G\alpha_{i/o}$-independent pERK1/2. In addition, 2-AG stimulated cAMP inhibition was significantly reduced in I2.43A and completely abrogated in S7.57E. We also evaluated receptor internalization and found that this response was unaltered in I2.43T and S7.57E, but increased in I2.43A. However, in the analysis of β-arrestin1 recruitment using a β-Galactosidase complementation assay, we found that ligand induced β-arrestin1 recruitment is reduced in I2.43T and S7.57E, but unaltered in I2.43A. We also evaluated $G\alpha_{q/11}$ signaling by measuring changes in intracellular calcium levels using Fura-2AM, and we found that neither WT nor mutated CB1 receptors increase intracellular calcium levels in response to 2-AG. These findings suggest that the I2.43A mutation induces β-arrestin 1 biased signaling, however I2.43T and S7.57E induce $G\alpha_{i/o}$-independent signaling that does not involve an increase in β-arrestin 1 recruitment. This indicates that stabilization of Y7.53 Chi1 dihedral in trans can bias CB1 signaling toward $G\alpha_{i/o}$-independent mechanisms. Further studies are required to identify the source of $G\alpha_{i/o}$-independent pERK1/2 induced by these CB1 mutations.

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DESIGN AND SYNTHESIS OF 3-AMINO-2-PHENYL INDOLE ANALOGS AS NOVEL ACHIRAL LIGANDS FOR ALLOSTERIC MODULATION OF THE CB1 RECEPTOR

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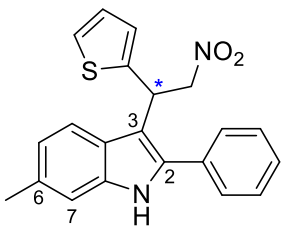
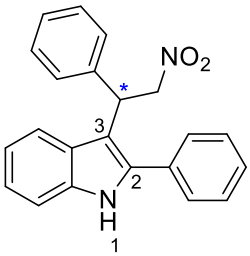
Previous *in vitro* and *in vivo* studies have shown that ZCZ011 is a positive allosteric modulator of the cannabinoid CB_1 receptor. The compound reverses nociceptive behaviors in murine models of neuropathic and inflammatory pain. Its antiallodynic effects depends on the CB_1 receptor. GAT211 is a close analog of ZCZ011. It was demonstrated to suppress the inflammatory nociception induced by complete Freund's adjuvant (CFA) and the neuropathic pain evoked by the cancer chemotherapeutic agent paclitaxel. While the CB_1 allosteric modulators represented by ZCZ011 and GAT211 showed promising pharmacological effects, both these modulators are racemic mixtures of two enantiomers. They possess a chiral center at the C10 position. Further investigations found that the two enantiomers of GAT211 behave differently. The (*R*)-enantiomer of GAT211 was reported to function as an allosteric partial agonist of the CB_1 receptor, while its (*S*)-enantiomer acts as a pure CB_1 positive allosteric modulator (PAM) with biphasic properties. These findings highlighted the need to develop achiral analogs of ZCZ011 and GAT211. Therefore, we designed and synthesized 3-aminao-2-phenyl-indole analogs with the aim to get rid of the chiral center from the structure. It was found that some of these novel analogs bind and modulate the CB1 receptor in a way similar to ZCZ011. Our synthesis and preliminary results in pharmacological characterization of this novel class compounds will be discussed.



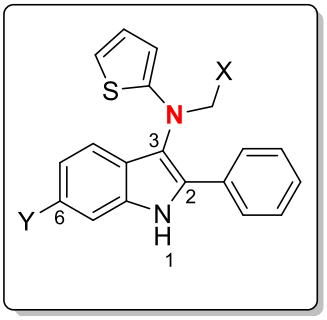
ZCZ011

racemate



GAT211

racemate



achiral

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A META-ANALYSIS ON THE EFFECTS OF CANNABIDIOL IN EXPERIMENTAL STROKE

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Background
Multiple *in vivo* studies indicate cannabidiol (CBD) improves outcomes in animal models of ischaemic-reperfusion injury. Given the accumulating pre-clinical evidence, a meta-analysis was undertaken to assess CBDs effects on *in vivo* models of focal ischaemic stroke.

Methods
Studies were systematically identified using Medline, Embase and PubMed. Infarct volume, neurological outcome and quality data were extracted. Data were analysed with Cochrane Review Manager using random effects models; results are expressed as either standardised mean difference (SMD) or mean difference (MD) with 95% confidence intervals [CI]. Publication biased was assessed using STATA.

Results
In focal ischaemic-reperfusion injury (8 articles, from 2 laboratories) induced by middle cerebral artery occlusion, CBD administered intraperitoneally in male ddY mice (age: N/A n: control 70, CBD 68) reduced infarct volume by a MD of 29.60mm^3 (Cl [34.94, 24.26], p<0.00001) and CBD administered via intracerebroventricular injections in adult male Wistar rats (age: 8-12 weeks, n: control 81, CBD 81) reduced infarct volume by a MD of 88.62mm^3 (Cl [101.98, 75.26], p<0.0001). CBD displayed a bell-shaped dose response curve in both species (range 0.83µg/kg-20mg/kg). Neuroscore evaluated sensorimotor functions as a marker of functional recovery. In 5 publications early neuroscore (24hrs) significantly improved in the CBD group (SMD -1.04, CI [-1.43, -0.65], p<0.00001; n: control 48, CBD 140), but later evaluations (336hrs, in mice only) revealed no significant improvement (SMD -0.92, CI [-1.96, -0.11], p=0.08; n: control 6, CBD 18). Publication bias was evident (Egger's statistic p=0.004) and median study quality was 2 (range 0-4/10).

Conclusions
CBD significantly reduces infarct volume and improved early functional outcome in experimental stroke in rodents. Further studies with longer follow up periods in aged, larger and female animal models of different species and with other co-morbidities are required.

Acknowledgements: RM is funded by an Artelo Biosciences PhD studentship.

THE PHARMACOLOGICAL EFFECTS OF SYNTHETIC OR PLANT-DERIVED CANNABIDIOL (CBD) ARE SIMILAR IN A RANGE OF HUMAN CELL LINES

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Introduction
Cannabidiol (CBD) can be derived from the Cannabis Sativa plant (Marijuana or Hemp) or synthetically produced. A number of studies have compared the efficacy of purified CBD vs. CBD rich extract, and have found differences in efficacy, generally referred to as the entourage effect. One published study has compared the efficacy of a synthetically produced CBD with a CBD rich extract (plant-derived), this however is not a like for like comparison, and has led some people to claim plant-derived CBD is superior to synthetically produced CBD (Gallily, Yekhtin, & Ondřej Hanuš, 2015). Therefore, the aim of this study was to compare the effects of purified CBDs to establish if there is any pharmacological difference in synthetic versus plant-derived compounds.

Methods
SkoV-3 cells (n=15, over 3 experiments) were used to assess CBDs (3 sources, 2 suppliers per source) anti-tumoural effect in proliferating or fully confluent cells (using resazurin assay). Pericytes (n=6, over 2 experiments) were treated with the CBDs and exposed to 4hrs oxygen-glucose deprivation (OGD) with IL-6 secretion and LDH activity measured as markers of inflammation. CaCo2 cells (n= veh 4, CBD 5, over 2 experiments) were treated with CBDs (10μM) and exposed to a 24hr inflammatory protocol (IFN-γ and TNF-α, 10 ng/mL) measuring transepithelial resistance (TEER) as a marker of intestinal barrier integrity. CBDs were compared to each other or vehicle using a one-way ANOVA or unpaired t-test. Data is presented as mean % change from vehicle.

Results
In proliferating SKOV-3 cells, all sources of CBD (10μm) significantly reduced proliferative capacity. In fully confluent SKOV-3 cells, all sources of CBD (50μm) significantly reduced resazurin metabolism by ~90%. In pericytes, all CBDs reduced post-OGD IL-6 increases to a similar extent. 4 out of the 6 CBDs tested significantly reduced LDH content 24hrs following OGD. In Caco-2 cells the 24hr inflammatory protocol lead to an average reduction of 24% in TEER. 4 of the 6 CBDs (10μm) tested significantly increased the speed and magnitude at which TEER recovered relative to its vehicle control. In each cell line and model, there was no statistical differences between CBDs.

Manufacturer (source)	Purity	Proliferating Skov-3 (resazurin, 48hrs, 10μM vs. veh)	Pericytes (IL-6, post-OGD, 100nM vs. veh)	Pericytes (LDH, 24hrs, 100nM vs. veh)	CaCo2 (TEER, AUC, 10μM vs. veh)
Medropharm (sativa)	98.6%	-35% ($p<0.0001$)	-68% ($p<0.05$)	-47% ($p<0.05$)	21% ($p<0.05$)
Flura fusion (sativa)	99.3%	-48% ($p<0.0001$)	-72% ($p<0.05$)	-50% ($p<0.01$)	10% (NS)
CBD depot (hemp)	98.7%	-40% ($p<0.0001$)	-75% ($p<0.05$)	-40% (NS)	9% (NS)
AliLab (hemp)	99.4%	-30% ($p<0.001$)	-72% ($p<0.01$)	-42% ($p<0.05$)	14% ($p<0.05$)
Logical (synthetic)	99.9%	-33% (<p 0.0001)	-69% ($p<0.05$)	-45% (NS)	20% ($p<0.01$)
THCpharm (synthetic)	99.9%	-31% (<p 0.001)	-57% ($p<0.05$)	-51% ($p<0.05$)	15% ($p<0.05$)

Conclusion These data suggest that there is minimal pharmacological difference between plant- and synthetically-derived CBD in human cell models when examining the anti-tumoral, anti-inflammatory, and anti-permeability effects of CBD.

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DEVELOPMENT OF A PERIPHERALLY RESTRICTED CB1 RECEPTOR ANTAGONIST FOR ALCOHOL INDUCED LIVER DISEASE

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Antagonists of peripheral type 1 cannabinoid receptors (CB1) have potential utility in the treatment of various diseases including alcoholic liver disease (ALD). Unfortunately, inhibition of CB1 receptors in the central nervous system (CNS) produces adverse effects including depression, anxiety and suicidal ideation. Otenabant is a potent and selective CB1 inverse agonist developed by Pfizer, but its advancement in clinical trials was halted because of its ability to penetrate the CNS and produce effects like rimonabant (2), a clinically approved drug that was eventually withdrawn. Structure-activity relationship (SAR) studies of otenabant would be useful in developing analogs with limited brain penetration and thereby, limited adverse effects. Recent crystal structures of hCB1 and docking studies with otenabant indicated that the piperidine group might be functionalized at the 4-position to access a binding pocket that could accommodate both polar and nonpolar groups. Therefore, we proceeded to examine the piperidine as a linker, which was functionalized with alkyl, heteroalkyl, aryl and heteroaryl groups using a urea connector. These studies resulted in orally bioavailable and peripherally selective compounds that were demonstrated to be potent inverse agonists of hCB1 with exceptional selectivity for hCB1 over hCB2. The lead compound from this series presented good ADME properties, clean selectivity profile against >40 high-risk receptor targets (SafetyScreen, Eurofins), and was advanced into *in vivo* efficacy studies in the Lieber DeCarli model of alcohol-induced steatosis. Once a day oral dosing with this lead compound blocked alcohol-induced liver steatosis in mice and reduced expression of several molecular biomarkers associated with hepatic inflammation. In conclusion, a promising peripherally selective CB1 receptor antagonist has been identified that is suitable for further clinical development for ALD and other disorders.

DEVELOPMENT OF AN INTRAOCULAR PRESSURE LOWERING, OPHTHALMIC FORMULATION CONTAINING THE Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC) PRODRUG, Δ^9-THC-VAL-HS (NB1111)

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The intraocular pressure (IOP) lowering activity of Δ^9-THC has been recognized since 1971, albeit through the systemic or inhalation route. However, development of a topical ophthalmic formulation of this very lipophilic molecule, that consistently and reproducibly reduces IOP, has been challenging. Over the last ten years we have developed a hydrophilic Δ^9-THC prodrug (Δ^9-THC-Val-HS; NB1111) with improved physicochemical characteristics. Several formulation approaches, including micellar solutions, lipid nanoparticles, melt-cast films and nanoemulsions were also investigated. Through various formulation and *in vitro* and *in vivo* preclinical studies, a combination of the prodrug and the nanoparticle or the nanoemulsion formulations were identified as the most promising formulations. Further refinement of the nanoemulsion formulation, through the selection of the type and concentration of the oil as well as the surfactant system and inclusion of Carbopol as a mucoadhesive and viscosity enhancer, was undertaken. The lead formulation consistently produced a drop in IOP in normotensive New Zealand white rabbits, following topical instillation. The maximum drop in IOP was about 20% from baseline and the duration of activity was more than 8h, outperforming commercial timolol and pilocarpine ophthalmic formulations. All animal studies were performed using University of Mississippi IACUC approved protocols.

The data suggests that NB1111 is a promising candidate for further development for the fight against glaucoma.

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POTENCY OF CANNABIS AND RELATED PRODUCTS IN THE USA DURING THE PERIOD 2008-2018

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Cannabis is the most widely used illicit drug in the world. Reports indicate that the potency of cannabis and cannabis preparations has been increasing worldwide. In the United States, through a nationwide potency monitoring program supported by NIDA, the University of Mississippi analyses cannabis samples seized by the Drug Enforcement Administration (DEA).

During this reporting period, 17,551 total cannabis specimens were analyzed from DEA laboratories, including 16,624 cannabis, 579 hashish, and 348 hash oil.

Our results indicate that the number of samples received over the last 5 years has decreased dramatically due to the legalization of marijuana either for medical or recreational purposes in many US states. The results also showed that the mean Δ^9-THC concentration has increased dramatically in cannabis over the period of this report, from 8.59% in 2008 to 16.12% in 2018. The mean Δ^9-THC:CBD ratio also rose substantially from 23 in 2008 to 113 in 2017 and then dropped to 57 in 2018.

The mean Δ^9-THC concentration of hashish samples has increased substantially from 21.96% in 2008 to 40.79% in 2017 and then dropped to 21.11% in 2018. There was a marked increase in the mean Δ^9-THC concentration of hash oil from 6.73% in 2008 to 58.02% in 2018. The implications of these trends will be discussed.

ACKNOWLEDGMENTS: The work was supported with federal funds from the National Institute of Drug Abuse (NIDA), National Institute of Health (NIH), Department of Health and Human Services, USA, under contract No. N01DA-15-7793.

INFLUENCE OF CHRONIC CANNABIDIOL ADMINISTRATION ON CARDIOVASCULAR PARAMETERS, ENDOCANNABINOID LEVELS AND OXIDATIVE STRESS IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS

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Cannabidiol (CBD), a non-psychotropic constituent of *Cannabis sativa L.* has been suggested to be beneficial in hypertension. **The aim of our study** was to examine the influence of chronic CBD administration on cardiovascular parameters, endocannabinoid levels and oxidative stress in spontaneously hypertensive (SHR) and normotensive Wistar Kyoto rats (WKY).

CBD 10 mg/kg or its vehicle was administered intraperitoneally once a day for two weeks. Cardiovascular parameters were measured in conscious animals telemetrically. Hearts and blood for functional and biochemical studies were isolated 24 hours after the final dose of CBD.
CBD did not modify blood pressure (systolic and mean) and heart rate that were higher in SHR than in WKY. In isolated hearts (Langendorff model) left ventricular pressure (LVP), the maximum rate of positive (inotropism) or negative (lusitropism) changes in LVP and RPP (rate-pressure product) were higher in SHR than in WKY and they were not changed by CBD. The inotropic and lusitropic (but not chronotropic) effects of isoprenaline (ISO; the non-selective β-adrenoceptor agonist) and stimulated by ISO decreases in coronary perfusion pressure (CPP) were diminished in SHR. CBD reduced the lusitropic and inotropic influences of ISO in SHR and WKY, respectively. In isolated left atria, the negative inotropic effect of the cannabinoid receptor agonist CP55940 was reduced in SHR. CBD did not modify basal force of contraction and positive inotropic effect of ISO, but impaired the negative inotropic effect of CP55940.

Hypertension was connected with the enhanced cardiac fatty acid amide hydrolase (but not monoacylglycerol lipase) activities and decreased cardiac (but not plasma) levels of anandamide, 2-arachidonoylglycerol and linoleoylethanolamide. The plasma (but not cardiac) levels of stearoylethanolamide (SEA) and dihomo-γ-linolenoyl-ethanolamide (DLE) increased. The cardiac and plasma levels of palmitoylethanolamide and oleoylethanolamide were not changed. CBD decreased the plasma level of SEA and DLE. Moreover, it reduced plasma and/or cardiac markers of lipid peroxidation (4-hydroxy-2-nonenal and 4-hydroxy-2-nonenal but not malondialdehyde) and protein oxidation (carbonyl groups).

Conclusions: We did not confirm that CBD might be useful in primary hypertension, since its two weeks administration did not diminished blood pressure in SHR. CBD only slightly improved the cardiostimulatory effects of β-adrenoceptor activation and changed plasma and/or cardiac endocannabinoid levels and markers of oxidative stress. However, other models of hypertension, CBD dosage and prolonged time of administration should also be examined.

Acknowledgement: Founded by the National Science Centre (Poland), research grant NCN 2015/19/B/NZ7/02270.

TOXICOLOGICAL PROFILE OF SELECTED TERPENOIDS PRESENT IN CANNABIS AND IN OTHER PLANTS – FOCUS ON BETA-CARYOPHYLLENE

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Introduction: The current development of new formulations based on *Cannabis sativa* (cannabis) extracts requires the manipulation of the chemical constituents of the different strains of the plant. Terpenoids, among them beta- caryophyllene (BCP), a dietary cannabinoid, are responsible for the aroma of cannabis and BCP is a CB2 ligand. They represent a useful group of compounds for modulating cannabinoid content of novel formulations targeting different systems of the body. Changes in the composition of the plant material may affect the toxicological profile of the formulation. Therefore, it is necessary to access the toxicity of these new formulations before developing them into commercial products.

Methods: Samples (1 lL) of essential oil were analyzed by gas chromatography/mass spectrometry (GC/MS) in a Hewlett Packard G 1800B GCD system with an HP-5971 gas chromatograph, with an electron ionization detector. The software used was GCD Plus ChemStation and the column was a Rtx 5MS Low bleed GC/MS column (30 m · 0.25 mm · 0.25 lm film thickness). For analysis, the column was kept at 50C for 4 min and then the temperature was programmed from 50C to 280C at 8C/min; inlet 250C; detector 280C; splitless injection/purge time 1.0 min; initial temperature 100C; and with initial time 4.0 min. The helium flow rate was 1 mL/min. Compound constituents were identified by comparison with standards and by the retention times, Kovats indices and by comparison with mass spectra from computerized libraries (HPCH2205, Wiley7N, and FENSC3).22 The terpenoids isolated by steam distillation as essential oil from each of the cannabis chemotypes gave the test samples whose toxicologic profile was characterized *in vitro*. Samples with different terpenoids content were tested for cell viability in RAW 264.7 cells. For each sample was tested 6 different concentrations (500, 250, 125, 62.5, 31.25 and 15.62 µg/ml) in quadruplicates. Control cells were treated with the same vehicle used to dilute the samples (Water or EtOH).

Results: Terpenoid content of different formulations did not alter the toxicological profile significantly.

Conclusion: This study has demonstrated an approach to assess cannabis toxicity profile based on their terpenoid profile. The different compositions of terpenoids vary according to their concentration in the formulation. The MTT assays showed that the terpenoids in different concentration did not present different toxicological profile even when the terpenoid content was doubled. The results point to a high degree of safety when adding different terpenoids including BCP in novel formulations based on cannabis.

AN ESTIMATION OF THE CB_1 RECEPTOR ANTAGONISM OF CANNABIDIOL ON α_1-ADRENOCEPTOR-MEDIATED CONTRACTIONS OF RAT VASA DEFERENTIA

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Electrical stimulation of the mouse or rat isolated vas deferens releases the cotransmitters ATP and noradrenaline that result in a short biphasic contraction; the first peak mediated by ATP acting at post-junctional $P2X_1$ receptors and the second by noradrenaline acting at post-junctional α_1-adrenoceptors. This bioassay has been helpful in determining the action of cannabinoids released endogenously (anandamide, 2-AG) and the pharmacology of cannabidiol. The aim of this study was to determine the actions of cannabidiol in the rat vas deferens under conditions of normal Mg^{2+} (1.2 mM) and where the complication of ATP release and its subsequent contraction is obviated in the presence of a selective $P2X_1$ receptor antagonist NF449 (Angus & Wright, Eur. J. Pharmacol. 769 (2015) 162-166).

Methods: Rat isolated vasa deferentia were mounted in organ baths in normal physiological salt solution containing NF449 10 μM and stimulated with a single electrical field pulse (SEFP; 150 V, 0.5 ms duration) every 30 min. The tissues were then bathed in either vehicle (DMSO 0.1%) or a concentration of cannabidiol (3, 10, 30 or 100 μM) for 1 h followed by a SEFP. Cumulative 0.5 log unit increments of the CB_1 agonist WIN55,212-2 (1-10,000 nM) were added at 30 min intervals following a SEFP. This protocol was repeated (in separate tissues) with the CB_1 receptor antagonist SR141716 (3-300 nM) replacing the cannabidiol pretreatment.

Results: In preliminary experiments we measured the peak responses to ATP- (phase 1) and noradrenaline- (phase 2) mediated contractions following a SEFP. In the presence of NF449, the contraction was increased by cannabidiol in a concentration-dependent manner (3-100 μM) with the noradrenaline-mediated contractions rising by up to 210% of the control (vehicle) contraction (100%). The EC_{50} of the cannabidiol effect was 10 μM. Subsequent additions of WIN55,212-2 (1-10,000 nM) decreased the contraction to zero. Despite the rise in contractions to cannabidiol, the concentration-response curves to WIN55,212-2 were right-shifted with a pK_B of 5.7 and aligned with the competitive model in the Clark analysis (i.e. slope 1 in Schild plot; Lew & Angus, Trends Pharmacol. Sci. 16 (1995) 328-337). Similarly, preincubation of separate tissues with the CB_1 antagonist SR141716 displaced the concentration-response curves to WIN55,212-2 to the right with an estimated pK_B of 7.8, without enhancing the contraction.

The rise in the SEFP-induced contraction in this assay, under our conditions of normal Mg^{2+} and ATP blockade, suggest that endogenous tissue anandamide or 2AG have an affinity for prejunctional CB_1 receptors that inhibit noradrenaline release. This background inhibition is antagonised by cannabidiol with an EC_{50} of 10 μM. In addition, the antagonism of the nerve response by WIN55,212-2 suggests in this assay that cannabidiol is acting as a competitive antagonist and the pK_B aligns with its K_i 4.4 μM in rat brain (CB_1). Alternate reports that cannabidiol is a negative allosteric modulator of CB_1 receptors in HEK293A cells gave poor estimates of pK_B in Schild analysis with minimal dextral shift of THC concentration-response but inhibition of Emax. We conclude that the functional rat vas deferens preparation is a viable bioassay for estimation of the pK_B of CB_1 receptor antagonists.

CANNABIDIOL BLOCKS THE HYPO-LOCOMOTIVE EFFECT OF Δ^9-THC THROUGH A GLUTAMATERGIC/DOPAMINERGIC-DISCRIMINATING MECHANISM

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Background: Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the two most prominent active molecules in cannabis. In humans, activation by THC of type-1 cannabinoid receptors (CB1R) in the central nervous system (CNS) elicits the classical intoxicated state associated to cannabis consumption known as "high". It has been proposed that concomitant administration of CBD can reduce the incidence of unwanted psychological side effects of acute THC such as paranoia, anxiety, or even psychosis. Although CBD has been characterized as a CB1R negative allosteric modulator *in vitro*, the molecular underpinnings responsible for this psycho-protective effect of CBD remain largely uncharacterized.

Objective and Methods: To further characterize the ability of CBD to modulate the effects of THC on the CNS, we investigated the combined effects of these molecules on two mechanistically distinct models of drug-induced hyperlocomotion by using i) MK-801, a N-methyl-D-aspartate (NMDA) receptor antagonist, and ii) D-amphetamine, a dopaminergic indirect agonist. Adult CD1 male mice pretreated with vehicle, CBD (1 and 10 mg/kg), THC (5 mg/kg) or a combination of both, were administered MK-801 or D-amphetamine and locomotor activity was subsequently recorded for 2 hours.

Results: THC was able to reduce spontaneous locomotor activity in control animals, as well as hyperlocomotion induced by both MK-801 and D-amphetamine, while CBD alone did not have any effect on locomotion. When combined, CBD was able to revert, in a dose-dependent manner, the hypo-locomotive effects elicited by THC in control animals and in animals treated with D-amphetamine but failed to do so in mice treated with MK-801.

Conclusion: Our results indicate that CBD selectively modulates the hypo-locomotive effect of THC through a mechanism that discriminates between glutamatergic and dopaminergic transmission. Further mechanistic experiments will help elucidate the implication of different potential mediators, such as CB1R or the serotonin receptor 5-HT (2A/2C).

THE PRESENCE OF CBD IN CANNABIS FLOWER MODERATES THE RELATIONSHIP BETWEEN BLOOD THC CONCENTRATION AND FATTY ACID AMIDE HYDROLASE (FAAH) GENOTYPES

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The endocannabinoid system (ECS) consists of lipophilic ligands (endocannabinoids (eCBs)) and cannabinoid receptors (CBRs) and involved in a diverse range of physiological processes. Essential to the ECS is fatty acid amide hydrolase (FAAH), an enzyme that catalyzes the hydrolysis of anandamide (AEA), an eCB and CB1 agonist. A single nucleotide polymorphism (SNP) in the *FAAH* gene (C385A) results in reduced cellular expression of FAAH and greater AEA activity. The wildtype (WT) *FAAH* allele is associated with an increased risk of developing cannabis use disorder (CUD) due to reduced AEA activity during abstinence. The two principally expressed cannabinoids produced by cannabis are the non-psychotropic cannabidiol (CBD), and the psychotropic Δ^9-tetrahydrocannabinol (THC). THC is a partial CB1 agonist, and chronic exposure to THC increases tolerance due to the desensitization of CB1. Acute THC exposure also leads to a reduction in circulating AEA, while CBD inhibits its intracellular degradation. With increasing recreational legality in the US and greater exposure to high THC strength products, it is important to determine if products with CBD could impact THC use differently in individuals based on their *FAAH* genotype. Thus, we observed differences in cannabis use by quantifying blood THC levels, an objective measure of intoxication, between genotypes after *ad libitum* administration of either a flower strain with both CBD and THC (10% CBD and 9% THC; CBD+THC) or a THC only strain (24% THC; THC+).

Methods: Current cannabis users in Boulder, Colorado, were recruited and asked to exclusively use either the THC+ or CBD+THC strain *ad libitum* for five days before an acute administration session in a mobile pharmacology lab. Demographic breakdown of the four user groups by genotype and strain: CBD+THC (AC: n=5; age *M*=33.2, *SD*=20.3; 100% male / CC: n=20; age *M*=35.3, *SD*=16.8; 45% male) and THC+ (AC: n=37; age *M*=31.3, *SD*=13.1; 51.4% male / CC: n=49; age *M*=30, *SD*=10.6; 65% male). Intravenous blood was obtained before and approximately 10 minutes after self-administration. Blood was used to assay *FAAH* and to quantify circulating THC. A 2-way factorial ANOVA was used to determine if the change in THC levels from before to 10 minutes after use differed depending on strain chemotype, *FAAH* genotype, or an interaction. Results: No significant main effects were observed for strain (THC+: *M*=135.9; CBD+THC: *M*=136.6) or genotype (CC: *M*=131.4; AC: *M*=144.1). However, there was a significant interaction, such that individuals with the mutant allele showed a greater increase in THC levels when using the THC+ vs. CBD+THC strain, and WT individuals showed the reverse (i.e., greater increase using CBD+THC vs. THC+) ($F(94)=4.29$, $p=.041$). In conclusion, individuals with the mutant allele may use less THC when CBD is present due to a potential compounding effect of both greater basal AEA and inhibition of AEA degradation by CBD. Similarly, WT individuals may use more THC when CBD is present due to the increase in AEA activity and greater CB1 agonism.

IN VIVO INVESTIGATION OF THE (*R*)- AND (*S*)-STEREOISOMERS OF THE CB_1 POSITIVE ALLOSTERIC MODULATOR (PAM) ZCZ011

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Cannabinoid type-1 (CB_1) receptor allosteric modulators represent a promising strategy for the treatment of neuropathic pain, with a reduced cannabimimetic side effect profile. In particular, the CB_1 PAM ZCZ011 reduces mechanical allodynia in a nerve injury mouse model of neuropathic pain and augments the pharmacological effects of CB_1 orthosteric agonists, but on its own, lacks CB_1 receptor mediated subjective and motor effects. The fact that ZCZ011 contains a chiral carbon provides the opportunity to determine whether the pharmacological effects of this molecule exhibit stereoselectivity. In the present study, we resolved the (*R*)- and (*S*)-enantiomers of ZCZ011 and tested whether they differentially augment the pharmacological effects of the CB_1 receptor orthosteric agonist CP55,940 in C57BL/6J mice. Accordingly, we tested whether ZCZ011 (40 mg/kg), its (*R*)-configuration (40 mg/kg), or its (*S*)-configuration (40 mg/kg), would alter the dose-response relationship of CP55,940 (0.1-3.0 mg/kg) in producing catalepsy (bar test), antinociception (warm-water tail immersion and hot-plate assays), and hypothermia. All three compounds significantly increased the potency of CP55,940 in producing antinociception and hypothermia by approximately 2-fold compared with vehicle pretreatment. The ZCZ011 racemate and its stereoisomers did not significantly differ from one another in producing leftward shifts in the CP55,940 dose-response relationship for these measures. In the hot-plate assay, only the ZCZ011 racemate pretreatment produced a significant increase in the potency of CP55,940 compared with the vehicle pretreatment, with a potency ratio (CL 95%) of 1.7 (1.1-2.8). In the catalepsy assay, only the (*S*)-stereoisomer significantly increased the potency of CP55,940 compared with the vehicle pretreatment, with a potency ratio (CL 95%) of 1.7 (1.2-2.6). Ongoing studies are evaluating whether other doses of ZCZ011's stereoisomers differentially alter the dose-response relationships of CP55,940 in these *in vivo* measures, as well as testing them in a mouse model of neuropathic pain. Collectively, these preliminary results provide minimal evidence of stereoselectivity between the (*R*)- and (*S*)-ZCZ011 stereoisomers in augmenting the potency of a CB_1 receptor orthosteric agonist in common *in vivo* pharmacological assays indicative of CB_1 receptor activity. Understanding the relationship between the cellular and *in vivo* effects of these stereoisomers will provide further insight into the CB_1 allosteric site(s) of action and contribute to further drug development of CB_1 allosteric modulators.

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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF DIARYLUREA BASED ALLOSTERIC MODULATORS OF CB1 RECEPTOR

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The cannabinoid 1 receptor (CB1R) plays an important role in many physiological processes such as pain, learning and memory, appetite and feeding behaviors, anxiety and depression. In consideration of the serious psychiatric side effects associated with the first in class anti-obesity CB1R inverse agonist/antagonist, rimonabant, that led to its withdrawal from market, our group has focused on developing negative allosteric modulators to modulate CB1R for therapeutic benefits while avoiding the adverse effects of orthosteric antagonists. We have demonstrated that our diarylurea-based RTICBM-74 is more effective in than the lead compound PSNCBAM-1 in attenuating reinstatement of cocaine-seeking behavior in rats, probably due to better metabolic stability. In this study, we describe the design, synthesis and evaluation of a new series of CB1R allosteric modulators by optimizing the middle phenyl ring with its substitution with a variety of heterocycles or alicyclic groups. All target compounds were characterized by MS, ^{1}H and ^{13}C NMR and HPLC.

These novel CB1R allosteric modulators were evaluated in the FLIPR calcium mobilization assay using CHO cells overexpressing human CB1R and the [^{35}S]GTPγS binding assay in either HEK cells overexpressing human CB1R or mouse brain preparations. These compounds reduced the E_{max} of the orthosteric CB1R orthosteric agonist CP55940 in calcium mobilization assays, as expected with negative allosteric modulators. Most compounds possessed low nanomolar IC_{50} values at CB1R without any significant activities at the CB2R. Their potencies in calcium mobilization assay correlate in general with the [^{35}S]GTPγS binding assays, though differences in potency ranking between these assays were noted with several compounds. The new compounds also showed better metabolic stability in rat liver microsomes than PSNCBAM-1. These results expanded our structure-activity relationship understanding of these diarylurea based CB1R allosteric modulators, facilitating the development of this class of compounds as potential medications for the treatment of drug addiction and CB1R-mediated conditions.

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DRUG DELIVERY AND FORMULATION OF CANNABINOIDS

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Optimized drug delivery and formulation of drug product remains one of the central problems in maximizing medical benefits of cannabinoids. The common delivery methods in recreational use involve inhalation by smoking of the whole plant parts or thermal vaporization of extracts, consuming edible food products, and oil-based tinctures. Lipophilic intra-oral formulations are used in several FDA-approved products on the market. In every instance, variable hepatic metabolism and low bioavailability present formidable barriers to overcome for increased and controlled exposure along with reduced adverse effects that is complicated by presence of other components of natural cannabis isolates, e.g., minor cannabinoids and terpenes.

There is also considerable interest in the application of different doses of and/or combination of CBD and THC to obtain a desired therapeutic benefit. Detailed pharmacokinetic analysis of absorption, distribution, metabolism and excretion of these medical cannabinoids in various and larger patients groups is still lacking and only beginning to emerge. Performed by mostly academic centers and limited by the pharma quality material supply, the value of these data will be closely tied to broader adoption of the FDA regulatory principles and related to CMC (chemistry, manufacturing, control) guidelines that include drug formulation and delivery.

This presentation will review state of the art advanced formulations for cannabinoid products and assess utility of nano and micro-emulsions, liposomes, inclusion complexes and related solubility enhancement approaches, along with other complex custom-tailored formulations. Comparative importance of different delivery routes, e.g. intra-oral, nasal, rectal, transdermal vs. traditional modalities is actively debated in current literature. Careful selection and fine-tuning of the proper physico-chemical characteristics of the formulated cannabinoids combined with appropriate body targeting will play a key role in enabling better-designed human clinical studies to yield more meaningful pharmacokinetic parameters and establishing PK-PD relations along with predictable dosing. This will in turn lead to unveiling a full potential of cannabinoids in treating multiple disease conditions for which there are still limited scientific evidence.

CHARACTERIZATION OF Δ⁹-TETRAHYDROCANNABINOLIC ACID AS A DUAL PPARγ/ CB_1 LIGAND. IMPLICATIONS IN RHEUMATOID ARTHRITIS

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Medicinal cannabis and purified cannabinoids have garnered worldwide attention since millions of patients can benefit from its medical properties. Remarkably, Δ⁹-THCA, the natural precursor of Δ⁹-THC, is an underexplored non-psychotropic phytocannabinoid that shows potent PPARγ agonistic activity. PPARγ is a member of a family of nuclear receptors able to regulate lipid turnover and metabolism and also mediates potent anti-inflammatory activities. Herein, we have investigated the role of Δ⁹-THCA on PPARγ signaling and its ability to modulate classic cannabinoid receptors (CB_1 and CB_2), including evaluation of its efficacy in murine collagen-induced arthritis (CIA).

Both Δ⁹-THCA and Rosiglitazone (RGZ) bind to the canonical binding site in the PPARγ ligand-binding pocket but differentially regulate PPARγ co-regulator binding, transactivation and target gene expression. We found that Δ⁹-THCA induced osteoblastogenesis measured by Alizarin staining and by the determination by qPCR of markers specific for osteoblasts (RUNX, SP7, IBSP, ALP). Our data also demonstrated that Δ⁹-THCA binds to CB_1 (Ki=251.6 nM) and CB_2 (Ki=505.5 nM), acting as a partial agonist for CB_1 (EC50=3.8 μM) and as inverse agonist for CB_2 (IC50=1.3 μM) in [^{35}S] GTPγS binding analysis. Interestingly, we have evidence that Δ⁹-THCA works as a positive allosteric CB_1 modulator in a pM range of concentrations, and against CP-55,940. *In vivo* experiments showed that Δ⁹-THCA (20 mg/Kg i.p.) greatly prevent collage type II-induced arthritis in DBA/1 mice. According to the evaluation criteria the clinical arthritis score in the CIA model group was significantly higher than that of the control group from day 21 onwards (P<0.01). The arthritis clinical score in Δ⁹-THCA-treated group was significantly less than that of CIA group (P<0.05). Paw inflammation measured by plethysmometry and weight loss in CIA mice were also alleviated by Δ⁹-THCA treatment. Immunochemistry analysis showed that Δ⁹-THCA prevented the infiltration of inflammatory cells and synovium hyperplasia (H&E staining), cartilage loss (Safranin O staining) and proteoglycan loss (Toluidine blue staining) in comparison to the untreated CIA group. Furthermore, the administration of Δ⁹-THCA significantly inhibited the mRNA expression of inflammatory genes such as IL-1β, TNFα and MCP-1 on knee joints. The efficacy of Δ⁹-THCA in CIA was greatly prevented by either SR141716A (CB_1 antagonist) or T0070907 (PPARγ antagonist). Finally, proteomic SWATH mass spectrometry analysis of plasmatic biomarkers demonstrated that Δ⁹-THCA is mediating its activity mainly through PPARγ and CB_1 pathways, but other pathways cannot be discarded.

In conclusion, our studies document the anti-inflammatory and osteoblastogenesis activities of Δ⁹-THCA highlighting its potential for the treatment of chronic inflammatory diseases such as Rheumatoid Arthritis.

IDENTIFICATION OF SEX, STRAIN, AND ENTOURAGE EFFECTS IN THE BEHAVIORAL AND PHYSIOLOGICAL RESPONSE TO CANNABINOIDS

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Recent policy changes are expected to increase the prevalence of cannabis use in the U.S. Marked variation among individual humans has been observed for acute and chronic responses to cannabis and cannabinoids suggesting that genetic factors play a key role in initial behavioral response, sustained use, and persistent effects. However, the precise genetic and biochemical mechanisms mediating behavioral and physiological responses to cannabis and its constituent components are unknown. This knowledge gap represents a critical barrier to progress in leveraging the therapeutic potential of cannabinoids and in identifying individuals at risk for adverse health consequences associated with use. Accordingly, our group has initiated a project to identify genetic factors mediating behavioral and physiological responses to cannabinoids following exposure to two of the major components of cannabis, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in male and female C57BL/6J (B6), DBA/2J (D2) and their recombinant inbred BXD progeny. We have developed a phenotyping pipeline that measures physiological responses (metabolic, hypothermic, analgesic, and motor traits) to THC (10 mg/kg) and cannabinoid mixtures [THC (10 mg/kg) + CBD (0.56 or 5 mg/kg)] during acute (1 day) or sub-chronic (5 days) exposures. We report significant and heritable dose, strain, and/or sex effects of CBD on THC response between the parents of the BXDs—B6 and D2—and identify heritable variation in the response to THC using a subset of the BXD cohort. We propose that physiological and behavioral responses to cannabinoids are driven by genetic variation in metabolic genes and effector proteins that regulate cannabinoid 1 receptor (CB1) signal transduction, termination of signaling, and trafficking. Future directions include the identification of key genes and pathways moderating individual differences in THC response and CBD modulation of the THC response using forward genetic mapping and systems genetics analysis in the well-characterized BXD genetic population.

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EVALUATING NOVEL CONTROLLED DEACTIVATION CANNABINOID AGONISTS WITH REDUCED TOLERANCE AND DEPENDENCE PROFILES

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Classical cannabinoids such as Δ^9-THC and its analogs display potent analgesic effects that are CB1 receptor (CB1R) mediated. However, they are also known to produce rapid tolerance along with dependence. Here we report the *in vivo* pharmacological properties of novel short-acting CB1R agonists, AM7438, and AM7410. In this study we show that AM7438 and AM7410 are potent analgesic drugs and have diminished *in vivo* tolerance and dependence profiles compared to their long acting congener, Δ^8-THC-DMH, a classical cannabinoid. We determined the potency and duration of action of AM7438, AM7410 and Δ^8-THC-DMH using the tail-flick latency analgesia assay in male CD-1 mice. The corresponding ED_{50} values (in mg/kg with 95% CI) were 0.21 (0.1692, 0.2767) for AM7438, 0.48 (0.33, 0.070) for AM7410 and 0.22 (0.0614, 0.353) for Δ^8-THC-DMH. Onset of effects for Δ^8-THC-DMH was slow with an extended time-course; with functional, perpetual *in vivo* half-life of 17 hours for its analgesic effects. Both AM7438, and AM7410 had quick onset of effect and short duration of action; with functional perpetual *in vivo half-life of 5 hours* for both AM7438 and AM7410 for their analgesic effects. We also evaluated the tolerance profiles of AM7438, AM7410 and Δ^8-THC-DMH in male CD-1 mice using the tail-flick analgesia and hypothermia assays. Animals when administered with Δ^8-THC-DMH (1 mg/kg) repeatedly over 6 days, developed tolerance rapidly as seen in both assays. Beginning day-2, Δ^8-THC-DMH produced no hypothermic or analgesic effects. AM7438 (1mg/kg) continued to produce significant analgesic effects and hypothermic effects until day-5 with a relatively slower onset of tolerance. AM7410 (3 mg/kg) produced significant analgesic and hypothermic effects until day-4 and thereafter had complete tolerance to its analgesic and hypothermic effects. Additionally, we looked at CB1R antagonist (rimonabant 10mg/kg) precipitated withdrawal by measuring head-shakes and paw tremors in mice treated repeatedly with either AM7438 (1mg/kg) or AM7410 (3 mg/kg) or Δ^8-THC-DMH (1 mg/kg). Notably, mice treated with the short-acting CB1R agonists AM7438, and AM7410 over 6 days displayed reduced rimonabant-precipitated withdrawal symptoms as compared to animals treated with Δ^8-THC-DMH. Based on these data, we propose that controlled deactivation cannabinoids represented by two of our lead compounds AM7438, and AM7410 may act as promising pharmacotherapies for treating pain without producing tolerance or dependence.

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MEDICAL CANNABIS: AVENUE TO ALLEVIATION OR PATH TO PROBLEMATIC USE?

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Although many have studied cannabis use disorders (CUD) among recreational cannabis consumers, it remains unclear whether medical cannabis (MC) patients develop symptoms or behaviors associated with problematic cannabis use. As the number of MC patients continues to grow, it is imperative to assess rates of potentially problematic cannabis use in this unique population of users. Given that MC patients use cannabis to achieve symptom alleviation rather than a primary goal of intoxication, we hypothesized that MC patients would exhibit few symptoms of problematic use.

As part of an ongoing, longitudinal study, 40 MC patients completed baseline assessments (cognitive testing, clinical ratings, treatment regimen information, multimodal neuroimaging, etc.) and follow-up visits after three months of treatment, and a subset have also returned after six (n=27) and twelve (n=14) months of treatment. In order to assess potential problematic cannabis use, patients completed the Cannabis Use Disorders Identification Test (CUDIT) at each visit. MC patients' CUDIT scores were also compared to CUDIT scores in recreational cannabis users, including a cohort of heavy users (>4 days of use per week; n=38) and a cohort of more "casual" users (once per month to twice per week; n=13).

Although CUDIT scores increased in MC patients over time, on average, ratings fell below the threshold for 'hazardous use' at each visit. Further analyses indicated that increased ratings were largely attributable to increased frequency of use rather than clinically significant increases in other, more problematic symptoms (e.g., failure to meet expectations, memory/concentration difficulties, using cannabis in hazardous situations). In addition, comparisons between MC patients and recreational consumers suggest that *heavy* recreational users demonstrate significantly higher levels of problematic cannabis use, while few differences were observed between medical patients and *casual* recreational users.

Overall, findings suggest that MC patients generally do *not* meet the threshold for 'hazardous' cannabis use, and exhibit fewer symptoms of problematic cannabis use relative to heavy recreational cannabis consumers. As increases in CUDIT scores are primarily driven by frequency of use in MC patients without clinically significant increases in other symptoms, future metrics should consider this distinction. These findings highlight the need for tools specifically designed to assess CUD in MC patients.

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ALKALOID-BASED CB1 RECEPTOR ALLOSTERIC MODULATORS

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The cannabinoid receptor-type 1 (CB1R) contributes to diverse pharmacologic effects and may be targeted by agonists or antagonists to influence various behaviors. For example, CB1R agonists reduce stress and anxiety, whereas CB1R antagonists may be useful probes of substance abuse and dependence disorders. Targeting of CB1Rs by direct, orthosteric agonists and antagonists have their disadvantages, however: many CB1R agonists are reinforcing, whereas CB1R inverse agonists are associated with anhedonia and suidicidal behaviors. CB1R allosteric modulators (CB1R-AMs) are proposed to have a "ceiling effect," whereby the adverse effects of orthosteric agonists and antagonists are diminished. In the case of CB1R negative allosteric modulators (CB1R-NAMs), such agents could attenuate the reinforcing effects of drugs of abuse without causing anhedonia. Two commercially available CB1R-NAMs (Org 27569, PSNCBAM-1) that have been the subject of intense chemical optimization have lipophilicity (LogP > 5.0) and aqueous solubility (≤ 1 mg/mL) profiles that may adversely impact central bioavailability. We sought to explore the structure-activity relationships (SAR) of a lesser-explored lead compound, Org 29647, which, unlike these other leads, contains a weakly basic tertiary amine that is capable of formulation as aqueous-solubilizing salts.

We synthesized a series of amide and urea analogs of Org 29647 predicted to have structural similarity to known CB1R-NAMs. *In vitro* evaluation (PathHunter, DiscoveRx) showed that *para*-halogenation (Br > Cl > F) improved potency in the urea series, and ring-constrained naphthyl groups were most potent in the amide series. The most active lead (CWC-1-002: *(R)-N*-(1-benzylpyrrolidin-3-yl)-2-naphthamide) showed CB1R-NAM activity against two synthetic CB1R agonists (CP-55,940 and WIN-55-212-2, pKb = 6.09 and 6.71, respectively) and the endocannabinoid, 2-AG (pKb = 6.49). CWC-1-002 showed similar potency against 2-AG compared to Org 27965 (pKb = 6.43). Physicochemical property analysis demonstrates that CWC-1-002 has lower LogP and topologic polar surface area (TPSA) compared to commercially-available CB1R-NAMs. A preliminary screen (NIMH Psychoactive Drug Screening Program) suggests CWC-1-002 potentially binds 5-HT, dopamine, and sigma receptors, all possible pharmacologic targets for medications to treat substance abuse and dependence disorders. Future studies will explore the SAR of this lead at these receptors with the ultimate goals of developing selective CB1R pharmacologic probes and medications to treat substance use disorders.

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BLADDER DISFUNCTION AS A CONSEQUENCE ON NEONATAL HYPOXIC-ISCHEMIC BRAIN DAMAGE: PROTECTIVE ROLE OF CBD

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Perinatal and early childhood asphyxia is a major cause of morbidity and chronic cognitive dysfunction. Cannabidiol (CBD) has demonstrated neuroprotective effects in different animal models of hypoxic-ischemic (HI) brain damage in newborns (Lafuente *et al*., 2011; Pazos *et al*., 2012). However, brain damage not only leads to cerebral injury but also to multiorganic dysfunction and it could have different consequences depending on the neurovelopment stage at the moment of HI insult. The aim of this proyect is to analyze cerebral consequences of HI-brain insult in neonatal *versus* young adult animals and to study the urinary tract secondary injury following brain hypoxia-ischemia.

To this aim adapted *Rice-Vanucci* model was used to develop the HI brain injury. Newborn (7 day-old) and young adult (30 day-old) C57BL/6J mice were submitted to left carotid artery electrocoagulation followed by exposure to hypoxia (8% oxygen) in a temperature-controlled chamber. Then vehicle (HV) or CBD (HC, 1mg/Kg) were administered (s.c.), while other animals remained as controls (SHAM). Animals were sacrificed 7 days after HI injury and histological, qPCR and Western-Blot analysis were performed. Bladder function was examined in an organ bath system coupled to electrostimulator for isometric force recording.

In vitro bladder function assays shown that electrical field stimulation (EFS) and carbachol induced frequency- and concentration-dependent contractions EFS-induced detrusor smooth muscle (DSM) contractions are enhanced in HV newborn mice comparing with respective controls, and the CBD treatment recovery back the DSM contractions induced by EFS. Carbachol-induced DSM contractions are decreased in HV newborn mice and CBD treatment partially recover DSM contractions induced by carbachol. In adult mice, no differences are found between groups in EFS- or carbachol-induced DSM contractions. At brain cerebral cortex level, it has been found an alteration in some Endocannabinoid System elements, more evident in infant mice. Several brain biochemical changes have been observed, however oxidative stress seems to have a pivotal role in extracerebral afectation. In conclusion, HI-induced extracerebral damage seems to be different depending on the neurodevelopmental brain stage. These results indicate that different therapeutic approaches could be necessary for infant and adult patients following a cerebrovascular accident.

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CWC-1-001 EXHIBITS CB1 RECEPTOR ALLOSTERIC MODULATORY EFFECTS

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Allosteric modulators of the cannabinoid receptor-type 1 (CB1R) have great therapeutic potential as they can modulate signaling of endogenous agonists through the receptor without exerting direct agonist or antagonist effects. Allosteric modulation therefore has the potential to exert therapeutic effects while avoiding adverse effects seen with compounds that directly bind to the orthosteric binding site. We have developed and are characterizing a novel allosteric modulator of the CB1R, CWC-1-001 (*(R)*-*N*-(4-bromophenyl)-*N*-[1-(phenylmethyl)-3-pyrrolidinyl]urea).

In a cell-based assay, CWC-1-001 inhibited CB1 receptor/beta-arrestin recruitment by CB1R agonists 2-arachidonoylglycerol (2-AG), WIN 55,2212-1, and CP 55,940 at micromolar concentrations. These effects were specific to the CB1 receptor and not seen for the CB2 receptor. In a radioligand binding assay, CWC-1-001 increased the binding affinity of radiolabeled CP 55,960. This suggests the compound binds to an allosteric site rather than the orthosteric binding site, with positive allosteric effects on agonist binding but negative allosteric effects on beta-arrestin signaling. To test the *in vivo* effects of the compound, a 50 mg/kg dose was administered by intraperitoneal injection to mice followed by testing of the tetrad effects of cannabinoids (antinociceptive and cataleptic state, hypolocomotion and hypothermia). Hypolocomotion and reduced body temperature were observed following drug administration; there were no significant effects on other components of the tetrad. Co-administration of CWC-1-001 with WIN 55,2212-1 (1 mg/kg) or CB1R antagonist rimonabant (3 mg/kg) showed no drug interactions.

These data indicate that the urea-substituted *N*-benzyl-3-aminopyrrolidine structure has the features of an allosteric modulator of the CB1R. However, our current *in vivo* studies indicate that CWC-1-001 has pharmacological effects that are not affected by CB1R orthosteric signaling.

ANTIINFLAMMATORY AND NEUROPROTECTIVE EFFECTS OF CANNABIDIOLIC ACID (CBDA) UNDER HYPOXIC CONDITIONS *IN VITRO*

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Previous work in our group has shown cannabidiol (CBD) can improve blood-brain-barrier (BBB) permeability secondary to oxygen-glucose deprivation (OGD) in an *in vitro* model of the BBB, partly through $5HT_{1A}$. The precursor to CBD, cannabidiolic acid (CBDA), is not well studied but is thought to act through similar mechanisms to CBD ($5HT_{1A}$ activation). We therefore hypothesised that CBDA would also be protective in an *in vitro* model of stroke. Thus, the aim of this study was to investigate the effects of CBDA in an *in vitro* model of the BBB using transepithelial resistance (TEER) as a measure of BBB permeability and cytokines as a markers of inflammation.

Experiments were performed on pericyte monolayers (passage 4-6), neuronal monolayers (P1) and in an *in vitro* BBB model containing astrocytes, pericytes, HBMECs and neurons in a transwell system. In oxygen-glucose deprivation conditions (OGD), the model was treated with CBDA or CBD (300 nM-3 μM), placed in glucose free medium, 0% O_2 environment (BD GasPak™ pouch) for 4hrs. Propidium iodide staining (PI) was carried out on coverslips containing neurons as a marker of neuronal cell death. Briefly, cells were washed with 1x PBS and incubated with 100 μL/mL of PI (ThermoFisher, UK) and incubated for 5 minutes in the absence of light. Images were Olympus CKX4 using Q-Capture Pro software at 10x objective. Pericyte and neuronal monolayers were also treated with 10 nM-10 μM CBDA prior to OGD. Reperfusion was established by returning cells to normal medium in normoxia (20% O_2) in respective increasing concentrations of CBDA or CBD. To probe mechanisms of action, pericytes were treated with CBDA (1μM) in the presence of receptor antagonists; AM251(CB_1) (100 nM), AM630 (CB_2) (100 nM), capsazepine (TRPV1) (1 μM), GW6471 (PPAR-alpha)(100 nM), GW9662 (PPARy (100 nM), (S)-WAY-100,630 (5-HT1A) (300 nM) and (GPR55) O1918 (1 μM) and a vehicle control. Media samples were analysed for IL-6, IL-8 and VEGF (R&D systems) by ELISA. Statistical analysis was conducted using either one-way ANOVA (monocultures) or two-way ANOVA, with Dunnett's multiple comparison test (BBB model).

In a four-cell model of the BBB, all concentrations of CBDA (300 nM-3 μM) offset decreases in TEER as a result of OGD. This was significant at 24hrs for CBDA 3 μM vs vehicle control ($P<0.05$). Although not significant, CBD was also able to offset decreases in TEER post OGD at 1 and 3 μM, but not 300 nM. Results from PI staining showed that CBDA was also able to protect the neurons; with distinctly less fluorescence in CBDA 1 μM treated wells vs vehicle control, indicating less neuronal cell death.

In pericyte monolayers, CBDA mediated reduction in IL-6 secretion in 24hr post OGD samples and, this effect was blocked only with application of antagonist S-WAY-100,630 ($p<0.0001$ normoxia vehicle vs WAY+CBDA, one-way ANOVA). When applied alongside antagonists AM251, AM630, capsazepine, GW6471 and O1918, CBDA 1μM still attenuated IL-6 secretion and was comparable to the normoxia vehicle (not significant one-way ANOVA). Therefore, suggesting that CBDA does not appear to work through these receptors. CBDA at 10 μM also significantly increased VEGF and IL-8 secretion in 24hr pericyte medium samples ($P<0.001$).

These data suggest that like CBD, CBDA is effective in reducing BBB permeability and inflammation in a cellular model of stroke. We have shown the anti-inflammatory effects of CBDA were inhibited by a $5HT_{1A}$ antagonist, but not by antagonism of CB1, CB2, TRPV1, the O-1918-sensitive CB receptor or PPARα.

CANNABICHROMENE ACTIVITY AT CB1 AND CB2 RECEPTORS VIA MULTIPLE SIGNALLING PATHWAYS

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Cannabichromene (CBC) is one of the most abundant phytocannabinoids (Turner et al. 2017). We recently reported that it is a low efficacy, Gαi-protein dependent CB2 receptor agonist; capable of inducing a CB2 receptor internalization that is independent of GRK2/3. This study was based on cannabinoid receptor-induced cellular hyperpolarization, through the opening of the G-protein coupled inwardly-rectifying potassium (GIRK) channels. However, cannabinoids receptors have been shown to regulate multiple cell signalling pathways including inhibition of cAMP, and recruitment of beta arrestin. Therefore, we investigated whether the specificity of CBC at CB2 receptors and lack of CB1 activation could be pathway specific.

Using HEK cells stably transfected with human CB1 and CB2 receptors, we examined the actions of CBC on cannabinoid receptor (CBR) inhibition of cAMP production and activation of beta-arrestin using the BRET assay in a BMGLabtech Pherastar plate reader, and Alphascreen SureFire kit respectively. CBC-mediated CB2 receptor desensitization was also studied in AtT20 cells stably transfected with human CB2 receptors using FLIPR membrane potential assay in a FlexStation 3.

CP55,940 inhibited adenylyl cyclase activation by Forskolin (FSK) (CB1, EC50 28nM; CB2 EC50 39nM) which is consistent as a standard non-specific CBR agonist. Cannabichromene did not activate CB1 receptors. At CB2, it caused a concentration-dependent inhibition of adenylyl cyclase activation by FSK with a maximal efficacy of 33% of 1uM CP55,940 at 10μM. CBC efficacy did not saturate at 30μM (50% of 1uM CP55,940). Upon continuous stimulation of CB2 receptors for 30min, it caused a signalling desensitization which is indicated by a 73±6% reversal of cellular hyperpolarization. This signal desensitization was independent of GRK2/3, a G protein receptor kinase.

The CB2 receptor selectivity of CBC is not pathway specific to GIRK channel opening. Therefore, it can be concluded that it is a CB2-selective agonist which is capable of inducing CB2 receptor desensitization. Based on this selectivity, CBC is a great candidate for further research as a potential anti-inflammatory agent for conditions such as chronic pain without concern for psychoactivity.

THE MARIJUANA-DERIVED TERPINE α-TERPINEOL REVERSES MECHANICAL ALLODYNIA AND THERMAL HYPERALGESIA IN A MOUSE MODEL OF NEUROPATHIC PAIN

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Pain is one of the most common reasons to seek medical attention and chronic pain is a worldwide epidemic. Under some circumstances, incoming protective nociceptive signaling is prolonged, leading to behavioral sensory alterations common to pathological neuropathic pain. We hypothesized that the marijuana-derived terpene α-terpineol would reverse behaviors associated with pathological neuropathic pain. We used the chronic constriction injury of the sciatic nerve (CCI) model of neuropathic pain, which produces robust increases in sensitivity to light mechanical touch, or allodynia, as assessed with von Frey filaments, and increased thermal sensitivity, or thermal hyperalgesia, as assessed in the hotplate test. To examine the involvement of cannabinoid receptors, we also tested this compound in mice that had undergone the CCI surgery that lack either functional cannabinoid type 1 receptors (CB_1R (-/-)) or mice lacking functional cannabinoid type 2 receptors (CB_2R (-/-)).

First, we assessed the ability of α-terpineol to reverse mechanical allodynia and thermal hyperalgesia. Male and female wildtype mice on a C57BL/6J background underwent CCI surgery with chromic gut suture used to ligate the sciatic nerve. Sham mice underwent surgical procedures but without nerve ligation. Starting seven days after surgery, mice were injected intraperitoneally (i.p.) with a single dose of vehicle (1 part ethanol / 1 part emulphor/ 18 parts saline) or α-terpineol (5.6, 10, 17.8, 32, 56 mg/kg) and 20 min later mice were tested for mechanical allodynia and thermal hyperalgesia. Mice in the CCI – Vehicle group displayed robust mechanical allodynia and thermal hyperalgesia when compared to Sham – Vehicle mice. The terpene α-terpineol produced dose-dependent reversal of mechanical allodynia at the doses of 10, 17.8, 32, 56 mg/kg, and dose-dependent reversal of thermal hyperalgesia at the doses of 32 and 56 mg/kg. Next, we tested α-terpineol at the dose of 56 mg/kg (i.p.) in male and female CB_1R (-/-), CB_2R (-/-) mice. In both genotypes, this dose was not sufficient to reverse mechanical allodynia, and only reversed thermal hyperalgesia in CB_1R (-/-) mice. This demonstrates that both cannabinoid receptors are necessary for α-terpineol's anti-allodynic effects at this dose. Meanwhile CB_2R are necessary for α-terpineol's anti-thermal hyperalgesic effects, with CB_1R being dispensable. These findings suggest that α-terpineol may be an attractive therapeutic for the treatment neuropathic pain, with a cannabinoid pharmacophore.

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COMPARISON BETWEEN DRAGONFLY CANNABIDIOL VS TOCRIS CANNABIDIOL IN COLORECTAL CANCER SPHEROIDS

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Cannabidiol (CBD) is an abundant cannabinoid from the *Cannabis sativa* plant family. CBD exerts anticancer activity in different types of tumour-derived cell lines *in vitro* and reduces tumour growth *in vivo*. CBD is freely available through internet sources, marketed as a food supplement. However, without regulation, the inconsistency of purity and efficacy of these products could bring any actual or potential therapeutic benefit of CBD into disrepute. In addition, the importance of 3D cell culture models in the validation of potential drug action has recently gained traction. In this study, we investigated the viability of colorectal cancer spheroids cultured in CBD purchased from Tocris (biotechne) vs DragonflyCBD purchased via the internet from https://www.boots.com/dragonfly-cbd-cannabidiol-oil-5-6-cbd-10ml-10258511.

Caco-2 and HT-29 colorectal cancer cells ($5x10^4$) were seeded into black round bottom ultra-low attachment 96-well plates (Corning) that were coated with Lipidure® (Amsbio). Spheroids formed over 3 days and imaged at T0 prior to addition of CBD (10μM). Tocris-CBD was dissolved in ethanol (10mg/mL) and DragonflyCBD came as 50mg/mL in unspecified oil). Plates were incubated for 72 hours with or without daily replacement of CBD before assay. The viability of spheroids was measured by CellTiter-Glo® 3D Cell Viability Assay (Promega) according to manufacturer's protocol 72 h after drug administration with a Tecan multiplate reader. Standard dose response experiments were performed with 2D cell culture protocols using Presto Blue™ (Invitrogen), 5μM – 5mM). Relative treatment efficacy was expressed as percent of the appropriate solvent control.

Single dose CBD did not reduce viability or spheroid size in either Caco-2 or HT29 spheroids. Daily administration of Tocris-CBD reduced HT29 (but not Caco-2) spheroid viability by approximately 25%. DragonflyCBD had no effect on any 3D spheroid, whether delivered in a single or daily dose. Interestingly, daily Tocris-CBD was able to disrupt both Caco-2 and HT29 spheroid structure, although this was observational and the implications therefore unknown. Using a standard 2D cell proliferation assay, Presto Blue™, there was a 2-fold difference in the efficacy of DragonflyCBD compared to Tocris-CBD (IC50 72 ± 4.1 μM vs 31.5 ± 5.2 μM)

Daily administration of pure CBD has significant impact on tumour spheroids *in vitro*, although dependent on cell line and nutrient availability. Unregulated CBD purchased online may have variable CBD content and potency does not correlate with defined product. Finally, it is critical that we develop better cellular models of cancer, because what appears to be promising in 2D models does not translate into the more physiologically relevant 3D model.

OPTIMIZED SYNTHESIS OF ZCZ011, RESOLUTION AND CHARACTERIZATION OF ITS ENANTIOMERS

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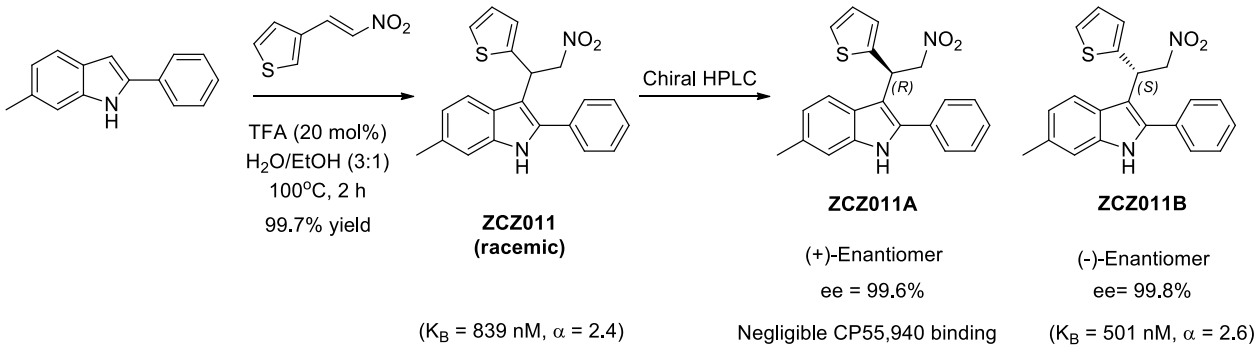
ZCZ011 is a positive allosteric modulator of the cannabinoid CB_1 receptor. It exhibits CB_1-dependent antinociceptive effects in murine models of pathological pain. ZCZ011 is a racemate carrying one chiral center. Whether its enantiomers functions differently in allosteric modulation of the CB1 receptor remains unknown. Investigation of GAT211, a homologous analog of ZCZ01, revealed that its (*R*)-enantiomer functions as an allosteric partial agonist of the CB_1 receptor and its (*S*)-enantiomer acts as a pure CB_1 positive allosteric modulator (PAM). This warrants the isolation and studies of ZCZ011 enantiomers. To this end, we first optimized the synthesis of ZCZ011 through various Lewis acid-catalyzed Michael addition reactions, and found that the reaction catalyzed by trifluoroacetic acid provided ZCZ011 in nearly quantitative yield (99.7%). Resolution of its enantiomers was achieved by chiral HPLC. The two enantiomers ZCZ011A and ZCZ011B were obtained with enantiomeric excess (ee) values greater than 99.5%. Recrystallization of the enantiomers individually in the mixture of MeOH/Acetone/Water (15:4:1) generated suitable crystals for structure determination by X-ray crystallography. X-ray crystallography analysis showed that the (+)-isomer of ZCZ011 has a (*R*)-configuration, and its (-)-isomer has a (*S*)-configuration. Preliminary data indicated that the (*R*)-isomer lost affinity to the CB_1 receptor, while the (*S*)-isomer of ZCZ011 showed an equilibrium dissociation constant K_B of 73 nM and potentiated CP55,940 binding with a binding cooperativity $\alpha = 1.64$. Preliminary assessment of their modulation of agonist-induced GTPγS binding, cAMP production and β-arrestin recruitment will be discussed. In mice, either isomer (5 mg/kg, ip) blocked rimonabant-precipitated Δ^9-THC (10 mg/kg, sc x 6 days) somatic withdrawal. ZCZ011 and ZCZ011A slightly increased immobility in the marble burying test.



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APPLYING MACHINE LEARNING TO CANNABINOID DRUG DISCOVERY WITH ASSAY CENTRAL

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In 2018 the Food and Drug Administration approved an oral cannabidiol (CBD) solution for treatment of two rare pediatric epilepsies, Dravet and Lennox-Gastaut syndromes. Both diseases are generally defined as a severe form of epilepsy beginning in infancy, and while Dravet syndrome is associated with a genetic mutation, the mechanisms of Lennox-Gastaut syndrome are still unknown. Collaborations Pharmaceuticals, Inc. aims to streamline the development of drugs for rare and neglected tropical diseases using our core technology, Assay Central. Thus, we are poised to apply our niche research to the cannabinoid field for other rare diseases.

Assay Central is a collection of predictive Bayesian and Random Forest models in a self-contained executable for non-experts to evaluate the likelihood of activity at a target of interest. This method aims to reduce superfluous synthesis, and by combining forces with many academic collaborators we have leveraged a broad, collective expertise to identify treatments for parasites (T. Cruzi), bacteria (M. tuberculosis), and viruses (Ebola, HIV). Using data available in the public domain such as ChEMBL and PubChem, we are able to expand our expertise to the endocannabinoid system. Using machine learning models of relevant receptors (CB1, CB2, GPR55, GPR18) we are able to predict target-specific lead compounds, as well as molecular properties and any potential liabilities.

Our business model is both scalable and replicable and applying it to the cannabinoid field is a natural step. We hope to develop new lead compounds for the treatment of other rare pediatric epilepsies like West syndrome.

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POTENT ANTI-INFLAMMATORY EFFECTS OF CANNABIDIOL (CBD) TO MODULATE INDUCED INFLAMMATION ON GINGIVAL KERATINOCYTES: *IN VITRO* STUDY

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Periodontitis is a chronic inflammatory disease affecting oral tissues characterised by a destructive inflammatory process affecting tooth-supporting tissues and resulting in periodontal pocket formation, alveolar bone resorption and, eventually, tooth loss. The continuous challenge of host immune and resident cells and their virulence factors, such as lipopolysaccharide (LPS), results in enhanced and uncontrolled secretion of cytokines. Recently, cannabinoids have been discussed widely due to their therapeutic properties showing promising results in the treatment of various chronic inflammatory diseases. The aim of this study is to evaluate the capacity of cannabinoids CBD to influence the secretion of inflammatory mediators in telomerase-immortalized human gingival epithelial (TIGK) cells induced by lipopolysaccharides (LPS) and flagellin.

The effect of different concentrations of CBD on the viability of TIGK cells was evaluated. TIGK cells were then treated with various determined non cytotoxic concentrations of CBD prior to being stimulated or not stimulated with LPS or Flagellin. The ability of CBD to modulate the release of interleukin (IL)-8 in TIGK cells was then evaluated at mRNA and protein level.

The results obtained showed that CBD was cytotoxic at concentration 100 μM but not cytotoxic at concentrations below 10 μM on TIGK cells. CBD was able to modulate the expression and production of pro-inflammatory cytokine IL8 induced by LPS/flagellin; it decreased the expression of IL-8 by 325 times at mRNA and by 60-70 % at protein level.

The ability of CBD to determine immunomodulatory effects could provide possible therapeutic applications in the field of periodontal research.

GENERAL SYNTHETIC APPROACH TO SELECTIVE FLUORESCENT PROBES FOR THE CANNABINOID RECEPTORS CB_1R AND CB_2R

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G-protein-coupled receptors (GPCRs) control a wide range of physiological processes and are the targets for many clinically used drugs. Two GPCRs of tremendous interest, which are involved in many of the main and fundamental functions of the human body, are the cannabinoid receptors type 1 and 2 (CB_1R and CB_2R). Intensive research to understand the physiological pathways of both CBRs also revealed their roles in numerous pathophysiological conditions, including e.g. chronic and inflammatory pain, diabetes type 2 and liver fibrosis. Understanding their molecular and cellular mechanism of action as well as their *in vivo* drug-target engagement are key to developing new drugs. Fluorescently labeled probes are a powerful tool to investigate the structure and function of these particular GPCRs in their native cellular environment.

In this work, an overview of our concept and the general synthetic approach of designing fluorescent probes addressing CB_1R and CB_2R will be presented based on highly selective and potent synthetic drugs. We will discuss the step-by-step process leading to an optimized synthetic fluorescent small molecule probe. In addition, we will also highlight optimization efforts regarding the placement and length of linker to reach the extracellular space of the receptor by methods like structure-activity-relationship (SAR) studies and *in silico* modelling. We believe that the general synthetic approach can be used as a toolbox for the development of new fluorescent probes to investigate cannabinoid receptors signalling in different tissues and cellular context.

A COMPARISON OF LIGAND-EVOKED SIGNALLING THROUGH MULTIPLE PATHWAYS (CAMP, CA^{2+}, ERK) IN CB_2 RECEPTOR RECOMBINANT EXPRESSION

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The best-established pathways for CB_2 cannabinoid receptor signalling are the inhibition of cAMP generation and activation of ERK through phosphorylation. Given the proliferation of chemotypes of CB_2 receptor ligands, we have investigated representatives of multiple chemotypes in these two assays, as well as for stimulation of calcium elevation, which is a much less well-defined pathway.

Compound	cAMP response (% forskolin)	Calcium response (% ATP)	ERK activation (% FBS)	ERK activation + 2AG (% FBS)
CP55940	-1 ± 6*	21.6 ± 2.2*	104 ± 2*	110 ± 10$^{\#}$
CP55940	6 ± 1*			
Fenofibrate	14 ± 4*	11.0 ± 2.4*	105 ± 4*	101 ± 5$^{\#}$
HU308	20 ± 2*	9.5 ± 0.7*	85 ± 3*	73 ± 8$^{\#}$
MDA19	23 ± 3*	3.4 ± 0.7*	87 ± 3*	69 ± 10$^{\#}$
L759656	63 ± 5*	0.8 ± 0.6	8 ± 2*	40 ± 6$^{\#}$
GW405833	81 ± 5*	0.1 ± 0.4	0 ± 3	0 ± 3$^{\#}$
CB65	105 ± 17	-0.6 ± 0.7	0 ± 2	5 ± 5$^{\#}$
SER601	106 ± 11	0.0 ± 0.4	36 ± 3*	37 ± 4$^{\#}$
COR170	115 ± 13	-1.7 ± 0.6	-1 ± 2	27 ± 8
GP2A	125 ± 4*	0.4 ± 0.7	-3 ± 2	4 ± 3$^{\#}$
AM630	140 ± 16*	-1.0 ± 0.6	-6 ± 3	-4 ± 4$^{\#}$
2AG				21 ± 8

*$P<0.05$ compared to the absence of cannabinoid ligand; $^{\#}P<0.05$ compared to 2AG alone

Analysing the cAMP inhibition profile, CB65, SER601 and COR170 were ineffective, while GP2A and AM630 appeared to act as inverse agonists. CP55940, fenofibrate, HU308 and MDA19 inhibited the forskolin cAMP response by >75 %, while L759656 and GW405833 were either low potency or low efficacy agonists. Based on the elevation of intracellular calcium ions, only CP55940, fenofibrate, HU308 and MDA19 evoked a significant effect. These four agonists also evoked significant phosphorylation of ERK. L759656 evoked a small but significant ERK phosphorylation, while SER601 evoked an unexpected elevation of ERK phosphorylation. None of the other agents evoked significant alterations in the levels of ERK phosphorylation.

When the same range of compounds were assessed in the presence of 1 μM 2AG, a further complex pattern was observed. As expected, AM630 blocked the 2AG response, while GP2A also appeared to act as an antagonist/inverse agonist. COR170 failed to alter 2AG-evoked ERK phosphorylation. CB65 and GW405833 appeared to act as antagonists, while combinations of CP55940, fenofibrate, HU308, MDA19, L759656 and SER601 with 2AG evoked responses greater than those to 2AG alone.

CP55940 is an apparently efficacious CB_2 receptor agonist independent of the pathway analysed, while fenofibrate, HU308 and MDA19 evoke a similar pattern, albeit less effective than CP55940. GW405833 appears to be a low efficacy agonist/antagonist, dependent on the signalling pathway studied. COR170 appears to be ineffective in any of the signalling pathways, while SER601 appears to be an ERK-biased agonist, at least compared to cAMP inhibition or calcium elevations.

PREDATOR ODOR STRESS ALTERS ENDOCANNABINOID SYSTEM PROTEIN EXPRESSION IN THE RAT BASOLATERAL AMYGDALA

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Individuals who experience life-threatening psychological trauma are at risk for developing neuropsychiatric pathologies that include post-traumatic stress disorder (PTSD) and drug addiction. Because PTSD and alcohol use disorder are highly co-morbid, it is critical to understand the biological basis for excessive alcohol drinking by humans with PTSD. The endocannabinoid (eCB) system is a modulator of the stress response, in part by regulating the activity of the amygdala and the hypothalamic–pituitary–adrenal axis.

Our lab is focused on understanding the long-term effects of stress on basolateral amygdala (BLA) eCB system protein expression and signaling changes, as well as understanding how these changes contribute to addiction-related behavior. Here, we used a predator odor model of traumatic stress in male Wistar rats to test the effects of stress on BLA eCB system protein expression over time. Using a predator odor stress conditioned place aversion model (bobcat urine), male and female rats were divided into groups (Avoider, Non-Avoider, unstressed Control) based on stress reactivity, as measured by avoidance of the odor-paired context. Rats were euthanized 2 or 16 days post-stress and the BLA was bilaterally extracted. Using Western Blot analyses, we measured the expression of endocannabinoid system components in this area.

Male rats exposed to predator odor presented a trend to increase the expression of DAGLα 16 days after stress, as well as a trend to decrease the expression of MAGL 2 and 16 days after stress. These results suggest that BLA eCB signaling may be involved in long-term stress effects on behaviors, including the higher post-stress alcohol drinking typically observed in Avoiders. We are currently testing our hypotheses that higher levels of 2-AG in BLA of Avoiders disinhibits BLA output by reducing GABAergic inhibition of BLA pyramidal neurons. Intra-BLA infusions of Rimonabant and D034 may reduce post-stress alcohol self-administration in Avoiders, providing a potentially new therapeutic strategy for reducing alcohol drinking in those living with the chronic effects of trauma.

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INTERPRETING THE STRUCTURES OF THE CANNABINOID RECEPTORS

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The recent release of multiple structures for the CB_1 and CB_2 cannabinoid receptors allows some insight into the ligand pharmacology associated with these targets. Thus far, there are published structures of the CB_1 receptor with two antagonists (taranabant and AM6538), two agonists (AM11542 and AM841), and a complex of the CB_1 receptor with G_i and the synthetic cannabinoid receptor agonist MDMB-FUBINACA in the presence of a positive allosteric modulator (ZCZ011). For the CB_2 cannabinoid receptor, this year a structure was published of a complex with the antagonist AM10257.

The antagonist-bound CB_1 structures reveal several unique structural features that contrast with the ligand binding seen in other GPCRs. In particular, the *N*-terminus forms a V-shaped loop that dips into the binding pocket and participates directly in ligand binding. The extracellular parts of helices I and II also extend outwards, creating a channel formed by helices I, II, and VII that leads to a significantly larger binding pocket. As expected, this binding pocket is highly hydrophobic, with the ligand itself binding deeper within the helix bundle and displaced laterally relative to the centre of the bundle. The structures of the bound CB_1 antagonists can generally be considered as triskelion in nature, with each of the three "arms" occupying a specific part of the binding pocket. In contrast, the agonist-bound CB_1 structures show that the *N*-terminus is not directly involved in ligand binding, instead forming a short α-helix that sits on top of the binding pocket. Relative to the antagonist-bound structures, the extracellular parts of helices I and II are displaced inward, in tandem with inward rotation of Phe170$^{2.57}$ and Phe174$^{2.61}$ that occupy the binding pocket. The result is a binding pocket that is markedly smaller when compared to the antagonist-bound structures. The ligands in the agonist-bound structures adopt a "C"-shaped conformation that is distinct and more central compared to the antagonists.

Studying the antagonist-bound CB_2 structure revealed that while the arrangement of the intracellular half of the helix bundle was similar to the antagonist-bound CB_1 structure, the extracellular half was distinct and shared more in common with the agonist-bound CB_1 structure instead. Similar to the antagonist-bound CB_1 structure, its *N*-terminus forms a short α-helix over the binding pocket and isn't directly involved in ligand binding. As the CB_2 antagonist AM10257 was rationally designed starting from the selective CB_1 antagonist rimonabant, it exhibits the same triskelion structure centred around a pyrazole core similar to AM6538. However, the ligand is bound in a conformation that matches more closely the CB_1 agonists rather than the CB_1 antagonists. This potentially represents a distinct mechanism of stabilizing the inactive state when compared to the CB_1 antagonists, which bind approximately 5 Å away from the twin toggle switch. The similarities in binding between AM10257 and CB_1 agonists are further reflected in its pharmacology, where it acts as a partial agonist at CB_1 receptors.

The distinct binding modes of CB_1 receptor antagonists and CB_2 receptor antagonists revealed through these structures provide a rational explanation for the high degree of subtype selectivity seen among cannabinoid receptor antagonists. The future resolution of an agonist-bound CB_2 structure would then provide a complete picture of the 'yin yang' relationship between CB_1 and CB_2 receptors and some of their ligands. This added detail would hopefully add towards the rational design of cannabinoid ligands to facilitate future therapeutic exploitation.

THE EFFECT OF OLEOYL-GLYCINE ON REINSTATEMENT OF PREVIOUSLY EXTINGUISHED MORPHINE PLACE PREFERENCE IN RATS

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Oleoyl glycine (OlGly), a newly isolated fatty acid amide, has a chemical structure much like other N-acylethanolamines, including anandamide, an endogenous cannabinoid of the body's endocannabinoid system. Recently, OlGly has been shown to interfere with nicotine reward and dependence in mice (Donvito et al, 2019). Using place conditioning procedures, we have shown OlGly interferes with the aversive properties of opioid withdrawal in rats, but did not influence the rewarding properties of opioids (Petrie et al, 2019). The current experiment sought to extend on these findings by determining the effect of OlGly on the reinstatement of a morphine conditioned place preference in Sprague Dawley rats.

Rats (n=36) received four conditioning cycles with subcutaneous injections of saline or 10 mg/kg morphine (24 h apart; counterbalanced order) 10 min before placement in a conditioning chamber with tactically distinct grid or hole flooring (counterbalanced) for 30 min. Following conditioning, the rats received 4 10-min test trials until the morphine place preference had extinguished. Twenty-four hr following the final extinction trial, the rats received a 10 min reinstatement test during which they were injected (i.p.) with VEH, 1.0 mg/kg, or 5.0 mg/kg OlGly 10 minutes prior to a priming injection of 2.5 mg/kg morphine 10 min prior to placement in the test chambers.

At doses of 1 and 5 mg/kg, OlGly did not interfere with the reinstatement of a previously extinguished morphine conditioned place preference. Taken together, these findings suggest OlGly only interferes with the aversive properties of opioid withdrawal, without interfering with the drug's rewarding properties. Therefore, OlGly may be a new therapeutic target for reducing the impact of opiate withdrawal.

Donvito, G, Piscitelli F, et al (2019) Oleoyl glycine reduces nicotine reward and withdrawal in mice. *Neuropharmacology*, 148, 320-331.

Petrie, GN, Wills, KL, Piscitelli, F et. al. (2019) Oleoyl Glycine: Interference with the aversive effects of acute naloxone-precipitated MWD, but not morphine reward in male Sprague-Dawley rats. *Psychopharmacology,* in press.

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CANNABIS EXTRACTS AS ANTI-TUMOR AGENTS: EVIDENCE FROM CANCER CELL LINES

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The *Cannabis* plant contains over 100 phytocannabinoids and hundreds of other components. The biological effects and interplay of these *Cannabis* compounds are not fully understood and yet influence the plant's therapeutic effects. Regardless the use of *Cannabis* as a palliative treatment in oncology, in the last decade, accumulating evidence indicates that cannabinoids might also have antitumor effects. In this study we assessed the antitumor effects of whole *Cannabis* extracts, which contained significant amounts of differing phytocannabinoids, on different cancer lines from various tumor origins. We first utilized our novel electrospray ionization liquid chromatography mass spectrometry method to analyze the phytocannabinoid contents of 124 *Cannabis* extracts. We then monitored the effects of 12 chosen different *Cannabis* extracts on 12 cancer cell lines. Our results show that specific *Cannabis* extracts impaired the survival and proliferation of cancer cell lines as well as induced apoptosis. Our findings showed that pure (-)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) did not produce the same effects on these cell lines as the whole *Cannabis* extracts. Furthermore, *Cannabis* extracts with similar amounts of Δ^9-THC produced significantly different effects on the survival of specific cancer cells. In addition, we demonstrated that specific *Cannabis* extracts may selectively and differentially affect cancer cells and differing cancer cell lines from the same organ origin. We also found that cannabinoid receptors were differentially expressed among various cancer cell lines and suggest that this receptor diversity may contribute to the heterogeneous effects produced by the differing *Cannabis* extracts on each cell line. Our overall findings indicate that the effect of a *Cannabis* extract on a specific cancer cell line relies on the extract's composition as well as on certain characteristics of the targeted cells.

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LOCALIZATION OF ENDOCANNABINOID SYNTHESIZING ENZYME NAPE-PLD DURING CEREBELLAR DEVELOPMENT

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The endocannabinoid system (ECS), which consists of cannabinoid receptors and enzymes involved in the synthesis and degradation of the endogenous cannabinoid signaling lipids, is highly expressed in the cerebellar cortex of adult humans and rodents, where it is involved in an array of functions ranging from short-term synaptic plasticity to regulation of microglia polarization. Endocannabinoid (eCB) synthesizing enzymes, acyl-phosphatidylethanolamine (NAPE-PLD) and diacylglycerol lipase alpha and beta (DAGLα/β), are the major contributors to the production of the eCBs anandamide (N-arachidonoyl ethanolamine, or AEA) and 2-arachidonoylglycerol (2-AG), respectively. 2-AG is found in the adult CNS at several fold higher concentration than AEA, and DAGLα is highly expressed in the adult cerebella. Conversely, expression and function of NAPE-PLD in the adult cerebella and during cerebellar development remains incompletely characterized.

Immunohistochemical characterization of NAPE-PLD localization during postnatal mouse cerebellar development was carried out in combination with cerebellar cell type markers. Specificity of staining was confirmed in littermate NAPE-PLD KOs. Our results reveal temporally, spatially, and cytologically dynamic patterns of NAPE-PLD expression during cerebellar development, laying the groundwork for a comprehensive investigation of specific cellular and molecular mechanisms regulated by AEA signaling during cerebellar development.

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CUMULATIVE EXPERIENCE FROM CLINICAL TRIALS IN ISRAEL: REALITIES AND CHALLENGES

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Tikun Olam is the largest medical cannabis provider in Israel since 2007. Since that time it initiated several clinical trials as well as preclinical studies of medical cannabis, all of them based on cannabis strains grown and cultivated in Israel, with varying concentrations of THC and CBD. The results of these studies will be presented as follows: 1) Retrospective studies analyzing the response of over 2000 patients to cannabis that clearly demonstrates the significant effect of cannabis on pain, nausea and sleep and also highlighting the positive effect on well-being and on decrease in use of medications. 2) Prospective and retrospective studies on inflammatory bowel disease (IBD) - both Ulcerative Colitis (UC) and Crohn's Colitis (CC) - demonstrating the significant beneficial clinical effect of CBD rich. cannabis in both diseases and in patients failing regular therapy. 3) Prospective studies of children with autism demonstrating very significant clinical improvement in behavior, cognition and social interaction in around 70 % of the participants in the trials.

The challenges that these clinical trials of medical cannabis have encountered will then be presented and discussed. These will include regulatory barriers, standardization of dosages, individual variation in response, whole plant versus pure chemicals, variation of plant source, proper control arms and placebos.

PERINATAL EXPOSURE TO THC DISRUPTS CEREBELLAR DEVELOPMENT

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The recent upsurge in legalization and positive public perception of cannabis has resulted in a higher prevalence of perinatal exposure, raising the urgency for better understanding the role of endogenous cannabinoid (eCB) signaling in brain development, and the neurodevelopmental consequences of perinatal exposure to phytocannabinoids.

Our results demonstrate a robust and dynamic expression of the eCB system in the cerebellum throughout development. Furthermore, using gross anatomical criteria, we show that eCB signaling plays a prominent role in cerebellar development, since genetic ablation of cannabinoid receptor 1 (CB1) leads to an increase in foliation of the anterior-most midvermis and decrease in midvermal cerebellar area, especially of the anterior region. Perinatal exposure (E17.5 – P2) to a low dose of the phytocannabinoid Δ-9-tetrahydrocannabinol (THC) alters cerebellar developmental trajectory in a similar fashion. This anatomical phenotype is accompanied by alterations in rotarod performance in THC-treated animals. Thus, our results indicate that perinatal exposure to THC alters the size and patterning of the cerebellar vermis and performance of a cerebellar-linked behavior.

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BODY MASS INDEX BUT NOT SLEEP IMPACTS THE ENDOGENOUS CIRCADIAN RHTYHM OF THE ENDOCANNABINOID ANADAMIDE IN HUMANS

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The prevalence of obesity has doubled over the last 25 years. Obesity increases the risk for heart disease, diabetes, depression, and many cancers, and costs the US healthcare system around $190 billion per year (~20% of healthcare expenditures). Recent studies suggest that obesity, metabolic disorders, and circadian rhythms are interconnected making therapeutic strategies that target the circadian clock a plausible mechanism to reduce weight. It has previously been established that anandamide (AEA) tone is elevated with increased body mass index (BMI) and that among young non-obese adults plasma levels of AEA exhibit a diurnal variation. We sought to determine the endogenous circadian profile of AEA and how this distribution may vary by BMI. Thirteen healthy participants (mean age, 51 years; 9 females; 8 non-obese [mean BMI, 24.5 kg/m^2]; 5 obese [mean BMI, 36.7 kg/m^2]) underwent a laboratory protocol that balanced eucaloric meals and sleep opportunities evenly across the circadian cycle (achieved by scheduling 10 identical, recurrent 5 h 20 min sleep/wake cycles in dim light thereby desynchronizing the circadian and behavioral cycles). Blood was sampled before sleep and upon awakening through a midline catheter. AEA was quantified by liquid chromatography-mass spectrometry. Salivary melatonin was used to assess circadian phase (phase marker = dim-light melatonin onset [DLMO]). Sleep quality was measured using polysomnography.

Average plasma AEA was lower in non-obese compared to obese participants (means: 0.4 pmol/mL versus 0.8 pmol/mL, respectively; p=0.024). There was an endogenous rhythm of plasma AEA and this was driven by obese participants: peak to trough range 0.65-0.96 pmol/mL; peaking in the biological afternoon (~2:45pm, ~18 hours after DLMO; p=0.01) with no significant rhythm in non-obese participants. Although the sleep opportunity was balanced across the circadian cycle with a 1:1 sleep to wake ratio, due to the circadian drive for alertness, sleep efficiency ranged from 0-98%. Despite previous findings of elevated levels of AEA following sleep loss, we found no correlations across the protocol between plasma AEA and sleep latency, sleep efficiency or amount of rapid eye movement sleep (REM). Further, in mixed model analysis, measures of sleep did not significantly alter the circadian model nor were the circadian rhythms of AEA different when measured immediately before or after sleep. Thus, the variation in AEA across 24 hours is modulated by the circadian system in obese individuals, whereas levels remain relatively constant in non-obese healthy adults. Future studies are needed to determine if the 1.5-fold increase of peak to trough in AEA in obese adults is related to a chronic increase in caloric intake that may account for increased BMI.

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CELL-SPECIFIC CB_2R DEFICIENCY ATTENUATES CHRONIC INTESTINAL INFLAMMATION

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We have previously demonstrated that the CB_2R ligand GP1a can treat established ileitis in a murine model of inflammatory bowel disease (IBD). Questions regarding the agonism or inverse agonism of GP1a, prompted us to examine the contribution of cell-specific deletion of CB_2R in our TNF-driven ileitis model. We focused on the contribution of *CNR2* expression by $CD4^+$ T cells because of their central role in IBD pathogenesis. We found that CD4-specific CB_2R deficiency significantly attenuated intestinal inflammation and decreased central memory T cell frequency in the ileum. Using single-cell RNA sequencing on intestinal $CD4^+$ T cells, we identified a unique metabolic gene signature on *Cnr2* expressing cells, which we validated with conventional PCR on flow-sorted CB_2R-GFP^+ cells. This signature was consistent with activation of the pentose phosphate pathway and suppression of T cell glycolysis, a process that may suppress T cell proliferation in the short term but ultimately promotes the persistence of long-lived memory T cells that contribute to the chronic nature of the disease. We demonstrated a direct impact on glucose handling also, with CB_2R agonist JWH133 increasing uptake of a fluorescent glucose analog and inverse agonism with GP1a suppressing it in isolated T cells *in vitro*, similar to what has been reported in microglia. CB_2R^+ T cells also displayed a significant increase in the gut-homing integrin α4β7 and with increased binding to its cognate ligand MAdCAM-1 *in vitro*. We then recapitulated these findings *in vivo* using a competitive homing assay in which fluorescently labeled CB_2R-deficient $CD4^+$ T cells displayed reduced homing to the ileum relative to littermate controls. Thus, chronic activation of the CB_2R on $CD4^+$ T cells may select for long-lived memory T cells with enhanced invasive potential resulting in the exacerbation of IBD.

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THE REALM OF CARING OBSERVATIONAL RESEARCH REGISTRY: EVALUATING THE HEALTH IMPACTS OF MEDICINAL CANNABIS USE

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Aims: Due to the rapid expansion of medicinal cannabis availability through legislative action rather than traditional drug development, uncertainties remain regarding the safety and efficacy of cannabinoids as therapeutic agents. A comprehensive understanding of the consumers and products involved in cannabinoid therapies could assist both public health and commercial interests in best serving this rapidly growing industry. This study was conducted in a convenience sample of individuals from across the United States that were registered with the Realm of Caring Foundation, a non-profit organization located in Colorado, USA. The aim of this study was to characterize basic demographic and health profiles of patients currently using cannabis products for therapeutic purposes versus a control group of individuals who were considering medicinal cannabis use, but had not yet started cannabinoid therapy.

Methods: Patients registered with the Realm of Caring Foundation completed web-based surveys from 2016-2018 that queried participants with respect to demographics, health problems, use of cannabis and other medication, and validated assessments of pain (Numeric Rating Scale), anxiety and depression (Hospital Anxiety and Depression Scale), sleep (Pittsburgh Sleep Quality Index for adults and the abbreviated Children's Sleep Habits Questionnaire; CSHQ for children), and quality of life (World Health Organization Quality of Life). Independent samples t-tests and chi-square analyses were used to assess differences between medicinal cannabis users and controls.

Results: Medicinal cannabis users (N=808) and controls (N=468) were demographically similar except for age (mean (SD) age 38 (20) years vs. 35 (21) years respectively). Medicinal cannabis patients predominantly used high cannabidiol (CBD) containing products. Compared with controls, medicinal cannabis users self-reported significantly better ratings of quality of life, satisfaction with health, sleep, pain, anxiety and depression compared with controls. Cannabis users also reported less concomitant medication use and fewer past month ER visits and hospital admissions than controls.

Conclusions: In a convenience sample of individuals with significant health problems, medicinal cannabis users reported significantly better health than controls on a number of health domains. This suggests that there may be some added benefit of cannabinoid use on current health care utilization and outcomes for certain health conditions. Additional research is needed to prospectively evaluate the impact of cannabinoid use on health-related outcomes and to identify specific health conditions and cannabinoid product characteristics that are associated with greater therapeutic benefit.

AN UP-SCALABLE COMBINED GENETIC-BEHAVIORAL APPROACH USING CB2-KO ZEBRAFISH LARVAE

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The cannabinoid receptor 2 (CB2) was previously implicated in brain functions including complex behaviors. Here, we assessed the role of CB2 in selected swimming behaviors in zebrafish larvae and developed an *in vivo* up-scalable whole-organism approach for CB2-ligands screening. Using CRISPR/Cas9 technology, we generated a novel null allele (*cnr2*^{upr1}) and a stable homozygote viable loss-of-function (CB2-KO) line. We measured in untreated wild type and *cnr2*$^{upr1/upr1}$ larvae photo-dependent swimming responses (PDR) and center occupancy (CO) to establish quantifiable anxiety-like parameters. Next, we measured PDR alteration and CO variation while exposing wild type and mutant animals to an anxiolytic drug (valproic acid, VPA), or to an anxiogenic drug (pentylenetetrazol, PTZ). Finally, we treated wild type and mutant larvae with two CB2-specific agonists (JWH-133 and HU-308) and two CB2-specific antagonists, inverse-agonists (AM-630 and SR-144528). Untreated CB2-KO showed a different PDR than wild type larvae as well as a decreased CO. VPA treatments diminished swimming activity in all animals but to a lesser extend in mutants. CO was strongly diminished and even more in mutants. PTZ-induced inverted PDR was significantly stronger in light and weaker in dark periods and the CO lower in PTZ-treated mutants. Finally, two out of four tested CB2-ligands had a detectable activity in the assay. We showed that larvae lacking CB2 behave differently in complex behaviors that can be assimilated to anxiety-like behaviors. Mutant larvae responded differently to VPA and PTZ treatments, providing *in vivo* evidence of CB2 modulating complex behaviors. We also established an up-scalable combined genetic-behavioral approach in a whole organism that could be further developed for high throughput drug discovery platforms.

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LOSS OF STRESS- AND EXERCISE-INDUCED INCREASES IN CIRCULATING 2-ARACHIDONOYLGLYCEROL CONCENTRATIONS IN ADULTS WITH PTSD

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The endocannabinoid (eCB) system is a modulatory system that is both altered by stress and mediates the effects of acute stress, including contributing to restoration of homeostasis. Earlier studies suggest that circulating eCBs are dysregulated in adults with post-traumatic stress disorder (PTSD); however, it is not known whether circulating eCBs remain responsive to stress. The purpose of this study was to examine eCB and psychological responses to physical (exercise) and psychosocial (Trier Social Stress Test) stressors, using a randomized, counterbalanced procedure in adults with PTSD and healthy controls (N = 20, mean age = 24, SD = 7 yrs). Results from mixed-design, repeated measures ANOVAs revealed significant increases ($p < .05$) in negative mood states (tension, negative affect, state anxiety) following exposure to psychosocial stress in adults with PTSD and healthy controls. On the other hand, exercise produced significant reductions ($p < .05$) in negative mood states (tension, depression, anger, total mood disturbance, negative affect, state anxiety), as well as significant increases ($p < .05$) in positive affect and vigor in both groups. N-Arachidonoylethanolamine (AEA) and oleoylethanolamide (OEA) increased significantly ($p < .05$) following exercise and psychosocial stress in both groups. However, only the control group exhibited a significant increase ($p < .05$) in 2-arachidonoylglycerol (2-AG) following exercise and psychosocial stress exposure. These data extend our current understanding of circulating eCB responsiveness in PTSD, and provide preliminary evidence to suggest that the eCB system is hypoactive in PTSD following exposure to physical and psychosocial stressors.

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IMPROVED WHITE MATTER INTEGRITY FOLLOWING THREE AND SIX MONTHS OF MEDICAL CANNABIS TREATMENT

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Diffusion tensor imaging (DTI) assesses brain microstructure and provides a quantitative measurement of the white matter fiber tract integrity in the brain. Fractional anisotropy (FA) measures white matter integrity by measuring direction-dependent diffusion of water along axon bundles. Mean diffusivity (MD) measures overall isotropic water diffusivity in all directions and is usually inversely related to FA. Previous studies of recreational cannabis users have demonstrated decreased FA and increased MD in cannabis users relative to controls, with earlier age of onset of cannabis use associated with reduced white matter integrity. To date, however, no studies have examined the impact of medical cannabis (MC) use on white matter integrity despite growing interest regarding medical applications for cannabis as well as increased access to MC products. In fact, 33 states and Washington DC have fully legalized MC and an additional 14 states allow limited access to some MC products.

As part of a larger, longitudinal study, patients interested in using MC for a variety of indications were assessed before initiating MC treatment (baseline) and returned for follow-up assessments following 3 and 6 months of MC treatment. DTI data were acquired on a Siemens Trio 3T magnet using a 12-channel phased array head coil in 30 noncollinear directions and 3 b-value diffusion weights of 0, 1000, and 2500 s/mm^2. Region of interest (ROI) analyses were used to determine FA and MD values of fiber tracts in the corpus callosum, and included bilateral assessment of the genu, anterior corona radiata, anterior limb of the internal capsule, and the external capsule.

Following 3 months of MC treatment, patients demonstrated significantly *increased* FA values bilaterally in the genu, anterior corona radiata, and external capsule as well as in the left anterior limb of the internal capsule relative to their baseline values. After 6 months of MC treatment, patients continued to demonstrate *increased* FA values bilaterally in the genu, anterior limb of the internal capsule, and external capsule as well as in the right anterior corona radiata relative to both their baseline and 3-month values. Although MD values were not significantly different between baseline and 3 months of MC treatment, following 6 months of MC treatment, patients demonstrated significantly *decreased* MD values bilaterally across all ROIs relative to baseline.

Data from the current study suggest white matter fiber tract changes following MC treatment and are particularly interesting given previous findings of *decreased* white matter integrity among recreational cannabis users. Taken together with previous findings of improved cognitive performance among MC patients after 3 and 6 months of treatment, these data suggest a differential impact of cannabis use on brain microstructure in MC patients, which may be related to differences between recreational and medical cannabis-using populations including age of onset of use, product choice, and frequency/magnitude of use.

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EFFECTS OF ATYPICAL CANNABINOIDS ON BREAST CANCER CELLS VIABILITY

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It is estimated that 1 in 8 women will develop breast cancer at some time during their life. Although the mortality rates associated with this disease are decreasing, many patients present resistance to current therapies despite initial responsiveness. New therapeutic approaches are therefore warranted for the management of these cases. Extensive preclinical research has demonstrated that cannabinoids, the active ingredient of Cannabis sativa, trigger antitumor responses (anti-proliferation, anti-metastasis, etc.) in different cancer models.

Breast cancer cell lines that are resistant to Paclitaxel, a chemotherapeutic agent, or sensitive to it were used in our study. Several cannabinoids were tested on these cell lines and two atypical cannabinoids exhibited anti-tumour effects. Here, we show that O-1602 and Abnormal Cannabidiol were able to effectively decrease breast cancer cells viability, even when these cells were resistant to Paclitaxel chemotherapy. The same cannabinoids were also investigated for their effects on cell migration, and displayed effects reducing cell migration in a concentration-dependent fashion. Finally, the cannabinoids O-1602 and Abnormal Cannabidiol induced apoptosis of Paclitaxel-resistant breast cancer cell lines.

Our results suggest that cannabinoids could potentially be useful in cancer therapy, not only for their palliative effects, but also for their anti-tumour properties.

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TESTING CANNABINOIDS IN HUMAN PANCREATIC CANCER MODELS IN COMBINATION WITH CURRENT CHEMOTHERAPY AGENTS

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A recent paper from our group has shown evidence that G-protein coupled receptor 55 (GPR55) promotes pancreatic cancer progression. GPR55 has been shown to be a putative cannabinoid (CB) receptor of the endogenous cannabinoid system and, in pancreatic cancer, the use of synthetic cannabidiol (CBD) increases the effect of chemotherapy in a mouse model of pancreatic cancer. These findings have highlighted the potential for phyto-cannabinoids extracted from the *Cannabis sativa* plant to be used in cancer therapy. The aim of our work is to assess whether different combinations of synthetic or naturally-derived phyto-cannabinoids, can improve the potency of conventional chemotherapies against pancreatic cancer *in vitro* and *in vivo*. The overall aim is to develop new, more potent therapeutic options for treating pancreatic cancer sufferers. In addition, we aim to better understand the mechanisms of action of phyto-cannabinoids as anti-cancer agents.

We have tested different *C. sativa* plant extracts, provided by Zelda Therapeutics, alone, and in combination with existing chemotherapies, in human pancreatic cancer cell lines. We have performed dose response experiments and cell-viability tests. In addition, we are conducting pre-clinical studies using a genetically modified mouse model, the KPC (KrasLSL.G12D/+; p53R172H/+; PdxCretg/+), which is a clinically relevant mouse model for pancreatic cancer. We aim to validate the safety and the dosage for using phyto-cannabinoids from plant extracts in order to develop guidelines for a new therapeutic option for cancer sufferers, in combination with the standard chemotherapy. *C. sativa* plant extracts contain a heterogeneous mixture of over a hundred lipid-soluble cannabinoids in different concentrations. Preliminary data show a synergistic effect for some combinations of extracts over corresponding mono-therapies. This study highlights the potential for plant-derived phyto-cannabinoids to be used in a novel adjunct therapy for treatment of pancreatic cancer. These findings are supported by anecdotal reports from cancer patients who after initially using plant-derived cannabis to reduce toxic side effects of chemotherapy have, in some cases, reported better than expected therapeutics outcomes. To better understand mechanism of action, we are investigating how cannabis interacts with different cannabinoid receptors on cancer cells and what impact his has upon downstream signaling pathways.

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THE EFFECT OF CANNABIS ON PERFORMANCE DURING NEUROPSYCHOLOGICAL TESTING

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This study was designed to evaluate the effects of cannabis on cognitive functioning during an acute stage of intoxication following a 10-minute period of smoking cannabis with 20% concentration of Tetrahydrocannabinol (THC), compared to a relative state of deprivation (Baseline) and a period of 2-3 hours delay without any further cannabis consumption (Recovery). Participants (n = 22, 59.1% men) were community volunteers with a medical marijuana license (M_{Age} = 36.0 years, $M_{Education}$ = 13.7 years). Average amount of daily cannabis consumption was reported to be 3.2 grams. A brief battery of neuropsychological tests [Boston Naming Test- Short Form (BNT-15), category fluency, Coding, Digit Span, Stroop and Trail Making Test] was administered three times (during Baseline, THC and Recovery).

Performance on most tests either remained stable or improved from Baseline to THC (Cohen's d: 0.49-0.65, medium effect). Mean performance continued to increase during Recovery (Cohen's d: 0.45-0.51, medium effect). Overall effects ranged from small (η_p^2: 0.029 – Digit Span) to extremely large (η_p^2: 0.283 – BNT-15 completion time). Interestingly, failure rates on performance validity indicators increased during THC, and dropped to zero during Recovery. Contrary to expectations (a V-shaped pattern of performance was predicted, with a notable decline during THC), our results provide no clear evidence of decline in test performance following cannabis consumption. However, until several potential confounding variables have been ruled out, it would be premature to interpret the findings as evidence that cannabis use is unrelated to cognitive functioning. Alternative explanations include learning effects and the deleterious effects of THC cancelling each other out or the complex relationship among pain, THC and cognitive performance. Future research using double-baselines, higher doses of THC, more comprehensive test batteries and recreational cannabis users with lower levels of tolerance is clearly needed to understand the effect of cannabis on cognitive functioning.

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HUMAN LEUKOCYTES DIFFERENTIALLY EXPRESS ENDOCANNABINOID-GLYCEROL LIPASES AND HYDROLYZE 2-ARACHIDONOYL-GLYCEROL AND ITS METABOLITES FROM THE CYCLOOXYGENASE-2 AND 15-LIPOXYGENASE PATHWAYS

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2-Arachidonoyl-glycerol (2-AG) is an endocannabinoid with anti-inflammatory properties. Blocking 2-AG hydrolysis to enhance CB_2 signaling has proven effective in mouse models of inflammation. However, the expression of 2-AG lipases has never been thoroughly investigated in human leukocytes. Herein, we investigated the expression of seven 2-AG hydrolases by human blood leukocytes and alveolar macrophages (AMs) and found the following protein expression pattern: MAG lipase (eosinophils, AMs, monocytes), CES1 (monocytes, AMs), PPT1 (AMs), ABHD6 (mainly AMs), ABHD12 (all), ABHD16A (all), and LYPLA2 (monocytes, lymphocytes, AMs). All leukocytes could hydrolyze 2-AG and its metabolites derived from cyclooxygenase-2 (prostaglandin E_2-glycerol (PGE_2-G)) and the 15-lipoxygenase (15-hydroxy-eicosatetraenoyl-glycerol (15-HETE-G)). Neutrophils and eosinophils were consistently better at hydrolyzing 2-AG and its metabolites than monocytes and lymphocytes. Moreover, the efficacy of leukocytes to hydrolyze 2-AG and its metabolites was 2-AG $\geq$ 15-HETE-G > PGE_2-G for each leukocyte. Using the inhibitors MAFP, JZL184, Palmostatin B, WWL70, WWL113, THL and ML349, we could not pinpoint a specific hydrolase responsible for the hydrolysis of 2-AG, PGE_2-G and 15-HETE-G by these leukocytes. Furthermore, JZL184, a selective MAG lipase inhibitor, blocked the hydrolysis of 2-AG, PGE_2-G and 15-HETE-G by neutrophils and the hydrolysis of PGE_2-G and 15-HETE-G by lymphocytes, two cell types with limited/no MAG lipase. Using an activity-based protein profiling (ABPP) probe to label hydrolases in leukocytes, we found that they express many MAFP-sensitive hydrolases and an unknown JZL184-sensitive hydrolase of ~52 kDa. Altogether, our results indicate that human leukocytes are experts at hydrolyzing 2-AG and its metabolites via multiple lipases and probably via a yet-to-be characterized 52 kDa hydrolase. Blocking 2-AG hydrolysis in humans will likely abrogate the ability of human leukocytes to degrade 2-AG and its metabolites and increase their anti-inflammatory effects *in vivo*.

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CANNABINOID RECEPTORS BIASED SIGNALLING – IN DEPTH PATHWAY ANALYSIS APPLYING A DIVERSE COMPOUND LIBRARY

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G-protein coupled receptors (GPCRs) are the largest class of membrane proteins in the human genome, and are known to mediate a wide range of cell signalling processes. The cannabinoid receptor type 1 (CB_1R) and 2 (CB_2R) are key elements of the endocannabinoid system, and represent one subgroup of the GPCR superfamily. Signalling impairment of these receptors is related to various inflammatory conditions, such as neuropathy, nephropathy, pruritus, osteoporosis, and Alzheimer's disease. Despite the evident potential of CB_1R and CB_2R as drug targets, molecular and functional mechanisms driven by receptor-ligand interactions remain poorly understood.

To get a better insight on cannabinoid receptor mode of action, a multidimensional ligand screen on CB_1R and CB_2R signalling was performed. In total, 35 ligands which are highly diverse with regard to structure and properties were tested, including endocannabinoids, phytocannabinoids, and synthetic compounds. G protein activation and β-arrestin recruitment were monitored via a bioluminescence resonance energy transfer (BRET) assay in live HEK293 cells. Subsequently, a concentration-dependent response was measured for all activated signalling pathways. A maximal ligand-induced response (E_{max}) and negative logarithm of half-maximal response concentration (pEC_{50}) were determined for each ligand.

We will disclose the identification of cannabinoid ligands with a wide range of efficacies and potencies, as well as ligands biased towards forming complexes with either G proteins or β-arrestin. Moreover, we could confirm that both receptors couple mainly to the $G_{i/o}$ family, with CB_1 being less selective and activating also $G_{12/13}$ and G_{15}. Most interestingly, we will communicate that the endogenous cannabinoid 2-arachidonoyl glycerol (2-AG) shows bias towards β-arrestin recruitment relative to tested synthetic and phytocannabinoids. Eventually, these new insights into the molecular basis of cannabinoid receptors signalling may be utilized to design new drugs which modulate only the therapeutically beneficial pathway.

REWARDING PROPERTIES OF HEROIN DURING ACUTE DOSING, TOLERANCE, SELF-ADMINISTRATION, DEPENDENCE, AND WITHDRAWAL: IMPACT OF FATTY ACID AMIDE HYDROLASE (FAAH) INACTIVATION

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Over the past several years, our lab has presented research on the reduced escalation of opiate intake, as well as reduced motivation to self-administer opiates under extended-access conditions. We have examined these findings primarily in the context of stress regulation. However, an alternative source of neurobiological regulation of opioid intake could be differential sensitivity to the rewarding properties, or the tolerance to reward-like effects with repeated exposure. Using both passive injection and active self-administration, in combination with either PF3845 inhibition or FAAH knockout rats, we have used the discrete-trial method of intracranial self-stimulation (ICSS) as a means of gauging reward activity and mood. Electrodes implanted in the medial forebrain bundle (an intermediary structure along the mesolimbic dopamine pathway) are an established model of measuring the rewarding properties of drugs, aversive properties of higher doses, and aversion of acute drug withdrawal.

PF3845 (2mg/kg) did not significantly alter ICSS thresholds alone, nor did FAAH knockout rats have significantly different baseline thresholds. Acute heroin dose-responses show that FAAH knockout rats appear more sensitive to the aversive properties of heroin at higher doses, while both genotypes show tolerance to the aversive effects following chronic heroin (1mg/kg s.c., 2x daily for 6 days). Daily pretreatment with PF3845 did not alter rewarding effects due to short-access 1-hr heroin self-administration, with both PF3845 and vehicle-treated animals showing lowered thresholds immediately post-session. However, as rats were transitioned to 12-hr self-administration, both intake of heroin and post-session ICSS measurements were elevated over time in vehicle-treated rats but were absent in the PF3845-treated rats. During protracted withdrawal, both groups showed similar and rapid recovery to pre-heroin baselines. Overall, the data suggests a selective difference in levels of aversion immediately following high heroin exposure, while not preventing the rewarding effects of lower doses, which may be one contributing factor in preventing the FAAH inhibited rats from engaging in excessive intake. This may provide a model with which to predict the effectiveness of other potential treatments for opioid addiction.

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CANNABIS USE DISORDER AND LONG-TERM POST-DISCHARGE SURVIVAL IN TRAUMA INPATIENTS

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Psychoactive substance use disorder (SUD) is a leading cause of death in the United States, yet few studies have quantitatively evaluated the long-term mortality risk associated with having a SUD, or the differential risk associated with various substances. We evaluated the long-term mortality risk associated with cannabis use disorder (CUD), compared with other SUDs, in a prospective cohort constructed by linking adult trauma inpatients interviewed between 1994-1996 (baseline) to the National Death Index, a national database of death certificates, to obtain mortality follow-up from 1994-2017.1,220 unselected, consecutive adult trauma patients admitted directly from the scene of injury to a single level-one trauma center in Baltimore, Maryland, USA for at least two days were approached; 1,118 consented and 1,099 could be matched to the NDI. SUD at baseline was determined by the Structured Clinical Interview for the Diagnostic and Statistical Manual III-R. Cox proportional hazards methods were used to calculate hazard ratios (HR) adjusted for age and tobacco use.

At baseline, 199 (19.7%) subjects had lifetime CUD, of whom 106 (53.3%) had current CUD. These subjects were predominantly men (83%), non-white (58.3%), 18-35 years old (76.4%), and had at least one other SUD (80%). Over 20-year follow-up, the commonest causes of death for those with lifetime CUD were injury (41.7%), cancer (20.1%), and infection (16.7%). There was no significant difference between those with lifetime CUD and those with no lifetime SUD in the all-cause death rate (24.1% vs. 24.9%) or mean survival time (20.3 years in both groups); aHR=1.28 (95% CI 0.9-1.8). There was also no significant difference in those with current CUD in death rate (21.7% vs. 24.9%) or mean survival time (20.7 vs. 20.3 years); aHR=1.36 (0.9-2.1). In contrast, lifetime cocaine use disorder (aHR 1.66 [95% CI 1.2 to 2.2]), alcohol use disorder (aHR 1.58 [95% CI1.2 to 2.0]), and opioid use disorder (aHR1.51 [95% CI 1.1 to 2.1]) were all significantly associated with increased all-cause mortality.

Conclusions: CUD, unlike alcohol, cocaine, and opioid use disorders, was not associated with increased mortality over a 20-year follow-up period in post-discharge trauma inpatients. Findings are limited by the absence of information on SUD diagnoses and substance use during the follow-up period.

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THE EFFECTS OF CROSS-GENERATIONAL Δ^9-TETRAHYDROCANNABINOL EXPOSURE ON NICOTINE RESPONSIVITY IN ADULT OFFSPRING

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An emerging area of preclinical research has investigated whether drug use in parents prior to conception influences drug responsivity in their offspring (i.e., a 'cross-generational' effect). In one such report, male and female rats administered THC during adolescence produced male offspring that as adults displayed greater motivation to self-administer heroin and had changes in mRNA levels encoding for cannabinoid and glutamate receptors in the striatum. The present experiment extended these findings by examining the effects of a parental THC history on nicotine self-administration and locomotor sensitization in their progeny. On PND 28-49, male and female Sprague Dawley rats were administered 1.5 mg/kg THC or vehicle IP every 3rd day. On PND 65 males and females within the same dose group were bred together. All pups were cross fostered to control dams and aged to adulthood.

EXPERIMENT 1: Adult THC (F1-THC) and vehicle (F1-VEH*)* offspring of both sexes acquired nicotine self-administration at 0.03 mg/kg/infusion on an FR1 schedule over 14 days. Subjects then underwent 3 days at an FR2, FR5 and PR schedule, followed by a dose-response assessment (0.003, 0.015, 0.06) and extinction procedures. There was clear evidence of nicotine self-administration in both male and female subjects, and the two lowest doses of nicotine produced significantly more responding than the highest dose. However, there was no effect of a parental history of THC on any endpoint.

EXPERIMENT 2: Male and female subjects (F1-THC and F1-VEH) were placed in activity chambers for 1 h with no injections (baseline day). On the following 5 days, they received 0 or 0.4 mg/kg nicotine s.c. and were again placed in the chambers for a 1-h session. After 5 days of drug abstinence, a nicotine challenge (0.4 mg/kg) was given to all subjects and locomotor activity (and its sensitization) was assessed for 1 h. Nicotine produced robust locomotor sensitization in both males and females such that subjects demonstrated significantly more activity on locomotor day 5 compared to day 1. Germ-line exposure to THC had no impact on nicotine-induced locomotor activity.

An adolescent THC history in dams and sires failed to alter nicotine self-administration or locomotor sensitization in offspring. These findings suggest that mild to moderate marijuana use in parents pre-conception does not alter the reinforcing properties of nicotine in their progeny.

KINEMATIC SIGNATURES OF CANNABINOID SIGNALING IN MICE: CP55,940

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One of most pronounced behavioral effects of cannabinoid CB1 receptor activation is profound inhibitory effect on locomotor activity. However, effects of cannabinoids on subtle motor behavior have not been extensively studied. We hypothesize that at low doses that do not produce significant somatic effects but are known to evoke interoceptive effects, cannabinoids will cause changes in body kinematics during natural behavior. Such changes could be revealed by precise analysis of movement in freely moving animals. Here we demonstrate a method that may enable detecting cannabinoid activity with higher sensitivity than measurement of somatic effects or general locomotor activity parameters, without extensive training, expert observer or movement restriction.

We utilized a high-resolution 3D kinematic tracking that allows analyzing variety of behaviors with high spatiotemporal precision. We applied marker-based high-speed real-time 3D motion capture system (Qualisys) to track movement trajectories of freely behaving adult male C57BL6 mice. The mice (n=4-10 per group, within-subject design) were administered a synthetic cannabinoid, CP55,940 (0.03, 0.1, 0.3 mg/kg) or vehicle (ethanol, Kolliphor® EL, saline, at 1:1:18 ratio), i.p. 15 minutes before the start of behavioral testing. Mice were behaviorally assessed a) in a familiar open field, b) in a novel open field, c) on a vertical wire mesh (climbing task) d) on a horizontal wire. Parameters measured included gait analysis (step height, length, width, velocity, duration etc.), 3D trajectories of markers, assessment of speed, distance and activity index (average velocity of all markers). All mice were subjected to standard testing for typical cannabimimetic effects (catalepsy, analgesia, hypothermia,) 30 min following injection.

With this approach we observed changes in locomotor activity and gait characteristics with low doses of CP55,940. Preliminary data indicate that mice took shorter steps at doses that did not lead to catalepsy, hypothermia, analgesia or decrease in total distance traveled. Low-level catalepsy, hypothermia, analgesia or significant reduction in distance traveled were observed only at 0.3 mg/kg. Interestingly, while marked reduction in distance traveled was observed following dose of 0.3 mg/kg in the open field, no reduction in locomotor activity was seen in the climbing task. Also, the effects of CP55,940 in climbing and open field tasks differed. At 0.1 mg/kg locomotion was enhanced (as measured by increased activity index and distance traveled) during climbing but such effect was not observed in the open field.

The results indicate that step characteristics is a sensitive indicator of kinematic alternation following CP55,940 and that cannabinoid signaling affects locomotor activity in a task-dependent fashion. Precise movement analysis as described here could be useful in development of cannabinoid-based treatments for neurological and pain-related disorders both to assess therapeutic effects and untoward effects on fine motor control.

NOVEL MONO AND BIFUNCTIONAL CANNABINOIDS RECEPTOR PROBES

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We report the design, synthesis and biochemical characterization of novel cannabinergic ligands with remarkably high binding affinities for the cannabinoid receptors and tight/irreversible binding characteristics. These molecular probes are currently being used in studies aimed at uncovering the binding motifs of classical cannabinoids with the CB1 and CB2 receptors by using the Ligand Assisted Protein Structure (LAPS) approach which combines the use of receptor mutants and mass spectrometric proteomic analysis Our ligand design relies on the incorporation of reactive groups at judiciously chosen positions within the classical cannabinoid structure including the aliphatic chain at C3 and the substituents at C11. Reactive groups included the electrophilic isothiocyanate, nitrate ester, and cyano groups, as well as the photoactivatable azido moiety all of which are capable of tight/irreversible interactions with the target protein. Incorporation of one reactive group results in mono functional probes, while incorporation of two reactive groups leads to bifunctional ligands which can carry either a single reactive group (homo-bifunctional) or two different reactive groups (hetero-bifunctional). The novel probes behave as potent CB1 agonists as evidenced by functional data while a representative nitrate ester probe is a potent analgesic in mice. The ligands are also of great utility in developing crystals of the CB receptor-ligand complex for structural analysis using x-ray crystallography.

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THE ROLE OF THE CANNABINOID RECEPTOR (CB_2R) AND SEX DURING A MOUSE *CANDIDA ALBICANS* INFECTION

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Currently, 33 states and the District of Columbia have enacted variations of Medical Marijuana Laws (MML) legalizing *Cannabis* (marijuana) for therapeutic, and in some states, recreational adult use. In 2015, it was reported that the prevalence rates of *Cannabis* use among US adults more than doubled since 2001. This trend is consistent with the increasing number of states with MML, and the growing majority of Americans who agree with the use of *Cannabis* for therapeutic purposes. Δ^9–Tetrahydrocannabinol (THC) shares the ability with endogenous cannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in activating the central cannabinoid receptors (CB1R) and the peripheral cannabinoid receptors (CB2R). The expression levels for CB2R gene in immune tissues has been reported to be 10-100 times higher than CB1R, suggesting CB2R as having an immunomodulatory role.

THC is known to reduce immune responses to infections by certain bacterial, viral, parasitic, and fungal pathogens. In particular, our lab has reported that THC has a suppressive effect on resistance to a secondary systemic infection by *Candida albicans* (*C. albicans*) in female mice. *C. albicans* is a fungus that is a member of the normal human flora, can cause opportunistic infections ranging from mild skin infections to invasive systemic candidiasis, and is the 4th leading cause of hospital-borne infections with mortality rates reaching 30%. Males and females have been shown to have marked differences in their immune responses and resistance to various microbial infections. To investigate the role of CB2R and sex during a systemic *C. albicans* infection, CB2R wild-type (CB2R-WT) and CB2R knock out (CB2R-KO) male and female mice (n=10/sex/genotype) were injected intravenously with $1x10^6$ *C. albicans* cells/mouse. Mice (n=7/sex/genotype) were monitored daily for signs of morbidity through Day 13. Three days (D3) after infection, the remaining mice (n=3/sex/genotype) were used to assess cytokine, corticosterone, and tissue fungal load.

We found that both CB2R-WT and CB2R-KO male mice lose more weight and show similar weight loss patterns with lower survival rates as compared to CB2R-WT and CB2R-KO female mice. In addition, we found that CB2R-KO female mice tend to lose less weight than female CB2R-WT and have higher survival rates when comparing both genotypes. Corticosterone levels, a hormone associated with stress, was not significantly different between groups throughout the study. Thus, sex, and perhaps to a lesser extent CB2R, seem to play a significant role in mouse response to systemic *C. albicans* infection.

INVESTIGATION OF THE MECHANISM OF CANNABINOID-INDUCED CANCER CELL SENSITIZATION AND CELL DEATH VIA THE ACTIVATION OF DR4 AND DR5 DEATH RECEPTORS IN THE LN-18 GLIOBLASTOMA CELL LINE

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Glioblastomas are the most common type of primary brain tumors of the central nervous system and one of the leading causes of death from solid brain tumors in children as well as in adolescents. These tumors harbor a high degree of genetic heterogeneity and a diverse range of pathological entities rendering anticancer treatments very challenging. Despite recent advances in neuroimaging, neurosurgery, radiotherapy and molecular understanding of tumorigenesis, the prognosis for patients with malignant glioblastomas remains very poor. Therefore, development and characterization of potential novel drug candidates for the treatment of glioblastomas are in high need.

Cannabinoid ligands have been reported to activate several signaling pathways that are involved in the control of cell proliferation, differentiation and survival, via both CB-receptor dependent and independent mechanisms. Though the underlying molecular mechanisms of cannabinoid-induced tumor cell death have not yet fully been understood, exposure to such cannabinoids can trigger apoptosis and increase mitochondrial or ER stress, eventually leading to cancer cell death.

Death ligands and their receptors have been shown to induce apoptosis in various tumor models. Molecularly, each of these receptors has their own signaling characteristics but the control of tumor cell death seems to be orchestrated at multiple levels. Death receptors are thought to be localized in lipid rafts, and possibly co-localized with CB receptors in the CNS, and such co-localization might enable cross-talk between the two systems. When their activation occurs, such co-localization might be important and necessary for the coordination and initiation of cannabinoid-initiated death signals. Two members of the death receptor superfamily, namely DR4 and DR5, are particularly interesting since they share structural and functional similarities, the same ligand TRAIL, and agonist antibodies developed for these death receptors have been shown to be well tolerated in animal models of cancer and in human clinical trials.

Our goal in this pilot study was to investigate the underlying molecular mechanisms of cannabinoid-induced cancer cell death using the non-selective WIN55212 and CP55940 ligands, with a special focus on a possible cross-activation of DR4 and DR5 receptors in the human LN-18 glioblastoma cell line. We determined the LD_{50} concentrations for these drugs, investigated the role and significance of G-protein coupling, TRAIL release, the effect of the lipid-raft disruptor β-methyl-cyclodextrin, and the activation and expression level of different kinases on cannabinoid-induced cancer cell death. Our findings suggest that WIN55212 and CP55940 exert their anticancer activity through a CB-receptor independent, but lipid-raft coordinated manner, and does not seem to directly involve the DR4 or DR5 death receptors.

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SMART PHONE APPLICATION FOR THE REAL-TIME ACQUISITION OF CLINICAL TRIAL DATA AND REAL-TIME CAPTURE AND RESPONSE OF ADVERSE EFFECTS AND COMPLIANCE ASSURANCE

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Pure Green, a USA cannabis pharmaceutical manufacturing company formulating cannabinoid-based medicines formulated a patent pending rapidly dissolvable tablets consisting of a non-psychoactive composition including cannabinoids and fatty acid amide in a rapidly dissolving sublingual tablet as a therapeutic treatment for the different modalities of pain. We helped to design and are implementing a smart phone application to capture reporting in a more timely and efficient manner.

Last year at the 2018 ICRS poster session we reported the success of a 16-person proof of concept trial using a sublingual tablet containing 5mg cannabidiol (CBD), 100 mg palmitoylethanolamide (PEA) and 4 curated terpenes for the treatment of mild to moderate pain. We wanted to include patients with moderate to severe pain as the rationale for developing a new iteration of pain-relieving sublingual tablets. The new tablet includes 4X the CBD of the original tablet-20mg CBD, 100 mg PEA, and 5 curated terpenes. We are currently conducting a multi-site study onboarding patients with multiple types of painful syndromes. The goal is to delineate if we can identify which painful states respond to treatments that include CBD, PEA and terpenes.

Adverse side effects with the use of any medication is always a concern. The severity of adverse side effects of even over-the-counter pain relievers are well described. PEA and CBD both enjoy a low rate of adverse effects. The composition of the tablet contains constituents that are under the FDA designation of GRAS-generally regarded as safe, along with CBD which has a favorable safety profile, especially at low doses. Even with the GRAS designation, safety is of paramount concern as we increased the CBD dose by 4X. We want to ensure that patients are safe and not experiencing any problems with the study material. We want to set up a mechanism where we can quickly intervene if needed and continue to have a robust clinical trial whose data can be useful in identifying the types of painful conditions that respond to certain cannabinoid formulations while continuously monitoring for adverse effects.

Patient compliance with data entry is paramount to capturing meaningful information. Relying on recall is not as favorable as real-time data entry. We included check-in reminders as part of the application design but again wanted to monitor the data entry on the back end to insure inputs were entered as instructed without interfering with the study results.

Methods: We are currently conducting a multi-site clinical trial including patients with chronic moderate to severe pain. Patients include both male and female, ages 21 or above.
The MB Clinical application (App) for smartphone was designed to capture anonymous, real-time symptoms, dosing, and outcomes. In addition, patients are able to include notes regarding other secondary responses (ex. sleep effects, appetite effects, mood effects) as well as adverse effects.

Patients were interviewed to be evaluated for participation. Once accepted into the study they receive a study package that includes instructions and study tablets. Patients are instructed to download the MB Clinical application (App) onto their smartphone. They are given a study code and a HIPPA compliant study ID that de-identifies them from anyone who has access to the back end of the application. Once the patient answers the initial questions, they are instructed to assess and document their pain on a pain scale score of 0-10 using VAS (visual analog scale) and enter it into the App prior to dosing. They are then instructed to take a tablet and place it under their tongue. The tablet will dissolve rapidly in about 60 seconds or less. The patient is to swallow any residual but is instructed to not eat or drink anything for 3-5 minutes post dose. They are then sent check-in reminders via the App every 10 minutes for the first 30 minutes, then at 45 minutes and then 60 minutes, then hour 2 and hour 3. They enter in their pain scale score in the App at each check in. The principal investigator and the clinical trial coordinator monitor the App. The App also contacts the principal investigator and the clinical trial coordinator in case of a reported adverse reaction.

Conclusions:
As the study is still being conducted, the App permits the principal investigator and the clinical trial coordinator to monitor the results of the patient inputs to ensure that the patients are safe, and that they are compliant with data entry. Early on we learned that there was some confusion with the data entry, and we were able to adjust the instructions to ensure compliance, rather than waiting until the end of the trial to learn that patients were unable to comply with the instructions.

We also are able to read the daily inputs and fortunately have not had any adverse reactions to the study material.

We look forward to sharing the results of this multi-site clinical trial at ICRS 2020.

MARIJUANA AND CANNABINOID RESEARCH PRODUCTS AVAILABLE FROM THE NATIONAL INSTITUTE ON DRUG ABUSE

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Provision of marijuana and cannabinoid research products is currently limited to a single source in the United States. NIDA provides a variety of chemotypes of marijuana and several marijuana extracts of varying THC, CBD, and other cannabinoid content to the research community. This poster will update conference participants on the varieties of compounds and preparations available from the NIDA Drug Supply Program and future plans for producing additional products for research.

ANALYSIS OF COVARIATES ASSOCIATED WITH SELF-REPORTED CANNABIS USE DISORDER SYMPTOMS

Alexus S. Longo and Linda E. Klumpers*

Tomori Pharmacology Inc, DBA Cannify

People suffering from Cannabis Use Disorder (CUD) have generally been studied after diagnosis by physicians. While this is the standard way to identify CUD patients, we wanted to explore potential covariates for a wider population and include people who do not seek professional help. Our aim was to examine covariates for self-reported CUD symptoms.

Data were collected from an online, self-selected survey (www.cannify.us, up to v0.2.5). In the survey, symptoms as described in the DSM-V criteria for CUD were given and the selection of two or more symptoms was used to indicate possible disordered use. Linear regression and logistic regression compared reported negative side effects and CUD symptoms. Logistic regression also compared reported cannabis administration methods and CUD symptoms. Independent two-samples t-tests were used to investigate potential gender differences in the average numbers of negative side effects and CUD symptoms reported.

Linear regression found a strong effect with a weak positive association between the number of negative side effects and CUD symptoms selected ($n=1342$, $\beta=0.273$, $p<0.001$, $R^2=0.092$). Logistic regression ($n=1342$) found that those who reported "lack of motivation" were 7% more likely to report ≥2 CUD symptoms than those who did not report that effect ($\beta=0.068$, $p<0.05$). This logistic regression also found associations between people who reported ≥2 CUD symptoms and depression (18.8% more likely to report ≥2 CUD symptoms than those who did not select depression; $\beta=0.172$, $p<0.01$); respiratory illness or discomfort (21.4% more likely; $\beta=0.194$, $p<0.05$); impaired attention, memory, or concentration (7.6% more likely; $\beta=0.073$, $p<0.05$).

An independent two-samples t-test did not find any gender differences in the number of CUD symptoms selected (males: $n=910$, $M=1.213$, $SD=1.764$; females: $n=431$, $M=1.355$, $SD=1.764$, $t(1339)=1.42$, $p>0.05$), but did find gender differences in the number of negative side effects selected (males: $n=1005$, $M=1.615$, $SD=1.933$, females: $n=508$, $M=1.972$, $SD=1.992$, $t(1511)=3.245$, $p<0.01$, Cohen's $d=0.183$).

For administration methods, logistic regression found that those who reported administering cannabis by "dabbing" were 22.9% more likely to have selected ≥2 CUD symptoms than those who did not select that administration method ($n=1344$, $\beta=0.206$, $p<0.001$).

Based on our data, specific administration methods and the number and type of reported negative cannabis side effects show a relation to potential disordered cannabis use. While we found gender differences in the number of reported negative side effects, we did not find differences in the number of CUD symptoms. This lack of a difference might contrast findings as described in the National Academy of Sciences (NASEM) Cannabis report from 2017 and should be further investigated.

MEDICAL CANNABIS, PAIN AND PTSD: PATIENTS-CENTRIC STUDIES IN TWO TORONTO HOSPITALS

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In Canada, medical cannabis has been available for individual consumption for medical reasons since 1999, while approval for medical cannabis derivatives such as cannabis oils recently occurred in 2016. We undertook an investigation in chronic pain at Toronto General Hospital (TGH), and in PTSD at Center for Addiction and Mental Health (CAMH)/TGH. Neither hospital provides authorization for medical cannabis to patients by policy, but many pain and PTSD patients obtain the necessary authorizations elsewhere in order to purchase medical cannabis from Canadian licensed producers.

Clinically-diagnosed pain and PTSD condition (n=22, each) using medical cannabis were recruited. Study involved, interviewing for an hour to document relevant clinical information, collecting sample(s) of medical cannabis, and collecting a blood sample at the time of interview, for a nominal cash remuneration for materials expenses.

Medical cannabis (or its derivatives) were subjected to quantitative and qualitative analyses for the phytocannabinoids and other phytochemicals. Interesting discoveries are that THC and CBD are quite variable (0-100% each) in both the pain and PTSD patient's cannabis samples when laboratory analyses were conducted. All cannabis samples are evaluated for their receptor responses at CB1 and CB2 receptors; cannabis extracts exhibited much moderated receptor responses than the pure phytocannabinoids. Interesting correlations between cytokine/chemokine markers (GM-CSF, IFN-a2, IL-12, IL-2, IL-3, IP10 and cortisol) and the plasma concentrations of phytocannabinoids were identified. Correlations between such clinically-relevant data, disease-specific clinical diagnosis scores and co-medications, from each cohort, along with chemical analyses of medical cannabis and their cannabinoid receptor profiles will be presented.

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MEDICAL CANNABIS DERIVATIVE PRODUCTS: PRODUCT QUALITY, BIOCHEMISTRY AND CLINICAL RELEVANCE

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In Canada, medical cannabis derivatives, i.e., products such as cannabis oils derived from medical cannabis (dried buds) were approved for commercial sales in December 2016. Patients who could not consume medical cannabis via smoking or vaping – or prefer to consume via non-inhalation methods – often opt to purchase such products. As a part of ongoing clinical studies, a number of cannabis oil samples were collected from chronic pain and PTSD patients for chemical and biochemical investigations in our laboratories at University Health Network. This report will present our findings specifically dealing with the analyses of cannabis oils used by these patients, their chemical composition and the associated variabilities in chemistry, in the context of receptor efficacy studies, potential impact on the product quality and patient experience.

We collected a total of 12 cannabis oil samples consumed by study participants (institutional authorizations issued by Research Ethics Board) at University Health Network. These samples were analyzed to profile for 64 phytochemicals qualitatively and four phytocannabinoids quantitatively, using LC-MS analysis method. All samples were then titrated for dose-response against CB1 and CB2 receptors for potential agonist and antagonist activities, which were then compared to the dose-response profiles of pure phytocannabinoids. In a cAMP functional agonist assay, most samples displayed higher efficacy in CB1 receptors over CB2. On the other hand, CB2 efficacy was higher in the antagonist assay. All but one sample contained ≤ 10 %w/v of total Δ^9-THC and total CBD. A number of samples contained higher quantities of phytocannabinoid carboxylic acids (Δ^9-THCA and CBDA), which are 2-3 fold weaker ligands at the cannabinoids receptors, than the corresponding decarboxylated phytocannabinoids. Heterogeneity introduced during the manufacturing of these cannabis derivatives is a concern for their clinical effects, and patient safety. Chemistry and receptor responses of these medical cannabis derivatives products obtained from pain and PTSD patients will be discussed in the context of clinical efficacy and safety.

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POST-TRAUMATIC STRESS DISORDER (PTSD) AND MEDICAL CANNABIS: CONNECTING 22 PATIENTS' DATA

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PTSD is a complex disease condition and could be induced by a variety of events considered traumatic for the patient. Typically, PTSD patients have a compromised quality of life and are under complex pharmaceutical care. Medical cannabis has been a very popular substance in the veterans' communities as well as among other PTSD sufferers because these patients reportedly experience higher quality of life. Since medical cannabis is not an approved drug with a drug identification number in Canada, nor is it a single substance, patients typically try various strains and forms of medical cannabis in order to identify a suitable choice for self-treatment. We undertook an investigation to understand any potential relationship between the chemistry of medical cannabis consumed by PTSD patients and clinical diagnosis.

Study participants obtained medical cannabis via physician authorizations and purchased the substances from licensed producers of medical cannabis in Canada. Patients were recruited with full consent and a comprehensive interview was conducted to administer PTSD diagnostic tests, collect information on their disease condition, dosing and any other co-medications (UHN REB#: 17-5180.0 and CAMH REB# REB#: 036/2017). A total of 31 cannabis samples comprised of dry plant, resins and oils were collected and were analyzed. From a 22-patient investigation, medical cannabis use amongst PTSD patients is variable from 1 - 4 varieties per patient. When all samples were analyzed, 77% of samples contained ≥ 20% total THC (i.e. THCA+THC) and 19% of samples contained ≥ 20% total CBD (i.e. CBDA+CBD). Interestingly in a cAMP functional assay, the samples displayed agonism in both CB1 and CB2 receptors, and most samples exhibited higher potency against CB1 receptors. In the antagonism assays, a slight preference towards CB2 receptors was observed. Interestingly, almost 40% of participants use only medical cannabis without any other concomitant medications. This presentation will discuss the study design, medical cannabis trends in the participants pool, and relationship to the clinical diagnosis.

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A PRAGMATIC INVESTIGATION INTO MEDICAL CANNABIS FOR CHRONIC PAIN

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Medical cannabis has been available to authorized patients in Canada since 1999. Patients experiencing chronic pain often resort to the self-titrating administration of physician's-authorized medical cannabis. We were interested in understanding the relationship between clinical diagnostic scores for pain, a "chosen" medical cannabis product by the patients, chemical compositions of those medical cannabis samples, their effects on the cannabinoids receptors, and the relevance to any concomitant medication. Patients were recruited at Toronto General Hospital, and we conducted interviews to collect relevant clinical information and diagnosis of the pain condition. We collected a small sample of medical cannabis products used by these study participants at the time of interview, and conducted complete chemical analyses, as well as cannabinoids receptor profiling. Here, we present the analyses of the data from 22 patients.

All patients obtained physician authorizations for medical cannabis and purchased the substances from licensed producers in Canada. Patients were recruited with full consent and a comprehensive interview was conducted to administer pain diagnostic scores, collect information on their disease condition, dosing and any other co-medications (REB#: 16-6375). A total of 28 cannabis samples comprised of dry plant and oils were collected from the 22 patients.

Medical cannabis use amongst chronic pain patients is variable from 1 - 3 varieties per patient. When all samples were analyzed, 57% of samples contained ≥ 20% total Δ^9-THC and 21% of samples contained ≥ 20% total CBD, implying a preference for THC-containing strains. When tested against CB1 and CB2 receptors, all samples displayed higher efficacy on CB1 receptors over CB2 as agonists. However, most samples displayed high CB2 receptor efficacy as antagonists. Overall, the general trends in cannabis chemical composition, their corresponding receptor activities and clinical pain parameters will be presented.

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PHARMACEUTICAL-GRADE CANNABIS IN CHRONIC PAIN PATIENTS WITH FIBROMYALGIA: PHARMACOKINETIC EFFECTS OF THREE CANNABIS VARIETIES

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Introduction: Given the growing number of chronic pain patients on opioid treatment and the associated addiction epidemic in both the US and Europe, effective pain treatment alternatives may possibly be found in the use of cannabis. In this experimental trial, we explored the analgesic and pharmacokinetic effects of pharmaceutical-grade cannabis in patients with chronic pain from fibromyalgia. Here we report on the results of the pharmacokinetic analyses.

Methods: We studied different cannabis varieties: Bedrocan (22.4-mg THC and <1-mg CBD), Bediol (13.4-mg THC and 17.8-mg CBD) and Bedrolite (18.4-mg CBD and <1-mg THC). All cannabis varieties were obtained from Bedrocan BV, The Netherlands. Twenty female fibromyalgia patients inhaled a single cannabis dose within 3-5 min, after which frequent arterial samples were obtained for 3 hours. Determination of the CBD, THC, 11-hydroxy-THC (11-OH-THC) plasma concentrations was performed using liquid chromatography with tandem mass spectrometer detection (LC-MS/MS).

Results: After inhalation of all 3 active treatments, CMAX and TMAX values of THC, 11-OH-THC and CBD were for Bedrocan: THC 82 ± 20 ng/mL at t = 5 minutes, 11-OH-THC 5 ± 3 ng/mL at 10 minutes, and CBD 0.2 ± 0.3 ng/mL at 5 minutes; for Bediol: THC 76 ± 35 ng/mL at t = 5 minutes, 11-OH-THC 5 ± 3 ng/mL at 10 minutes, and CBD 80 ± 29 ng/mL at 5 minutes; and finally for Bedrolite: THC 13 ± 5 ng/mL at t = 5 minutes, 11-OH-THC 0.9 ± 0.5 ng/mL at 10 minutes, and CBD 155 ± 57 ng/mL at t = 5 minutes. The plasma concentrations observed following treatment with Bedrocan and Bediol were associated with effective pain relief.

Discussion: These data indicate an important pharmacokinetic interaction between THC and CBD with greater THC plasma concentrations when CBD is present in the inhalant. We explain these findings by either an increased absorption of THC in the lung or the inhibition of THC metabolism, both induced by CBD, or the conversion of CBD into THC. Further studies are needed to explain the mechanism of our findings.

Conclusions: In a population of chronic pain patients, the inhalation of three cannabis varieties produced effective plasma concentrations of CBD and THC. In the presence of CBD, THC concentrations were 50% greater than expected. This first study suggests that Bedrocan and Bediol may be attractive alternatives for the treatment of chronic pain.

Withdrawn

PATIENT FOCUSED INVESTIGATION OF THE ENTOURAGE EFFECT, EXPERIENCE, AND BENEFITS TO PUBLIC SAFETY AND THE EMERGING THERAPEUTIC MARKETPLACE

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There have been several studies in recent years citing the health benefits and improved therapeutic value of co-administration of THC with CBD.[1-4] Very few have examined the patients' interpretation and experience based on using formulated and target methods. Solowij et. Al. reported that low doses of CBD when combined with THC enhanced, while high doses of CBD reduced the intoxicating effects of THC.[5] Furthermore, they reported that enhancement of intoxication by low-dose CBD was particularly prominent in infrequent cannabis users and was consistent across objective and subjective measures.

Thus, an examination of inhaled tailored cannabis medicines with varying ratios of THC and CBD was carried out to provide a deeper look into the symphony of entourage effects.[6] Cannabis botanical medicines were bred, and grown to contain specific ratios of THC to CBD, while others were bred to be nearly devoid of THC and had ratios of CBD:CBDV and CBGA and turned into rolled cannabis cigarettes. Patients/customers were blindly paneled to examine the impact of CBDA and CBDGA on the overall 'cannabis experience' using THC alone as the baseline. In particular, we examined the varying ratios of THC:CBD and THC:CBG and the impact on mood, cognition, subjective intoxication, anxiety, and short term memory loss. 21 individuals with varying historical cannabis use patterns participated in this investigation. Three different terpene profiles were examined. Each terpene profile was paired with three different THC:CBD ratios and were employed to investigate mood enhancement, anxiety, subjective intoxication, and memory impairment.

Across all terpene profiles, more than 66% of the participants reported decreased intoxication and decreased anxiety with increasing CBD content. While CBD:CBDV cultivars showed next to no anxiety and very little intoxication, this was to be expected considering the THC content was so low (> 0.3% THC). Additionally, there seemed to be a correlation with respect to mood elevation and a ratio of THC:CBD of 2:1, and the most extreme improvements were evident with the terpenes Limonene and B-Caryophyllene specifically. Interestingly, high THC samples negatively impacted the mood of novice users, and conversely, high CBD content negatively impacted the mood of heavy users. Remarkably, approximately half of the subjects saw improved memory test scores with increasing CBD concentration. As a result, the link between preventing short-term memory loss and improving mood were shown to be strongly correlated with increasing CBD levels and decreasing THC levels in infrequent or novice cannabis users.

1. Yucel M, Lorenzetti V, Suo C, Zalesky A, Fornito A, Takagi A, Lubman DI, Solowij N. Hippocampal harms, protection, and recovery following regular cannabis use. 2016 Transl Psychiatry 6: e710
2. Englund A, Freeman TP, Murray RM, McGuire P. Can we make cannabis safer? The Lancet Psychiatry 2017; 4: 643-648
3. Englund A, Morrison PD, Nottage J et al. Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampaldependent memory impairment. J Psychopharmacol 2013; 27: 19–27
4. Henquet C, Kuepper R. Does cannabidiol protect against the negative effects of THC? The britishjournal of psychiatry 2010; 197: 259-260
5. Giese MW, Lewis MA, Giese L, Smith KM. Development and validation of a reliable and robust method for the analysis of cannabinoids and terpenes in cannabis. J AOAC Int 2015; 98: 1503–1522
6. Lewis MA, Russo EB, Smith KM. Pharmacological Foundations of Cannabis Chemovars. Planta Medica 2018; 4: 225-233

DIRECT MEASUREMENT OF ACTIVITY-DEPENDENT ENDOCANNABINOID MODULATION IN BRAIN SLICE USING THE NOVEL GENETICALLY-ENCODED FLUORESCENT SENSOR GRAB$_{eCB}$

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The endocannabinoid (eCB) system is a major source of synaptic modulation and thus involved in regulating neural functions and behavior. Although many mechanisms underlying eCB mobilization have been elucidated, direct measurement of eCBs on time scales supporting synaptic modulation has yet to be accomplished. Such ability will allow for many new investigations on eCB signaling logic as it relates to neural plasticity, behavior, and pathophysiology. We will report on a novel genetically encoded biosensor, based on a CB1 receptor scaffold, which is being implemented in a brain slice photometry technique to study eCB mobilization kinetics, neural activity rules supporting eCB generation, and neurochemical pathways underlying eCB synthesis and degradation. Preliminary data shows that, in the striatum, eCB signals can be generated by intrastriatal electrical stimulation and are blocked by CB1 receptor antagonists. The putative eCB transient is slow with rise and decay times spanning several seconds, consistent with conceptual models of eCB mobilization. An eCB transient can be generated by a single stimulation and is modulated by stimulation frequency and duration. Transients evoked by train stimulation are attenuated by Group I mGluRs and iGluR antagonists, which is consistent with 2-AG synthesis through the Gq-dependent signaling cascade and an eCB synthesis pathway dependent on postsynaptic drive. We are continuing experiments on characterizing the physiological and biochemical rules governing eCB generation in the basal ganglia and are initiating *in vivo* fiber photometry experiments to measure eCBs in the context of behavior.

THE ABSENCE OF MONOGLYCERIDE LIPASE (MAGL) INFLUENCES RETINAL FUNCTION AFTER PHOTIC INJURY

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Ocular tissues express all the components of the endocannabinoid system (ECS), including cannabinoid receptors (CB1 and CB2), endocannabinoid ligands (2-arachidonoyl glycerol (2-AG), 2-arachidonoyl ethanolamide), and enzymes responsible for their synthesis and degradation. The pharmacological inhibition or genetic deletion of degradation enzymes, including monoglyceride lipase (MAGL), modulates ECS activity and therefore may affect normal retinal physiology as well as pathological states. Previous studies indicate that genetic and chronic pharmacological inactivation of MAGL results in increasing 2-AG levels and inducing CB1 receptor desensitization, thus leading to functional and behavioral tolerance. Excessive light exposure has been shown to accelerate oxidative stress, photoreceptor cell death, and lead to retinal damage and vision impairment**.** Using a light-induced retinal injury model, we sought to investigate whether MAGL deficiency alters retinal activity and cell survival.

Two months old, dark-adapted wild-type (WT) and MAGL knockout (-/-) mice in a C57Bl/6 background (n = 4 –8/ group) were anaesthetized, and with dilated pupils, exposed to 18,000 lux of white fluorescent light for 6 hours. Electrophysiology assays were performed 2 days before and 5 days after light induced retinal degeneration (LIRD). To evaluate the effect of MAGL in retinal function in a scotopic environment, differences of parameters V*max* (value of the maximum amplitude), *n* (slope of the intensity-response function), and *k* (response sensitivity) of the Naka-Rushton hyperbolic equation, were analyzed. Animals were then sacrificed, and the eyes were removed for examination of photoreceptor layers at various time intervals, from 0 to 21 days after light induction.

No difference was observed in a- and b- wave ERG amplitudes between WT and MAGL -/- mice. Five days after LIRD, ERG results showed significant drops of a- and b- wave amplitudes in MAGL -/- mice. Using the Naka-Rushton equation, intensity-response functions indicated that the mean a- and b- wave Vmax were reduced by 33% and 32% respectively in MAGL -/- mice. In contrast, we found no change in ERG amplitudes 5 days after LIRD in WT mice. These results indicate that MAGL -/- mice are vulnerable to light damage, which may result in the earlier onset of retinal cell death.

The loss of retinal activity in MAGL -/- animals after light-induced injury demonstrates that MAGL plays an important role both in regulating CB1 receptor signaling and in determining the survival of these injured retinal cells.

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THERAPEUTIC CANNABINOIDS IN ANXIETY AND DEPRESSION: RESULTS FROM AN OBSERVATIONAL RESEARCH STUDY

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Cannabis is used recreationally for both its mood-enhancing and relaxing effects, and the endocannabinoid system plays a demonstrable role in modulation of mood and anxiety. However, cannabis/cannabinoids are not currently approved in most states for the treatment of depression or anxiety. Pre-clinical literature involving acute cannabinoid administration, particularly cannabidiol (CBD), is strongly suggestive of an anxiolytic and antidepressant effect, but research in humans is limited. The aim of this study was to examine the health impact of regular cannabis product use among individuals with anxiety and/or depression compared with controls who did not use cannabis.

Study participants were a sub-group of individuals in a medicinal cannabis observational research patient registry that were either current cannabis users (n = 55) or non-using controls (n = 33) that listed anxiety or depression as the primary health condition for which they were, or were considering medicinal use of cannabis. Participants reported demographic information, cannabis product and prescription medication usage, and completed self-report questionnaires assessing mood and anxiety, sleep, and quality of life via web-based surveys. Follow-up surveys containing the same questionnaires were completed at three-month intervals.

No demographic differences were found between cannabis users and controls at baseline, and rates of antidepressant and anxiolytic medication usage were similar. The majority of therapeutic cannabis users reported regular use of high-CBD products. Compared with controls, cannabis product users reported significantly lower anxiety and depression on the HADS, were significantly more likely to score within the 'Normal' range on the HADS for depression, and were significantly less likely to score within the 'Severe' range for depression and anxiety at baseline. Cannabis users also reported significantly better sleep, and had significantly higher scores in the 'Physical' and 'Psychological' domains of the WHOQOL-BREF. A significant reduction in anxiety, but not depression, was observed in a small sample of individuals that initiated cannabis product use after completion of their baseline survey (n = 8).

Medicinal cannabis users reported significantly less anxiety and depression than non-users, and those that initiated cannabinoid therapy reported reduced anxiety compared to baseline. These results suggest promise for the use of medicinal cannabinoids in this population, but additional, placebo-controlled research is required to ascertain the reliability and validity of these findings.

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PREVALENCE AND IMPACT OF PTSD SYMPTOMS ON SLEEP AND CANNABIS ABSTINENCE IN AN URBAN CANNABIS TREATMENT TRIAL

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Among individuals seeking treatment for Cannabis Use Disorder (CUD), co-occurring PTSD is associated with more severe withdrawal, difficulty quitting, and coping-oriented use. Most prior research has been conducted in military populations. We investigated the prevalence/severity of PTSD and its relation to cannabis cessation and sleep quality in an urban sample of individuals seeking treatment for CUD.

Treatment-seeking heavy cannabis users in Baltimore (n=127) were recruited for a 12-week pharmacotherapy clinical trial (zolpidem or placebo). The PTSD Checklist (PCL-C; DSM-IV) and Pittsburgh Sleep Quality Index (PSQI) were administered at intake and post-treatment. A PCL-C cutoff score of >30 was used to identify individuals likely to have PTSD. Urine analysis determined abstinence. A two-tailed t-test assessed group differences in PSQI scores and baseline cannabis use. Logistic regression was used to determine whether PCL-C score predicted abstinence. Alpha was 0.05 for all analyses.

Prior trauma was reported by 70% of study participants and 95% indicated that they used cannabis to improve sleep. A PTSD diagnosis was probable (PCL-C score > 30) in 21% (N=19) of those with past trauma; 15% of total sample. This group had significantly higher PSQI scores (worse sleep quality) than other participants. No differences were observed between the probable PTSD group and other participants on intake cannabis use characteristics (# of days used and grams smoked in past 30 days, years of regular cannabis use) or cannabis abstinence during the trial. PCL-C score did not predict the likelihood of abstinence during the trial. PTSD symptom severity at the end of treatment did not differentially change from intake among abstainers versus non-abstainers.

While individuals with PTSD reported greater sleep difficulty, contrary to studies of military/veteran samples, the presence of PTSD was not associated with differential cannabis use behavior or CUD abstinence during treatment.

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WE DON'T RINSE CANNABIS: EXPOSURE AND HEALTH EFFECTS OF RESIDUAL PESTICIDES IN CANNABIS

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Sales of both medical and recreational cannabinoid-based products have proliferated throughout the United States, yet a gold standard for acceptable pesticide use is lacking. Regulators, healthcare providers, and the general public would benefit from an understanding of best practices around pesticide use in the production of cannabis.

From the review of the literature the most commonly reported contaminants of cannabis preparations were microbes, heavy metals and pesticides. Similar types of contaminants have been reported in complementary alternative medicines including herbal, Ayurvedic and Chinese traditional medicines. Growers have an economic incentive to produce large yields and high quality plants, and may resort to pesticides to achieve these outcomes. Chemical residues present on cannabis can be pyrolyzed and transferred across the blood brain barrier via the mainstream smoke and ultimately into the lung of the end user.

Studies have connected human health effects and environmental impact with pesticide use for many decades, but evidence-based policy guidelines have not been implemented. Overall, 19 commonly reported pesticides belonging to different chemical classes have been identified including o-phenylphenol, bifenazate, cypermethrin, imidacloprid, propamocarb, propiconazole and tebuconazole. Robust methods of analysis for the quantification of pesticides in cannabis have been developed. State analytical laboratories are capable of testing for residual solvents, heavy metals, and other dangerous compounds. Early data collection has successfully validated 66 pesticides in cannabis leaves, 55 in dried cannabis flowers, and 79 in cannabis oil. Yet the Environmental Protection Agency (EPA) requirement for pyrolysis studies for products intended to be smoked, like cannabis, has left a policy vacuum. As a pioneering jurisdiction, Colorado faces a considerable knowledge gap.

In our presentation, we will discuss the state of the evidence of chemical stressors used for pest control during the cultivation of cannabis, the health effects of these exposures (both occupational and end user hazards), and particularly harmful pesticides. Pesticides represent an underestimated and under-documented health risk for consumers and cultivation staff. We will propose an evidence-based standard for adoption by state and future federal regulators.

THC CONTENT AND NUMERIC SUBJECTIVE HIGHNESS REPORTED IN A REDDIT ONLINE CANNABIS COMMUNITY 2010-2018

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Background: Increasingly liberal cannabis policy worldwide has coincided with a growth in availability of diverse cannabis products. These products have varying levels of THC content that is often greater than what is administered in laboratory studies. In this context, people who use cannabis often turn to social media venues to access peer-generated information and to share their experiences. One such venue is Reddit, where users post to public topic-specific forums and interact with one another using pseudo-anonymous usernames. In one popular cannabis-themed forum, people post about experiences with cannabis, sometimes including a number from 1-10 in brackets indicating how high they are – similar to other subjective effect scales for pain and intoxication. We previously reported that mean subjective highness was significantly greater for posts that mentioned dabbing, butane hash oil extract terms, and edible terms compared to smoking (Meacham et al., *Drug Alcohol Depend,* 2018, 188:364-369). Further study of this collection of reported cannabis use and its effects in naturalistic settings may elucidate the relationship between potency and subjective effects in an era where cannabis is becoming more varied and accessible.

Methods: We examined keyword mentions and distributions of potency (mg, % THC, and THC:CBD ratios/cannabis chemotypes) and numeric subjective highness (from 1-10) in 2.6 million post titles in a popular cannabis subreddit from January 1, 2010-December 31, 2018. We then assessed correlations between potency values and numeric subjective highness, expecting that as potency increased numeric subjective highness would also increase.

Results: There were 2,846 posts that mentioned THC content in the post title, the proportion of which increased from .017% of posts in 2010 to .514% of posts in 2018 (a 30-fold increase). Across all years, the distribution of % THC values (N = 771) exhibited a bimodal distribution, with peaks of % THC in the 30s and 90s. There were far more mentions of numeric subjective highness (13.4% of all post titles), with a normal distribution (mean 7.0, SD 1.8). Though there were very few posts that mentioned both potency as percentage and subjective highness (N=32), correlation between % THC and numeric subjective highness was positive (Spearman *rho* = .22, p = .23).

Conclusions: Across 9 years of posts to a popular cannabis subreddit, mentions of THC content were infrequent yet increasing. The clearest signal of potency was found for % THC, with a distribution matching what is advertised in U.S. legal markets (e.g., 20-30s% for flower, 70-90s% for extracts). While there was little overlap between posts mentioning THC content and subjective highness, this overlap may continue to increase. Alternatively, people who are able to get cannabis in a legal market, where THC content is tested and labeled, may be less likely to engage in peer-to-peer information sharing in an anonymous online forum. Future research will examine other features of posts that contain a subjective highness rating, such as quantity, setting, and sentiment.

IN VITRO EVALUATION OF THE EFFECT OF CANNABIDIOL AS AN ADJUVANT THERAPY FOR PAEDIATRIC BRAIN TUMOURS

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Brain tumours are the leading cause of cancer related death in children and are one of the most challenging childhood cancers to treat. These patients have a poor prognosis with limited treatment options and high recurrence rates. Recent evidence suggests there may be anti-tumoral properties of cannabinoids, and of cannabidiol (CBD) in particular. *In vitro* studies have shown that CBD can decrease cancer cell proliferation and/or induce apoptosis or autophagy in a wide range of models. *In vivo*, CBD reduces the growth of tumour xenografts in mice with additive effects when co-treated with temozolomide. With its lack of side effects, including a lack of psychoactive side effects, it could quickly be re-purposed as an anti-cancer drug.

We evaluated the effect of CBD on paediatric brain tumour cell lines; pHGG (SF188) ependymoma (BxD-1425EPN) and human astrocytes, with cells grown as 2D monolayers and 3D spheroids in both hypoxic and normoxia conditions. The EC_{50} for each cell line was initially established using a resazurin metabolism assay. The EC_{50} in 5-day 2D experiments with SF188 was 14.8µm 14.6µM with BxD-1425EPN cells. In 3D spheroids the respective EC_{50} values were 15µM and 14.8µM, thus there was no significant difference in EC_{50} with respect to cell culture conditions. At these CBD concentrations astrocytic cell death was insignificant. The lactate dehydrogenase (LDH) assay showed increased cell death in all cell lines in the presence of CBD in normoxia and hypoxia except for astrocytes incubated for 24 hours with CBD which showed limited cell death. Western Blot showed an increase in Lc3b expression (autophagy) after 24 hours incubation with CBD in both BxD-1425EPN and SF188 cells but not at 5 days. PARP expression (apoptosis) was increased after 5 days incubation with CBD only. Astrocytes showed a small increase in PARP after 5 days CBD incubation but no increase in LC3b at any time point. 3D spheroids decreased in size by approximately 20% when cultured in CBD compared to cells only after 5-day exposure. In hypoxia, SF188 and BxD-1425EPN cells showed decreased cell death after 24 hours and 5 days when compared to normoxia. The EC_{50} increased slightly to 17.7µm (SF188) and 15.8µm (BxD-1425EPN) in 24-hour experiments and to 15.1µm (SF188) and 15.2µm (BxD-1425EPN) in 3D 5-day experiments. No significant cell death was observed in the astrocytes after 24 hours. After 5 days some cell death was seen to have occurred in hypoxia, however, the rate of cell death was lower than in the SF188 or BxD-1425EPN cells.

In summary, in both 2D normoxic and hypoxic conditions as well as in 3D models, there is evidence that CBD causes cell death in paediatric brain tumour cells by autophagy following short term (24 hour) and apoptosis following long term (5 day) exposure and that though some apoptosis is seen in normal astrocytes, their rate of cell death is significantly lower than in the tumour cells providing further evidence for CBD as a potential treatment for paediatric brain tumours.

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AGONIST-INDUCED SURFACE UPREGULATION OF CANNABINOID RECEPTOR 2 (CB_2)

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GPCRs are typically expressed on cell surfaces where they respond to extracellular ligands, initiating downstream signalling cascades. The responsiveness of a cell to ligand is therefore reliant on cell surface receptor density. Cannabinoid Receptor 2 (CB_2) mediates cannabis-induced immunosuppression and is a promising drug target for modulating inflammation.

Whilst CB_2 is expressed at the cell surface, a sizeable proportion is retained intracellularly. Although surface human CB_2 (hCB_2) internalises in response to acute stimulation by a variety of agonists, we have observed that with sustained stimulation hCB_2 repopulates the cell surface resulting an apparent reversal of the initial internalisation; a phenotype which challenges the traditional paradigm of GPCR desensitisation and endocytosis. This phenomenon may be attributable to lipophilic CB_2 ligands having the ability to traverse the plasma membrane and act as pharmacological chaperones which "mobilise" intracellular hCB_2 (i.e. induce surface upregulation) by potentiating delivery of receptors to the cell surface.

In addition, mutation of a di-lysine 'KK' motif in the hCB_2 cytoplasmic C-terminal tail near abolishes basal cell surface delivery of hCB_2. Total expression levels are fairly equivalent, indicating that the mutant is likely inhibited from exiting the synthetic pathway. Despite this, the mutant receptor is still mobilised in response to sustained agonist stimulation, suggesting that basal surface delivery and agonist-induced delivery are likely distinct processes.

The current study set out to further investigate the mechanisms behind this agonist-induced mobilisation via the use of various receptor signalling and trafficking pathway inhibitors. Immunocytochemistry was performed on HEK293 cells stably expressing HA-tagged hCB_2 or KK mutant hCB_2, and quantified by automated imaging and analysis. Trafficking pathways examined for their role in this process include Golgi export and protein synthesis, and signaling pathways include those downstream of $G\alpha_i$, $G\alpha_q$, and $G_{\beta\gamma}$.

Results showed that hCB_2 mobilisation was not sensitive to treatment with pertussin toxin ($G\alpha_i$ inhibitor), U73122 (PLC inhibitor), GF109203X (PKC inhibitor) or Gallein ($G_{\beta\gamma}$ inhibitor) and is therefore not downstream of $G\alpha_i$, $G\alpha_q$, or $G_{\beta\gamma}$ activation. Conversely, it was sensitive to Brefeldin A (disrupts Golgi export), Monensin (inhibits vesicle acidification) and Cycloheximide (inhibits protein synthesis) indicating that that in addition to requiring uncompromised intracellular transport/receptor export, protein synthesis is also a requirement. Whether this is synthesis is of hCB_2 itself, or an adaptor/chaperone protein is yet to be determined.

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EFFECTS OF THE CANNABINOID RECEPTOR 1 GENE ON FEAR EXTINCTION NEURAL CIRCUITRY IN HEALTHY ADULTS

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Anxiety disorders are characterized by an inability to extinguish conditioned fear responses. Rodent studies have consistently found evidence for the endocannabinoid system in successful fear extinction. Moreover, recent studies in humans have shown that genetic variants of *CNR1*, the gene that encodes for cannabinoid type 1 receptor, has been linked to fear extinction success and anxiety symptoms. For instance, using a fear potentiated startle paradigm, individuals with the A/A genotype show impaired extinction of fear potentiated startle compared to G-allele homozygotes, suggesting a potential mechanism that may increase risk for anxiety disorders. However, it is unknown whether the *CNR1* variant effects the underlying neural mechanisms of fear extinction. Indeed, cannabinoid type 1 receptors are highly expressed in brain regions that are essential for fear extinction, including the amygdala (AMYG), hippocampus (HPC), and medial prefrontal cortex (mPFC). The present study examines the effect of *CNR1* on fear extinction neural circuitry.

Twenty-nine healthy adults (ages 19-33, 16 female) underwent a novel two-day fear extinction paradigm during functional magnetic resonance imaging (fMRI). One day 1 participants underwent fear acquisition followed by fear extinction learning. Approximately 24 hours later all participants underwent a recall test of fear extinction learning. Participants were genotyped for a polymorphism located within the promotor region of *CNR1* (rs2180619). Skin conductance responses (SCRs) were recorded as a marker of conditioned fear. SCRs and fMRI response in extinction-related neural circuitry (i.e., AMYG, HPC, mPFC) was compared between gene groups (A-allele carriers vs. GG homozygotes).

Overall, participants showed intact fear extinction learning and recall, evidenced by sustained low SCRs at the end of extinction to the beginning of recall. Surprisingly, there were no differences in SCRs between A-carriers and GG-carriers during fear extinction learning or recall. However, there was an effect of *CNR1* on neural activation during recall, such that A/A carriers showed higher activity in the AMYG and mPFC during extinction recall, as compared to GG homozygotes. These results link variation in endocannabinoid signaling to disruptions in fear extinction neural circuitry, which has been postulated as an important mechanism in the pathogenesis of anxiety disorders.

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PRELIMINARY RESULTS FROM A STUDY ON THE MOLECULAR PREDICTORS OF THE EFFICACY OF ELECTROCONVULSIVE THERAPY (ECT) IN MAJOR DEPRESSION PATIENTS

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Background: ECT is an effective procedure indicated for patients with treatment resistant depression. Although most patients display some degree of recovery, 32-52% do not respond or remit at all. Considering the possible side effects and the considerably high cost of treatment it is important to identify sub-populations who would benefit the most from ECT. In the current study we seek to identify predictive molecular markers in the blood of depressed patients who are responsive to ECT. In addition, we aim to examine the molecular changes induced by ECT (baseline to post-treatment) in order to elucidate the biological mechanisms underlying the therapeutic effects of this procedure.

Method: Forty patients with the diagnosis of treatment resistant depression are being recruited from the ECT unit of the Sheba Medical Center. Participants undergo psychiatric and psychological assessments, using the Montgomery-Asberg Depression Rating Scale (MADRAS) and Clinical Global Improvement and Severity Scales (CGI-S, CGI-I). Similar psychiatric assessments are conducted before the first ECT treatment, and during ECT visits 7 and 12. Blood samples are taken before the first treatment and on the final treatment. Collected blood samples include whole blood, serum and isolated PBMCs for molecular analyses.

Results: Patients are now in different stages of the clinical trial. Preliminary results show mixed results in PBMC gene expression of the cannabinoid-related GPCRs: GPR18 and GPR55, and molecular markers of inflammation (CRP levels), before and after ECT as a function of treatment outcomes. Additional preliminary results from a proteomic analysis will be presented.

SUBSTITUTING CANNABIS FOR ALCOHOL: THE IMPACT OF LEGALIZATION

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Preliminary research has demonstrated a "substitution effect" whereby cannabis replaces the use of a potentially more harmful substance, such as alcohol. Given the recent change in the legalization status of cannabis, there is increasing public interest on the effect that increased access to and availability of cannabis presents, particularly on the use of alcohol. The present study seeks to examine substance use patterns prior to and directly following cannabis legalization to evaluate the impact of the policy change. Post-legalization data is forthcoming.

Participants were 565 undergraduate students (63.5% female; M=19.35, SD=7.34) reporting past cannabis and alcohol use. Participants completed an online survey on substance preference and substitution patterns.

Overall, prior to legalization, 39.3% (n=222) preferred cannabis to alcohol, 50.6% preferred alcohol (n=286), and 8.8% (n=50) had no preference. The intentional substitution of cannabis for alcohol was endorsed by 24.3% (n=137) of individuals and 56% (n=316) of individuals reported that they drank less alcohol when they used cannabis. With regard to concurrent use, 23.5% (n=133) of individuals reported that cannabis and alcohol “mixed well together” and 49% (n=277) disagreed with the statement. Finally, few respondents (4.3%, n=24) indicated that cannabis was associated with drinking more.

Pre-legalization data suggests that individuals substitute cannabis for alcohol and reduce alcohol consumption when cannabis is available. Notably, nearly a quarter of resondants indiciated that they liked the effects of the two together, suggesting that this may represent a potential risk for harm. Data collection for post-legalization responses is ongoing with scheduled completion March 2019. Given the major shift in cannabis policy in Canada and heightened public interest, examining the substitution effect and co-use of alcohol and cannabis is warranted. Moreover, examining these patterns pre-and post-legalization can provide important preliminary evaluation of Canadian cannabis policy.

CHRONIC USE OF Δ-9-TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD) ON CEREBRAL GLUCOSE METABOLISM IN PEOPLE WITH MULTIPLE SCLEROSIS – A PILOT STUDY

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Approximately 50% of people with Multiple Sclerosis (PwMS) use cannabis. Cannabis contains Δ-9-tetrahydrocannabinol (THC), which may cause executive dysfunction and acute psychosis, and cannabidiol (CBD), which is anxiolytic and may mediate THC effects. How these substrates influence cerebral blood flow and glucose metabolism in healthy adults has been studied, but how chronic use of THC and CBD affects cerebral glucose metabolism in PwMS remains unclear and was investigated with an exploratory analysis. Nineteen PwMS (Relapsing-remitting; mean ± SD: Patient Determined Disease Steps = 2.4 ± 1.7; age = 50.7 ± 14.6) underwent cerebral [^{18}F]-fluorodeoxyglucose positron emission tomography. Participants provided cannabis use logs and products labels and were stratified into non-users (no cannabis; n=9), THC-users (THC ≥ 0.3%; n=6) and CBD-users (THC < 0.3%; n=4). Global-mean normalized relative regional metabolism for 78 volumes of interest were calculated and analyzed with *t*-tests and effect sizes (Cohen's **d**). Compared to non-users: 1) THC-users had increased glucose uptake of the left superior temporal gyrus posterior part, the left inferiolateral remainder of parietal lobe, and right posterior temporal lobe ($p < 0.039$, $\mathbf{d} > 1.17$); 2) CBD-users had reduced glucose uptake of the left parahippocampal and ambient gyri, the left amygdala, the left hippocampus, the left fusiform gyrus, and the right nucleus accumbens ($p < 0.032$, $\mathbf{d} > 1.31$). THC-induced hypermetabolism of left temporal areas has previously been associated with executive dysfunction and psychosis, which are existent problems in PwMS that may be exacerbated by THC use. CBD-induced hypometabolism of left medial temporal areas may positively affect the MS comorbidities of anxiety and depression. This pilot study highlights the need to discriminate between THC and CBD in cannabis research.

ANTICANCER EFFECTS OF CANNABINOID ON HUMAN PROSTATE CANCER CELL LINE

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One in nine men will develop prostate cancer (PCa) over the course of their lifetime. It is the second most commonly diagnosed cancer in American men and the second leading cause of cancer death, despite recent advances in radiotherapy and chemotherapy. Cannabinoids, the active components of Cannabis sativa Linnaeus (marijuana) and their derivatives have received renewed interest in recent years due to their diverse pharmacologic activities such as cell growth inhibition, anti-inflammatory effects and tumor regression. Objective of our study was to evaluate cannabinoid receptors (CB1 and CB2) expression and anti-proliferative effect of cannabinoid in human prostate cancer cells. We used two androgen independent human PCa cell lines (PC3 and D145) and normal prostate cancer cell line RWPE to characterize the expression of cannabinoid receptors using conventional and quantitative PCR. We found positive expression of cannabinoid receptor CB1 in both PCa cell lines and normal prostate cell line, however CB2 receptor was not found in any of the cell line. To find the anti-proliferative effect of cannabinoid we used two endocannabinoids (2AG and AE), two synthetic cannabinoid agonists (2MAF and WIN55-212-2) and two phyto cannabinoids (CBD and THC). PC3 cells were incubated for 48 hrs with the drugs in five different concentrations, (10, 20, 40, 80 and 160 μmol). We used media as a control and looked at the anti-proliferative effect of cannabinoids using MTT assay. We found significant dose dependent inhibition of cell proliferation with endocannabinoids 2AG and AE, synthetic cannabinoid 2MAF and WIN55-212-2 and phyto cannabinoids CBD and THC. Our results suggest that cannabinoids could be developed as a novel therapeutic agents for the treatment of prostate cancer.

INPATIENT PRESCRIPTION CANNABINOID UTILIZATION RATE IN DIFFERENT SURGICAL COHORTS IN THE UNITED STATES: A POPULATION-BASED STUDY

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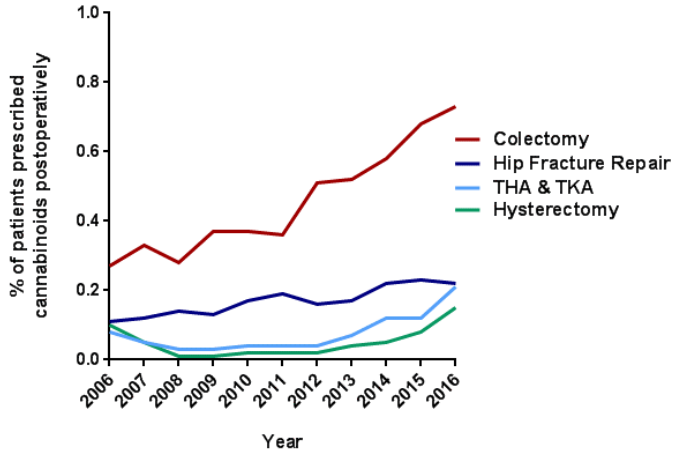
Dronabinol, synthetic Δ-9-tetrahydrocannabinol, has been FDA-approved in the United States for over 30 years for the treatment of nausea and vomiting due to chemotherapy and anorexia in AIDS patients. However, data on the perioperative utilization of dronabinol from a population perspective among different surgical cohorts is largely lacking. The timing of this study provides estimates of inpatient use of FDA-approved cannabinoids, as states continue to legalize medical and adult-use cannabis.

In this retrospective cohort study, data on patients who underwent surgeries of either total hip arthroplasty (THA), total knee arthroplasty (TKA), hip fracture repair, colectomy, or hysterectomy from 2006 and 2016 was extracted from the nationwide all-payer Premier Perspective Healthcare Database. The study was approved by the institutional review boards and exempt from consent requirements. The main outcome was cannabinoid utilization, defined by standard billing codes for dronabinol or nabilone. Chi-square tests were used to assess the incidence of cannabinoid utilization.

Overall, we identified 1,885,976 joint arthroplasty patients (THA/TKA), 526,414 hip fracture, 218,201 patients who underwent colectomies, and 1,573,269 hysterectomy patients. Among these, dronabinol was prescribed to 1,565 (0.08%) THA/TKA patients, 918 (0.17%) hip fracture patients, 976 (0.45%) colectomy patients, and 669 (0.04%) hysterectomy patients. The annual rate of dronabinol use in all cohorts increased over time. While analysis of various high-volume surgical cohorts showed that dronabinol utilization was low, an increase over time was observed. The highest rate was found in patients who underwent colectomies and the lowest among hysterectomy cases.



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BLUNTING THE PAIN: EXPLORING THE IMPACT OF MEDICAL CANNABIS TREATMENT IN PATIENTS WITH CHRONIC PAIN

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Currently, 33 states and Washington D.C. have legalized full medical cannabis (MC) programs, while 14 additional states allow limited access to MC products. Chronic pain is among the most common indications for MC use. Cannabis contains a number of cannabinoids that modulate the activity of the body's endocannabinoid system (ECS), which regulates mood, appetite, memory, and pain. The ECS has been implicated in both anti-inflammatory processes and analgesia, and previous studies have demonstrated improvements in pain tolerance and sensitivity following acute treatment with cannabis, suggesting that long-term treatment with MC may alleviate symptoms associated with chronic pain conditions. The aim of this study was to evaluate pain, clinical state, quality of life, and conventional medication use in patients with chronic pain following 3 and 6 months of MC treatment.

As part of a larger observational, longitudinal study, patients with chronic pain were assessed before initiating MC treatment and after 3 and 6 months of MC use on measures of pain, clinical state, quality of life, and conventional medication use, including opioids, non-steroidal anti-inflammatory drugs (NSAIDS), and over-the-counter (OTC) analgesics. In addition, pilot analyses comparing pain ratings in MC patients to a small group of treatment-as-usual (TAU) patients who endorsed chronic pain were also completed.

Following 3 months of MC treatment, patients reported primary symptom reduction evidenced by lower self-reported pain ratings on the Visual Analog Scale (VAS), Numerical Rating Scale (NRS), Pain Distress Scale (PDS), and Pain Disability Index (PDI). At 6 months, these ratings remained lower than baseline ratings. In contrast, TAU patients' ratings either stayed the same or *increased* at 3 and 6 months compared to baseline. In addition, MC patients exhibited improvements on self-reported depressive symptoms (Beck Depression Inventory [BDI]), sleep (Pittsburgh Sleep Quality Index [PSQI]), and several measures of quality of life following MC treatment. Finally, total opioid dose and total OTC analgesic dose per week were lower after initiating MC treatment compared to baseline.

These findings provide evidence that MC may be a valuable treatment option for patients with chronic pain, and may be viable as adjunctive or substitution treatment for conventional medications that often do not provide full symptom relief and are associated with negative side effects. While it is extremely important to assess ecologically valid products that consumers have access to in the marketplace, it is also of note that more rigorous clinical trial models are needed to address the impact of specific MMJ products and individual cannabinoids on variables related to pain, conventional medication use, and clinical state.

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THE ENDOCANNABINOID SYSTEM AS A PREDICTOR OF FEAR EXTINCTION LEARNING - AN FMRI STUDY

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Fear- and anxiety-related disorders have been intensely studied in psychiatric research. To enhance therapeutic progress, different aetiological models have been established, with basic Pavlovian conditioning and extinction paradigms up front. With the increasing legalisation of medical cannabis, its potential to treat anxiety-related disorders has become of great interest. As previous research has mostly focused on animal models, translational approaches in basic human research are mandatory (Papini et al., Biol. Psychol. 104 (2015) 8-18). Lutz et al. (Nat. Rev. Neurosci. 16 (2015) 705-718) and Hillard (Neuropsychopharmacology 43 (2018) 155-172) have postulated that endocannabinoids, particularly anandamide (AEA) and 2-arachidonoylglycerol (2-AG) play an important neuromodulatory role in fear-related processes. AEA is degraded by fatty acid amide hydrolase (FAAH), and the single-nucleotide polymorphism (SNP) rs324420 in the FAAH-coding gene has been demonstrated to modulate fear extinction learning (Dincheva et al., Nat. Commun. 6 (2015) 6395).

In the present 'learning-extinction-retention' functional magnetic resonance imaging (fMRI) study, 52 healthy men underwent a fear conditioning paradigm involving unpleasant thermal stimuli coupled with geometric figures to investigate the neural signals during fear learning (day 1), extinction learning (day 2), and retention of the latter (day 3). To elucidate the role of circulating plasma AEA and 2-AG in these three processes, blood samples were taken and analysed using liquid chromatography-mass spectrometry, and FAAH genotyping was performed.

Replicating the findings of recent meta-analyses (Fullana et al., Mol. Psychiatry 21 (2015) 500-508; Fullana et al., Neurosci. Biobehav. Rev. 88 (2018) 16-25), we observed brain activation associated with learning and extinction of fear in the anterior insula, dorsal anterior cingulate cortex (dACC), and the ventral striatum. Across participants, baseline (day 1) AEA-, but not 2-AG-levels, significantly correlated with the degree of extinction learning in the dACC and the right anterior insula (whole-brain analysis, voxel-level $p < 0.001$, cluster-level $p < 0.05$, FWE-corrected). Using the anterior insula as a seed region, we analysed baseline resting-state fMRI data to further explore AEA's putative modes of action. Highly significant functional coupling, negatively correlating with the degree of extinction, was found exclusively with the right hippocampus (whole-brain analysis, voxel-level $p < 0.001$; cluster-level $p < 0.05$, FWE-corrected). This means, the stronger the coupling, the lower the magnitude of the neural fear extinction signal. As with fMRI activations, individual baseline AEA-, but not 2-AG- levels, were negatively correlated with insula-hippocampal coupling indices (peak voxel at [28, -24, -18]; z-score = 3.35; $p = 0.002$, FWE-corrected for search volume), suggesting that individual availability of AEA could have influenced the neural network mediating extinction learning. Confirming previous literature (Dincheva et al., Nat. Commun. 6 (2015) 6395) regarding the FAAH polymorphism, mean plasma levels of AEA were significantly ($t(50) = 2.87$, $p = 0.006$) higher in the 18 participants identified as carriers of the A allele ($[AEA]_{CA} = 0.49 \pm 0.16$ pmol/ml) compared to the 34 individuals that were homozygous for the C allele ($[AEA]_{CC} = 0.38 \pm 0.13$ pmol/ml). However, the neural extinction signal did not significantly differ between FAAH genotypes.

In a nutshell, our setup, designed to advance the transfer of animal research findings regarding neural extinction learning, confirmed the hypothesis of an essential role of endocannabinoids – particularly AEA – in the neurobiology of anxiety-related disorders in humans. As a clinical proof of concept, patients with stress or anxiety disorders and lower AEA levels might be pre-treated with AEA-enhancing drugs to promote extinction learning prior to cognitive behavioural therapy interventions.

STANDARDIZATION OF CANNABIS DOSE FROM USER-DERIVED RATINGS; ESTABLISHING THE INDEX OF CANNABIS EQUIVALENCE (ICE)

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Regulatory liberalization and commercialization have resulted in increasing availability of diverse cannabis products. Standardization of dose units across modes is necessary to guide policy, research, and education. Previous attempts at standardizing cannabis dose have focused on herbal cannabis, and as such establishing consistency across other modes remains a research priority. The present study describes the development and preliminary validation of the Index of Cannabis Equivalence (ICE); a measure designed to establish user-derived equivalency across different modes and forms of cannabis use.

Adult Canadian cannabis users (n = 221; 76% Female, M_{age} = 19.49 (1.58)) reported experience with joint, pipe, vaporizer, bong, concentrates, and/or edibles and what they considered to be a 'low', 'medium', and 'high' dose for each. Users also reported their tolerance level and perceived level of cannabis knowledge. Data collection is ongoing with a target of N > 500 for time of presentation (June 2019).

Preliminary analyses indicated acceptable interrater reliability across subscales (ICC = .70 - .80). Responses indicated consistency across participants, particularly at low doses, with 78% identifying a low dose as 1-3 *puffs of a joint*, 85% as *2.5-5 mg of edible,* and 62% as ¼ *dab of concentrate.* Ratings of high doses were less consistent with 34% endorsing a high dose as 8-10 *puffs of a joint* as, 28% endorsing *1 dab of concentrate*, and 25% endorsing 10-15 *mg of edible*. Higher reported tolerance was associated with endorsing larger doses as being *high*.

The ICE represents an initial step to establishing standard cannabis dose across different modes of use. Responses were more consistent at the low dosages and for smoked herbal cannabis. Using puffs on a joint as an anchor point, results suggest that 1-3 puffs is perceived to be equivalent to a 2.5-5mg edible or a ¼ dab of concentrate. These results have methodological implications for measuring cannabis dosage in naturalistic settings. Further research is required to more reliably establish dose units for cannabis as have been developed for other psychoactive substances such as alcohol and opioids.

THE EDUCATION, KNOWLEDGE, AND PRACTICE CHARACTERISTICS OF CANNABIS PHYSICIANS: A SURVEY OF THE SOCIETY OF CANNABIS CLINICIANS

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Medical cannabis use has increased in recent years despite being a federally illegal drug in the United States. States with medical cannabis use laws require patients to be certified by physicians. However, little is known about the education, knowledge and practice characteristics of physicians who recommend and supervise patients' use of medical cannabis. The current study assessed how US physicians who practice cannabis medicine are educated, self-assess their knowledge, and describe their practice. In fall 2017, a 57-item, electronic survey was sent to all members of the Society of Cannabis Clinicians. Because California has had legalized medical cannabis for longer than any other state, we analyzed responses for 14 items between California and non-California physicians.

Of 282 surveyed, 133 were eligible, and 45 completed the survey. Of those, multiple medical specialties were represented. Only 1 physician received formal graduate education in cannabis medicine, but physicians gained knowledge through conferences (71%, 32/45), the medical literature (64%, 29/45), and websites (62%, 28/45). Just over half (56%, 20/45) felt there was sufficient information to practice, and of those who indicated, most (78%, 29/37 and 76%, 28/37) felt knowledgeable about cannabinoids and the endocannabinoid system, respectively. There was a wide variation in the number of cannabis recommendations provided by physicians (median 1200, IQR range 100-5000), and most (69%, 31/45) provided condition-specific treatment (69%, 31/45) and dosing recommendations (62%, 28/45). The majority (81%, 30/37) of physicians received referrals from mainstream medical providers. No differences were found between California and non-California physicians, except more women were from California (P=.02).

The use of medical cannabis continues to increase in the US and globally. All states that allow medical cannabis require a physician's recommendation, yet few states require specific clinical training. Findings of the current study suggest the need for more formal education and training of physicians in medical school and residency, more opportunities for cannabis-related continuing medical education for practicing physicians, and clinical and basic science research that will inform best practices in cannabis medicine.

Acknowledgements: The authors are indebted to the Society of Cannabis Clinicians for their administrative support and to the members of the research committee who contributed to this project.

DIFFERENCES AND SIMILARITIES IN RECREATIONAL AND MEDICINAL CANNABIS USERS IN THE NY-METRO AREA: A COMMUNITY SURVEY

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Aims: Cannabis is legal for medicinal purposes, and proposed for legalization for recreational purposes, in New York State (NYS). However, little is known about the association between cannabis use patterns and demographic factors, and medicinal vs recreational cannabis use, from the NY-Metro area (NYS' most populous region). The aim of this analysis was to compare self-identified medicinal and recreational cannabis users on these factors.

Methods: A 39-item original survey, targeting adult primary cannabis users (at least weekly) from Long Island and NY, was deployed via Facebook©. The full sample (n=891) was adult (M=33.2 years, SD=8.6), largely male (62.3%) and employed (67.6%), educationally diverse, and exhibited a racial composition representative of the region. Cannabis user type was identified via a 60/40 endorsement threshold on a 100-point Likert scale item anchored by "only medicinal" and "only recreational" (medicinal users: N=143, observed M=70.6 [SD=8.8]; recreational users: N=152; observed M=27.1 [10.4]). Group comparisons were accomplished via independent sample t-tests and chi-square tests.

Results: Relative to the recreational group, the medicinal group exhibited greater ($p<0.05$) frequencies of self-reported: 1) female biological sex (39.4 vs 25.7%), 2) nonsocial cannabis use (35.0 vs 16.4%), 3) lack of other substance use (38.5 vs 17.1%), 4) incidence of lifetime psychiatric diagnoses (65.5 vs 34.9%), and 5) obtaining cannabis in-person (47.6 vs 34.2%), from a dispensary (21.0 vs 8.6%), and using credit/debit card payment (9.8 vs 2.0%). Relative to the medicinal group, the recreational group exhibited greater ($p<0.05$) frequencies of reported: 1) male biological sex (73.0 vs 60.6%), 2) mixed (social and nonsocial) cannabis use (75.7 vs 62.9%), 3) alcohol use (69.7 vs 47.6%), and 4) obtaining cannabis via text message (50.0 vs 33.6%), and from a private dealer (65.1 vs 51.0%). The groups did not differ ($p>0.10$) on other reported demographics or other cannabis-related variables (e.g., current: non-oral administration routes, strain type, weekly frequency/amount; or lifetime: age of onset, and presence of cannabis withdrawal or use disorder).

Conclusions: The overall sample composition was reflective of the NY-Metro area. Almost all participants endorsed some degree of mixed medicinal/recreational cannabis use, with only 16-17% of the sample endorsing at least 60% of one-use type or the other. The groups were similar on most measures of demographic and cannabis use characteristics, but differed on biological sex ratio, methods to obtain cannabis, the sociality of their cannabis use, and presence of other substance use. These findings are relevant to the debate in NYS around cannabis.

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MARIJUANA/CANNABIS IMPAIRED DRIVING: RETINAL DYSFUNCTION OF RODS, CONES AND GANGLION CELLS

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Background: Marijuana/Cannabis causes changes in vision and perception and thus has consequences related to driving tasks. Cannabinoid receptors have been identified throughout all layers of the retina including the ganglion cell layer, the inner plexiform layer, the inner nuclear layer, the outer plexiform layer, the outer nuclear layer and photoreceptor layers. There are reports in the literature identifying functional deficits related to marijuana use that are likely to originate at the photoreceptor level which include color deficits. Other reports of functional loss related to motion and contrast perception likely originate in the nuclear or ganglion cell layers. There is every indication that cannabinoids have multiple mechanisms of action within the retina. IMMAD, LLC is developing technology that efficiently measures several functional aspects of vision processing in relation to marijuana use. This development is funded through a National Institutes of Health and National Institutes on Drug Abuse SBIR Phase I contract.

Methods: Using the prototype of a product developed by IMMAD, LLC – the Headborne IMMAD Test or HI-Test, participants were measured at baseline and then within twenty minutes of having self-dosed using their own cannabis product. HI-Test is a vision-based test projected to optical infinity by using a conventional virtual reality goggle and smartphone with a Bluetooth response button. The participant responds when they see a set of five-degree squares with stripes of either moderate or low spatial frequency alternating temporally at 20Hz. The test uses either grey scale or chromatic stripe (blue/yellow) with matched luminance.

Results: The data remains limited. A chromatic target of moderate/high spatial frequency is specifically measuring central cone function. The five-degree higher spatial frequency target is only measuring macular function and there are no rod receptors in this region. Only low spatial frequency targets were used outside the macular region. A chromatic target of low spatial frequency outside the central region can be used for measuring at other retinal levels including either the plexiform layer or ganglion cell layer. A peripheral target of either chromatic or grey scale measures rod or retinal layers other than photoreceptors. We are identifying deficits in the central region with a temporal alternating target. There are also deficits elsewhere with trends in the superior region with the temporally alternating target.

Conclusions: The retina provides an opportunity to better understand both structure and function in live human beings when it comes to neuroprocessing in general. Given that there are cannabinoid receptors throughout the multiple layers of the retina, using the retina to investigate the effects of both chronic and acute use of marijuana is an efficient way to better understand the dysfunction that can occur in neuroprocessing and neurotransmission. The strategy of using a quick, simple and objective means to assess the retina is not only of benefit to research but also to law enforcement and may have clinical applications as well.

A HUMAN ABUSE POTENTIAL STUDY TO EVALUATE THE SUBJECTIVE AND PHYSIOLOGICAL EFFECTS OF CANNABIDIOL COMPARED TO DELTA-9-TETRAHYDROCANNABINOL AND ALPRAZOLAM IN AN INPATIENT SETTING

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NIH (NIDA and NCATS), in collaboration with CSS-FDA, conducted an inpatient human abuse potential study to evaluate the abuse potential of cannabidiol (CBD). This was a single-dose, randomized, double-blind, placebo- and active-controlled crossover study enrolling healthy volunteers who were recreational drug users. The study was performed to provide clinical data that would contribute to the determination of the HHS recommendation for scheduling of CBD under the Controlled Substances Act (CSA).

Forty-three subjects were qualified to participate in the Treatment Phase and were randomized to 1 of 6 treatment sequences in which they received the following acute treatments: CBD 500 mg, CBD 1000 mg, THC 2.5 mg, THC 30 mg, alprazolam 1.5 mg, and placebo. One subject withdrew on Day 13 due to personal reasons. The remaining 42 subjects received each of the treatment conditions and completed all visual analog scale (VAS) assessments for at least 8 hours after administration of each of the 6 treatments. The duration of the Treatment Phase was 18 inpatient days.

The study's primary objective was to evaluate abuse-related subjective responses of CBD 500 mg and 1000 mg in comparison with the positive controls (THC 30 mg and alprazolam 1.5 mg) and placebo. Secondary objectives were to determine the safety, tolerability, physiological response, and pharmacokinetics of CBD, THC, and alprazolam.

The positive control drugs (THC 30 mg and alprazolam 1.5 mg) produced statistically significant increases on positive VAS measures compared to the placebo, which validates the study. On the primary study endpoint of Drug Liking VAS (maximum value; Emax), CBD 500 mg and 1000 mg produced responses that were statistically similar to placebo. CBD 500 mg and 1000 mg produced responses on all other VAS measures that were statistically significantly lower than the positive controls. Acute administration of CBD 500 mg and 1000 mg was well tolerated without any significant adverse events (AEs). No deaths, serious AEs or unexpected AEs, were reported during any phase of the study.

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IDENTIFYING AND EVALUATING ABUSE-RELATED EVENTS IN CLINICAL TRIALS EXAMINING CANNABIS-DERIVED SUBSTANCES

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As pharmaceutical development of cannabis and research into its potential therapeutic benefits continues to advance, the medical community and regulatory authorities are increasingly more accepting of cannabis and cannabis-derived substances for medical use. However, regulation and scheduling of cannabinoids, as with other psychoactive drugs, requires a careful balancing of their potential medicinal benefits with their potential for abuse.

Efforts to develop individual cannabinoid molecules such as tetrahydrocannabinol (THC) and cannabidiol (CBD) into approved medicines have provided evidence that the individual components of cannabis may have distinct therapeutic applications, with varying subjective effects that result in vastly different abuse potential profiles. Additionally, variations in formulation or route of administration may dramatically affect a drug's risk for abuse.

Therefore, a critical component of any cannabinoid pharmaceutical development effort must include a comprehensive assessment of the product's potential for abuse in the context of its therapeutic effects. As cannabis-derived products are assessed for their potential to treat diseases such as cancer, pain, epilepsy, and depression, it is necessary to define whether resulting subjective effects (if any) are signals that the drug has a risk for abuse, or if those effects are desirable in treating the intended indication.

While nonclinical abuse related studies can act to inform clinical study design, sponsors pursuing development and regulatory approval of cannabis-based products must systematically collect and assess abuse and withdrawal related adverse events in all phases of clinical development as required by FDA per its finalized Guidance on the Assessment of Abuse Potential of Drugs. Among the various components of a complete submission outlined in the finalized guidance are new standards for the monitoring of safety and efficacy trials, which can provide important information on abuse within target populations and rates of diversion. Findings from these studies can provide insights into the need for a risk management plan as well as guide design of such a plan.

This presentation will highlight the use of the MADDERS® system (Misuse Abuse and Diversion Drug Event Reporting System), which was developed in accordance with recommendations put forth by FDA to provide a systematic, standardized, and prospective approach to more accurately assess and classify potentially abuse-related events associated with CNS-active drugs in clinical trials. This presentation will also offer insights and guidelines for evaluating and contextualizing abuse-related events in pharmaceutical cannabinoid development in order to support appropriate approval, labeling, scheduling, and risk management.

PRELIMINARY OVERVIEW OF SELF-REPORTED ADVERSE EVENTS IN MEDICINAL CANNABIS USERS: RETROSPECTIVE DATABASE ANALYSIS ACCORDING TO PRODUCT POTENCY COMPOSITION AND FORM

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Cannabis is the most commonly consumed illicit drug globally. It is used for therapeutic, medicinal and recreational purposes. In response to newly implemented Canadian laws legalizing possession and use of Cannabis for recreational purpose, the consumption of cannabis may increase further. Legalization is also expected to lower the stigma attached to the use of Cannabis, and individuals may thus feel more comfortable to: 1. inquire with their health-care practitioner about its applications and effects; and 2. report the presence of adverse events when they do occur. Canadian licensed producers are required to report all adverse reactions to the Canada Vigilance Program, as stated in the *Compliance and Enforcement of the Access to Cannabis for Medical Purposes Regulations*. Monitoring the adverse events associated with Cannabis is further complicated by the variety of available formats (ingested, inhaled, sublingual, etc.), forms (dried flower, oral oil, and soft gel) and the potency of the primary active compounds, tetrahydrocannabinol (THC) vs. cannabidiol (CBD). Cannabis is used medicinally for a wide range of applications, however research to date has yet to identify specific products best suited for specific symptoms or conditions. The objective is to present the findings of a retrospective observation data set in support of the development of safety guidelines for cannabis products and the establishment of a global drug monitory program. We aim to describe self-reported adverse events (AE's) according to different product forms, among Canadian medicinal cannabis users.

A retrospective observational analysis was applied to the Canopy Growth Corporation (CGC) Customer Care database which collected self-reported AE's between January 2017 and November 2018 (22 months). The target population is adults (≥18 years of age), living in Canada who purchased and used Cannabis products based on their medical prescription, and self-reported AE's to the CGC Customer Care division by phone. Primary outcome measures included: self-reported AE's and level of seriousness of the AE. The analysis demonstrates the incidence of AE's (serious and non-serious) by product: BLUE (38.6%), YELLOW (35.3%), RED (11%), and ORANGE (8.1%), stratified by form: dried flower, oral oil, and soft gel. Non-serious adverse events (NSAEs) were coded in 14 MedDRA System Organ Class (PT level), including Hypersensitivity (23.3%), Other (17.5%), Discomfort (9.9%), Diarrhoea (7.2%), and Dizziness (5.3%).

Prospectively collected data such as the CGC Customer Care database provide important real-world evidence on safety and effectiveness of medical cannabis use. While conclusions about efficacy are limited, we have valuable insights on the current safety of products and the incidence of self-reported adverse events. Further research is necessary to investigate the causality of these adverse events according to potential explanatory variables, and the chemistry/pharmacodynamics of the THC and CBD composites.

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EFFICACY, SIDE EFFECTS, AND PATTERNS OF MEDICAL CANNABIS USE AMONG PATIENTS WITH CHRONIC LOW BACK PAIN

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Although cannabis is widely used for the treatment of chronic pain, there is very sparse data about the products patients consume and their patterns of consumption. The most widely used inventory for measuring cannabis use (Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory, or DFAQ-CU) was not designed to capture the medically relevant features of cannabis use. Thus, we created the Inventory of Medical Cannabis Use (iMCU) to measure the consumption methods, frequency, side effects, and phytochemical features of the cannabis products that patients use to alleviate their symptoms. Study participants were enrolled in Pennsylvania's medical cannabis program. At the time of their initiation into the program, participants were provided with education about various consumption methods, pharmacokinetics, and the types of cannabis products that they would subsequently be able to purchase at a retail dispensary. In this observational study, participants purchased products from state licensed retailers, in accordance with their needs and preferences. The first follow-up was conducted at 3 months after the initiation of cannabis therapy. The iMCU was utilized to capture details of cannabis use, and the analgesic efficacy of cannabis was assessed with the ODI (Oswestry Disability Index), PROMIS (Patient-Reported Outcomes Measurement Information System), and VAS (Visual Analog Scale). At the first follow up, back pain intensity was significantly decreased in patients aged 18-59. Forty-three percent of patients reported that cannabis relieved their symptoms without producing intoxication. Potent THC products (inhaled vapor) were most commonly used at nighttime, to promote sleep. Our preliminary results indicate that the iMCU is sensitive to the patterns of cannabis use by medical patients, however they also highlight the difficulty for patients to accurately report the doses and phytochemical makeup of the products in their regimen. This work highlights the importance of physician involvement in symptom management and provides a framework for understanding the efficacy of specific cannabis formulations and administration routes for analgesia.

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DELTA-9-TETRAHYDROCANNABINOL MODERATES THE EFFECTS OF AVOIDANCE SYMPTOM SEVERITY DURING FEAR EXTINCTION IN TRAUMA-EXPOSED INDIVIDUALS

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Avoidance of stimuli associated with a traumatic event can lead to the development of trauma-based disorders and perpetuate the severity of symptoms. Indeed, avoidance can interfere with the ability to extinguish conditioned fear responses, one of the hallmarks of trauma-based disorders. Recent data from our lab suggests that an acute dose of Δ9-tetrahydrocannabinol (THC), prior to fear extinction, facilitates recall of extinction learning by increasing activation in corticolimbic brain regions. However, it is unknown if THC can facilitate fear extinction in individuals with high avoidance symptoms. The present study examines the effect of avoidance symptoms on fear-related neural activation during extinction memory recall and how THC moderates that relationship. 60 trauma-exposed adults (ages 18 – 60) participated in a randomized, double-blind, placebo-controlled, between-subjects design and completed a novel Pavlovian fear-extinction paradigm using virtual reality coupled with FMRI. During fear acquisition, two conditioned stimuli (CSs) were presented: two CS+s paired with an aversive unconditioned stimulus (US) and one CS- never paired with the US (safety cue). Before fear extinction, participants were administered an oral capsule containing either 7.5 mg of THC or sugar (PBO). During fear extinction, one CS+ was extinguished (CS+E), while the other was not (CS+U). 24 hours later, all CSs were presented during recall of extinction learning. Avoidance symptom severity scores were measured with the Clinical Administered PTSD Scale-5.

Participants given THC had higher medial prefrontal cortex (mPFC) activation during recall of extinction learning (CS+E, $t(32.59) = 2.093$, $p < .05$) compared to those given PBO. Moreover, THC was a significant moderator in the relationship between avoidance symptom severity and vmPFC activation during recall of extinction learning ($\Delta R^2 = .17$, $\Delta F(1,56) = 11.58$, $p < .005$; $b = .45$, $t(56) = 3.35$, $p < .005$). Specifically, when participants were given THC, vmPFC activation increased with increasing avoidance symptom severity scores. However, vmPFC activation decreased with increasing avoidance symptom scores in participants given PBO. This data suggests THC modulates fear-related neural activation during extinction memory recall and may be most beneficial to trauma-exposed individuals with high avoidance symptom severity.

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CANNABINOID USE IN A POPULATION BASED SURVEY OF ADULT ATHLETES

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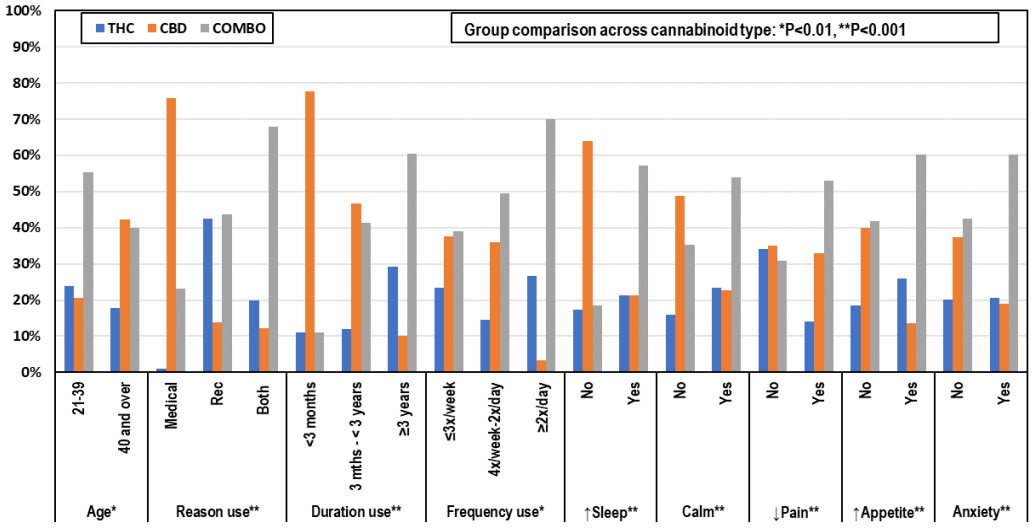
Tetrahydrocannabinol (THC) and cannabidiol (CBD) have been used for medicinal and recreational purposes. THC is primarily known for its psychogenic effects while CBD is purportedly an anti-inflammatory and analgesic, however it may be the synergistic effect of THC and CBD that provides the major benefit (National Academy of Sciences, 2017). Little is known about factors that impact cannabinoid choices and specific cannabinoid subjective effects in adults, thus the current study aimed to understand these aspects of cannabis use.

The Athlete PEACE Survey used mainly social media and email blasts to recruit and SurveyGizmo to collect data. 1,161 (91.1%) of the 1,274 athletes taking the survey completed it. Current cannabis use was evaluated by asking "In the past two weeks, have you used marijuana (including THC and/or CBD)?" and cannabis type used was assessed by asking "What do you primarily use THC, CBD, or both?". Cannabis patterns of use, benefits, and adverse effects were reported. 301 athletes (26%) currently use cannabis of which 61 (20.3%) use THC only, 101 use CBD only (33.6%) and 139 (46.2%) use both THC and CBD (Combo). Younger athletes use THC and Combo more often than older athletes (P=0.001) and athletes with pain lasting more than three months use CBD more often than those with no pain or acute pain (P<0.05). Athletes using cannabis recreationally predominantly use THC (42.5%) while those who use it both medically and recreationally prefer Combo (67.8%) (P<0.001). Novice users choose CBD (77.8%) while more experienced users favor Combo (60.3%) (P<0.001); the most frequent users also mostly use Combo (69%). Subjective effects are impacted by cannabinoid type. Specifically, THC and Combo were more effective for sleep and calm (both P<0.001) and caused an increased appetite (P<0.001) and anxiety (P<0.05) compared to CBD only. CBD and Combo improved pain more than THC (P<0.001). In conclusion, about 26% of adult athletes use cannabis with almost half choosing a combination of THC and CBD. Age, reason for use, and frequency and duration of use impact cannabinoid choices. The cannabinoid used impacts subjective effects to cannabis.



IMPACT OF THE ENDOCANNABINOIDOME ON SMALL INTESTINE EPITHELIAL PERMEABILITY

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The endocannabinoid system is composed of cannabinoid CB1 and CB2 receptors, their two main endogenous lipid ligands anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG) and enzymes involved in their biosynthesis and catabolism. The endocannabinoidome, encompasses anandamide, 2-AG, their congeners as well as dozens of related-converting enzymes and receptors. It is a pleiotropic system which is involved in several aspects of human physiology and pathology. The role of the endocannabinoidome in the gastrointestinal tract (e.g. small intestine) is not yet well known, but it is suggested to mediate environmental factors (e.g. gut microbiota, diet). The aim of this study is to investigate the impact of the endocannabinoidome on intestinal epithelium permeability, a key aspect of small intestine epithelium integrity. Organoid cultures (n=4) were derived from small intestinal crypts of C57BL/6J mice. Organoid cultures were pretreated (24h) with fatty acid amide hydrolase inhibitor (URB 597, 1-10 μM) and monoacylglycerol lipase inhibitor (JLZ 184, 1-10 μM) to increase levels of ethanolamine and glycerol mediators. The impact of these treatments on the epithelial barrier integrity was then investigated using fluorescent-labeled dextran molecules (FITC-dextran) of 4 kDa (detectable in the DAPI channel) and 10 kDa (detectable in the GFP channel) simultaneously in the same organoid cultures. Quantification of fluorescent-labeled dextran entering the organoid lumen was measured using a Cytation 5 image reader. The ratio DAPI/GFP was calculated for each treatment as a reflect of epithelial permeability of organoids; a higher ratio corresponding to a lower permeability.

We observed that organoids are permeable to dextran molecule of 3 kDa and slightly less permeable to dextran molecules of 10 kDa. Compared to control organoid cultures (ratio: 4.64±0.20), cultures incubated with 1 μM (ratio:8.03±0.27) or 10 μM (ratio:12.39 ±0.21) of JLZ 184 presented an elevated DAPI/GFP ratio. A dose response was also observed for URB 597 with 1 μM (ratio:5.07±0.25) and 10 μM (ratio:9.58 ±0.23). In conclusion, we have developed and validated an assay to assess the involvement of the endocannabinoidome in the regulation of epithelial permeability and underlying mechanisms. We have observed that increased overall endocannabinoidome signaling reduces epithelial permeability in organoids.

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ROLE OF 2-ARACHIDONOYLGLYCEROL IN STRIATAL LONG-TERM DEPRESSION AND ETHANOL PREFERENCE / DRINKING BEHAVIOR

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The striatum has important roles in action initiation and control, as well as the rewarding effects of alcohol. Endocannabinoid (eCB)-dependent long-term synaptic depression (LTD) is a well-characterized form of synaptic plasticity in striatum that may play a role in the hedonic (goal-directed) and automatized (habitual) behaviors controlled by this brain region. There are two types of eCBs, arachidonoylethanolamine (AEA) and 2-Arachidonoylglycerol (2-AG), which differ in their biosynthesis/degradation pathways and receptor binding affinity. Both subtypes of medium spiny neurons in striatum, indirect-projecting (iMSNs) and direct-projecting (dMSNs) contain the endocannabinoid signaling machinery and can undergo endocannabinoid mediated LTD. However, the type of eCB (i.e. 2-AG or AEA) mediating this form striatal synaptic plasticity is unclear. To probe the role of 2-AG in the induction/expression of LTD and the rewarding effects of alcohol, we generated Cre-LoxP conditional knockout mice (2AG KO) in which the 2-AG-synthesizing enzyme diacylglycerol lipase α (DAGLα) is deleted in dMSNs.

LTD induced by bath application of the group I metabotropic glutamate receptor agonist DHPG was intact in both d- and iMSNs in the dMSN-2AG KO mice, suggesting that 2AG is not involved in this form of eCB-mediated LTD. Experiments are underway to examined the role of 2-AG in HFS-induced LTD. To assess ethanol consumption and preference in the dMSN-2AG KO mice, animals were provided with increasing concentrations of alcohol in an intermittent two-bottle choice paradigm. On day 1 of drinking, male dMSN-2AG KO mice showed decreased ethanol consumption and preference. Although male dMSN-2AG KO mice consumed amounts of alcohol similar to controls with repeated exposure, there was a trend towards decreased alcohol preference in the KO mice. There were no genotype differences in alcohol consumption and preference in female dMSN-2AG KO mice. Thus far, our findings indicate that 2-AG is not involved in DHPG eCB mediated LTD, but may play a role in alcohol consumption and preference in a sex-dependent manner.

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ADAPTATIONS IN CANNABINOID RECEPTOR SIGNALING IN THE VENTROLATERAL PERIAQUEDUCTAL GRAY DURING PERSISTENT INFLAMMATION

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Chronic pain is increasingly prevalent, yet the primary treatment option, opioids, lack long-term efficacy and are highly addictive. Cannabinoids produce analgesia but the mechanism of is not well understood. The ventrolateral region of the periaqueductal gray (vlPAG) is a critical site for cannabinoid-induced analgesia, so our laboratory is examining cannabinoid receptor signaling within this region under normal conditions and following inflammatory pain. Persistent inflammation induced by Complete Freund's Adjuvant (CFA) induces a shift in cannabinoid signaling from the cannabinoid 1 (CB1) receptor to the cannabinoid 2 (CB2) receptor within the rostral ventromedial medulla (RVM), a region just downstream of the vlPAG. In the present study, cannabinoid receptor function after inflammation induced by CFA injection into the hind paw is examined in the vlPAG using slice electrophysiology and pharmacology. Male and female Sprague Dawley rats (PN 30-60) were used to determine potential sex differences within the vlPAG cannabinoid system.

In naïve animals, the non-specific cannabinoid receptor agonist WIN 55,212-2 (3μM) suppresses GABA release as measured by miniature inhibitory post-synaptic currents (mIPSCs). This effect was reversed by Rimonabant (3μM) and recovered above baseline, suggesting a CB1 receptor-mediated tone within the vlPAG of naïve animals. Consistent with previous findings in the RVM, this effect was abolished 5-7 days following CFA injection into the hind-paw. Current studies are investigating CB1 receptor desensitization and downregulation as potential mechanisms for loss of CB1 receptor inhibition of mIPSCs. In addition, CB2 receptor activity in the vlPAG was investigated in naïve and CFA-treated animals. Similar to the findings in the RVM, neither superfusion of the CB2 receptor-selective agonist AM1241 (3μM) nor the CB2 receptor antagonist SR144,528 (3μM) alter GABA release within the vlPAG. In contrast to findings in the RVM, there was no evidence of CB2 receptor effects in the presence of CFA. These data indicate that cannabinoid receptor signaling is different between vlPAG and RVM. Future work is investigating the possibility that CB1 receptors are expressed on specific brain circuits impinging on the vlPAG. Results from these projects will elucidate novel mechanisms of pain that could aid in the future treatment of pain and development of analgesic drugs.

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DELETION OF 12/15 LIPOXYGENASE LEADS TO ALTERED REMODELLING DURING WOUND REPAIR

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12/15-Lipoxygenase (LOX) is expressed by infiltrating immune cells and involved in innate immune and inflammatory responses. Here, we evaluate the role of this enzyme in the cellular orchestration of wound healing using a murine model. Full thickness (4mm) wounds were caused in wildtype and 12/15-LOX$^{-/-}$ mice, then wound closure measured and skin harvested at various time points (up to 14 days) post wounding and characterised using several approaches.

Although skin closure times were comparable to wild type mice, the underlying healing process was revealed to be significantly altered in mice lacking 12/15-LOX. First, 12/15-LOX$^{-/-}$ skin showed significantly reduced macrophage numbers (approximately 200 fewer per skin section). Although no differences were seen in neutrophil number, a four fold increase in the number of neutrophil extra cellular traps was observed in 12/15-LOX$^{-/-}$ day 4 wounds. Evaluation of epithelial to mesenchymal transition, revealed that the stem cell marker SSEA-3 was significantly increase (approximately five fold) in 12/15-LOX$^{-/-}$ wounds. The migration distance of proliferating keratinocytes into the wound was greater (approximately 208 μm) in 12/15-LOX$^{-/-}$ wounds. 12/15-LOX protein expression was strongly induced in macrophages at day 1 in wild type wounds, returning to non-wounded levels by day 14 post wounding. Lipidomic analysis revealed a temporal elevation in eicosanoids in wildtype and 12/15-LOX$^{-/-}$ wounds. Further analysis revealed that a subset of lipids including 15-HEPE, 14-HDOHE, 17-HDOHE, and 13-HOTrE were substantially increased in day 1 wildtype wounds but not in 12/15-LOX$^{-/-}$ wounds. By comparison elevation in prostaglandin levels (induced by wounding) were seen in both wildtype and 12/15-LOX$^{-/-}$ wounds.

These data indicate that 12/15-LOX exerts complex effects on the tissue repair process and is essential for the normal physiological response to wounding. The absence of the enzyme leads to a dysregulated phenotype. Our studies uncover novel control checkpoints that could be harnessed to improve wound healing in human populations.

TARGETING CANNABINOID TYPE 2 RECEPTORS TO SUPPRESS ANTIRETROVIRAL-INDUCED NEUROPATHIC NOCICEPTION

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The development of a painful peripheral neuropathy is the most common neurological complication associated with human immune deficiency virus (HIV) infection, affecting approximately one third of people living with HIV. HIV-associated peripheral neuropathy is typically due to two causes- the result of toxic viral protein byproducts, or the use of antiretroviral drugs such as 2'-3'-dideoxycitidine (ddC). Despite improving overall health of HIV-infected individuals, antiretroviral-induced neuropathic pain remains a significant source of morbidity and a detriment to quality of life. Currently available treatments for the clinical management of chronic pain fail to provide adequate symptom relief, indicating the need for novel treatment strategies. Several reports indicate the use of smoked cannabis in clinical populations provides relief from symptoms. However, agents found in cannabis that target cannabinoid type 1 receptors (e.g. Δ^9-tetrahydrocannabinol) produce problematic side effects such as impairments in learning and memory, a potential for abuse, and the development of tolerance to their antinociceptive effects. Previous reports from our laboratory indicates that agents that target cannabinoid type 2 receptors (CB2) display antinociceptive efficacy, but lack the problematic side effects associated with CB1 agonists such as tolerance and physical dependence. However, the impact of CB2 agonists on antiretroviral-induced neuropathic pain have not been previously described. Moreover, the cell types that express CB2 receptors are incompletely understood whether they are dynamically regulated by ddC remains unknown. Therefore, the current studies investigated the impact of the CB2 agonists AM1710 and LY2828360 on neuropathic pain induced by the antiretroviral drug ddC. We also examined cell populations that contain CB2 receptors that may contribute to their antinociceptive efficacy. To aid in the detection of CB2 receptors in different cell populations, we generated a transgenic mouse ($CB2^{f/f}$) in which enhanced green fluorescent protein is under control of the CB2 promoter (Lopez et al. (2018) J. Neuroinflammation 15: 158). Furthermore, in this mouse line the CB2 gene is flanked by *lox*P sites allowing for conditional deletion of CB2 receptors from cells expressing Cre recombinase. We, therefore, removed CB2 receptors from excitatory neurons by crossing $CB2^{f/f}$ mice with mice expressing Cre recombinase under the control of neuronal helix-loop-helix protein ($Nex^{cre/+;}CB2^{f/f}$). CB2 was also removed from peripheral sensory neurons by crossing $CB2^{f/f}$ mice with mice expressing Cre recombinase under the control of the actin regulatory/binding protein advillin ($advillin^{cre/+;}$ $CB2^{f/f}$). AM1710 and LY2828360 dose dependently decreased ddC-induced mechanical and cold allodynia in WT mice, but failed to alter hypersensitivity in global CB2 knockout mice. The antinociceptive efficacy of AM1710 and LY2828360 were preserved in $CB2^{f/f}$ mice, but absent in $Nex^{cre/+;}CB2^{f/f}$ and $advillin^{cre/+;}CB2^{f/f}$ mice, suggesting that removal of CB2 receptors from peripheral sensory neurons eliminated the antinociceptive efficacy of CB2 agonists. The results of these studies suggest that CB2 receptors in peripheral sensory neurons are necessary for the antinociceptive effects of CB2 agonists in antiretroviral-induced neuropathic pain induced by ddC. Finally, using flow cytometry, we also observed populations of CD45 positive immune cells that expressed eGFP, and consequently CB2, in lumbar spinal cord, sciatic nerve and dorsal root ganglia derived from vehicle and ddc-treated $CB2^{f/f}$ mice.

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THE INFLUENCE OF 2-AG ON CHEMOKINES EXPRESSION IN FIBROBLAST-LIKE SYNOVIOCYTES STIMULATED WITH TNFα

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Modern concepts of osteoarthritis (OA) have been changed by new imaging phenotypes demonstrating complex and multi-tissue pathologies involving cartilage, subchondral bone and (increasingly recognized) inflammation of the synovium. The soft tissue lining the spaces of joints consists mainly of fibroblast-like synoviocytes (FLS), which release hyaluronic acid and lubricin to synovial fluid, but they may also secrete molecules taking part in development of inflammation, such as chemo- and cytokines. Since acute inflammation can contribute to the long-term development of OA joint pain through inter alia chemoattractants (such as CXCL1, CCL5 and CCL7), the ability of endocannabinoids to modulate this process and to influence the subsequent progression of persistent OA pain is an interesting research approach. Moreover, as the concentration of 2-arachidonyl glycerol (2-AG) is elevated in synovial fluid of RA and OA patients, we aimed to test the influence of 2-AG on modulation of inflammation in FLS.

Firstly, the optimal concentration of endocannabinoid was assessed. Human FLS isolated from OA patients were stimulated with 2-AG for 24 h. Based on LDH assay we choose for experiments the non-toxic (1 μM) concentration of 2-AG. Next, the cells were stimulated with 10 ng/ml of TNFα for 24 h, after that time 2-AG was administrated for another 24 h, and mRNA was isolated. Expression of *CXCL1, CCL5, CCL7* and *PTSG2* genes were measured with qPCR method.

We observed that after 48 h of TNFα stimulation expression of *CXCL1, CCL5* and *CCL7* genes increased and 2-AG administration significantly upregulated this process. The same pattern was observed in expression of *PTGS2* gene. The obtained results may be related with CB2 receptor activation by 2-AG and/or increasing of metabolism of that endocannabinoid to prostaglandins glycerol esters by COX2 enzyme.

In summary, in our experiments 2-AG caused pro-inflammatory action and predict that the CB2 present in the synovium may be an important player for the treatment of inflammation associated with OA. Additional studies are necessary to explain mechanism of that phenomenon.

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NO CANNABINOID RECEPTOR MEDIATION OF ANTINOCICEPTION PRODUCED BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Limited evidence suggests that some non-steroidal anti-inflammatory drugs (NSAIDs), in particular cyclooxygenase (COX)-2 inhibitors, may exert anti-inflammatory and pain-relieving effects in part via interaction with the endocannabinoid system. The purpose of the present study was to determine whether the antinociceptive and anti-inflammatory effects of a COX-2 inhibitor (celecoxib) or a non-selective COX inhibitor (ketoprofen) were mediated by CB1 or CB2 receptors. Given previously observed sex differences in various aspects of the endocannabinoid system, both females and males were tested. Inflammation and pain were induced by injecting complete Freund's adjuvant into one hindpaw of adult Sprague-Dawley rats. Two and ½ h later, either vehicle, rimonabant (1 mg/kg), or SR144528 (1 mg/kg), was injected i.p.; immediately thereafter, either vehicle, celecoxib (10 mg/kg), or ketoprofen (3.2 mg/kg) was injected i.p. (experimenters blinded to antagonist condition). Mechanical allodynia, heat hyperalgesia, biased weight-bearing, and hindpaw thickness (an index of edema) were assessed at 0.5, 1.5, 3.5 and 6 h post-injection.

Celecoxib and ketoprofen produced anti-allodynic, anti-hyperalgesic, and anti-edematous effects, and also increased weight-bearing on the inflamed paw in both sexes. Celecoxib restored weight-bearing to a greater extent in males than in females. In contrast, both drugs reduced edema to greater extent in females than males. Pretreatment with cannabinoid antagonists had little to no significant effect on any NSAID effect in either sex: rimonabant partially blocked the effect of celecoxib on weight-bearing in males only. These results suggest that, for the most part, acute antinociceptive and anti-inflammatory effects of systemically administered COX inhibitors are not mediated by CB1 or CB2 receptors in rats of either sex.

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EFFECTS OF Δ^9-THC AND MONOACYLGLYCEROL LIPASE INHIBITORS ON PAIN-STIMULATED AND PAIN-DEPRESSED ACUTE PAIN BEHAVIORS IN MICE

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Preclinical behavioral studies play a major role in the evaluation of cannabinoids and other classes of candidate analgesics. In any preclinical assay for analgesic drug development, a putative pain stimulus is delivered to a research subject with the intent of producing a pain state, and some behavioral response is measured as evidence of that pain state. Drugs are then administered to evaluate their effectiveness to block the pain behavior. Two major classes of pain-related behaviors are pain-stimulated and pain-depressed behaviors, which can be defined as behaviors that increase or decrease, respectively, in their rate, intensity, or frequency after delivery of a pain stimulus. Drugs that block both types of pain behavior are more likely to be effective clinically than drugs that do not. Here we report on a novel preclinical procedure to assess candidate analgesic effects on a panel of pain-stimulated and pain-depressed behaviors in mice. Intraperitoneal administration of dilute lactic acid (IP acid) serves as an acute noxious visceral stimulus in male and female ICR mice (N=6/sex) to produce four pain-related behaviors: stimulation of stretching and facial grimace and depression of rearing and nesting. Additionally, drug effects on nesting in the absence of IP acid provide a measure of drug potency and effectiveness to produce general behavioral disruption. Studies are underway to compare the effects cannabinoids (Δ9-tetrahydrocannabinol and the monoacylglycerol lipase inhibitors JZL184 and MJN110) with effects of two clinically-effective positive controls (ketoprofen and oxycodone) and two active negative controls that are not used clinically as analgesics but that produce general motor depression or stimulation (diazepam, amphetamine). Drug effects on each endpoint are first evaluated by 2-way ANOVA followed by a Holm-Sidak post-hoc test, with dose as a within-subjects factor and sex as a between-subjects factor. Data are then pooled for males and females and evaluated by 1-way ANOVA followed by a Dunnett's post-hoc test, with dose as a within-subjects factor. IP acid alone (0.1-0.32% in water, 10 ml/kg) produced a concentration-dependent increase in stretching and facial grimace and decrease in rearing and nesting. There were significant main effects of acid concentration on all endpoints, but no significant main effect of sex and no sex X concentration interaction on any endpoint. An acid concentration of 0.32% is being used for all drug studies. The cyclooxygenase inhibitor ketoprofen (0.1-32 mg/kg) dose-dependently alleviated all IP acid-stimulated and acid-depressed behaviors at doses that produced no disruption of nesting in the absence of acid. In contrast to ketoprofen, THC significantly decreased IP acid-stimulated stretching, but did not alleviate IP acid effects on facial grimace or rearing, and evaluation of THC effects on nesting are in progress. Studies with the other compounds are also in progress. Results will provide a profile of effects on pain-stimulated and pain-depressed behaviors produced by cannabinoids, positive controls, and negative controls. It is expected that determination of drug-effect profiles across a range of pain-stimulated and pain-depressed behaviors will improve preclinical-to-clinical translation of candidate analgesics and facilitate prioritization of candidate analgesics for advancement from preclinical to clinical studies.

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INTRAOCULAR PRESSURE LOWERING EFFICACY OF Δ^9-TETRAHYDROCANNABINOL VALINE HEMISUCCINATE LOADED NANOEMULSION IN A NORMOTENSIVE RABBIT MODEL

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We have recently shown the effect of Δ^9 – Tetrahydrocannabinol Valine Hemisuccinate (THC-VHS) on the intraocular pressure (IOP). Initial formulations studied effectively reduced the IOP of rabbits to a greater extent than the marketed formulation of Timolol and Pilocarpine. These formulations were, however, restricted by drug loading capacity and the duration of action. Thus, the current study was undertaken to optimize nanoemulsion (NE) and corresponding mucoadhesive (NEC) formulations that could increase the duration of activity by increasing retention or increasing penetration or both. THC-VHS NE and NEC formulations were prepared and optimized using homogenization followed by probe sonication method. Further, the effect of a single dose administration of Timolol or the optimized THC-VHS formulations on the IOP profile of normotensive New Zealand white rabbits were determined. Fifty microliters of the formulations were administered to the left eye and the contralateral eye remained untreated. IOP was measured at predetermined time intervals. All animal studies were undertaken using UM IACUC approved protocols.

The Timolol formulation exhibited a drop in IOP of about 15% from the baseline with a 150-minute duration of activity. The THC-VHS formulation with drug loads of 0.5%, 1.0%, and 2.0% demonstrated a higher drop in IOP (20-25%) and a dose dependent duration of activity. Based on the observations, the 1.0% drug load formulation was selected for further trials. A viscosity enhancer/mucoadhesive agent was added to the optimized formulation (NEC formulations) which extended the residence time. IOP in rabbits receiving the THC-VHS NEC formulations remained about 15% below baseline even after 8h following topical administration. A comparative study with THC in the NEC formulation showed a shorter duration of activity with the IOP returning to baseline within 5h. Thus, the THC-VHS NEC formulation was superior to the other formulations tested in terms of both duration of activity as well as intensity IOP drop.

DEVELOPMENT OF ENDOCANNABINOID TRANSPORT INHIBITORS FOR THE THERAPEUTIC TREATMENT OF PAIN

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Fatty acid-binding proteins (FABPs) act as critical modulators of endocannabinoid signaling by facilitating intracellular transport, and subsequent inactivation, of the endocannabinoid anandamide. The FABP isoforms expressed in the central and peripheral nervous systems have subsequently emerged as potential therapeutic targets for the treatment of pain. Previous work by our group has led to the identification of SBFI-26, a novel small molecule competitive FABP inhibitor that elevates brain anandamide levels and exhibits considerable efficacy as an antinociceptive and anti-inflammatory agent in diverse rodent models. In an effort to increase the efficacy of our lead drug, we used the truxillic acid core chemical structure of SBFI-26 as a scaffold to design and synthesize a multitude of new chemical derivatives. The principal goals were to develop compounds that display improved aqueous solubility, increased FABP5 target affinity, and increased selectivity against off-target brain-expressed FABPs (i.e. FABP3 and FABP7). Secondary goals were to generate compounds that showed potent *in vivo* efficacy, reduced cytotoxicity, and improved metabolic stability.

Functional groups to be incorporated onto the truxillic acid scaffold structure were selected based on computationally predicted binding free energies to target and off-target FABPs utilizing the recently resolved co-crystal structures of FABP5 and FABP7 in complex with SBFI-26 (PDB: 5UR9 and 5URA). High scoring compounds were subsequently synthesized and subjected to fluorescence displacement binding assays to assess their *in vitro* affinity towards purified human FABPs. Select compounds were further assessed for cytotoxicity, metabolic stability, and biological efficacy in the Hargreaves test of inflammatory hyperalgesia.

To date, over one hundred novel compounds have been generated and tested through this multi-collaborative drug discovery effort. Three compounds were chosen as new leads for advancement based on each of our primary selection criteria; SBFI-81 (potent target affinity, moderately good selectivity, but poor aqueous solubility), SBFI-102 (excellent target affinity and hydrophilicity, but poor FABP selectivity), and SBFI-103 (highly selective, but little improvement to solubility and target affinity). All three of these lead compounds resulted in significant analgesic effects when administered to mice (20 mg/kg, i.p.), though only SBFI-102 exhibited antinociceptive efficacy that exceeded that of SBFI-26. SBFI-102 was further demonstrated to exhibit high *in vitro* plasma stability and relatively low cytotoxicity in several normal and immortalized human cell lines. These studies have allowed us to gain considerable insight into chemical moieties that facilitate selective binding of small molecule inhibitors to FABP5. Future efforts will focus on using this knowledge to generate new SBFI-102 analogs that further improve upon target potency and selectivity.

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COMPARISON OF THE NEUROPROTECTIVE ACTIVITY OF CANNABIGEROL DERIVATIVES IN HUNTINGTON'S AND PARKINSON'S DISEASE MODELS

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Neuroprotective properties of plant-derived and synthetic cannabinoids have been extensively studied in different preclinical models of neurodegeneration. The neuroprotection observed in models of Parkinson's disease (PD) and Huntington's disease (HD) resulted from the pleiotropic activities exhibited by this class of compounds. We have previously shown that VCE-003.2, a cannabigerol (CBG) aminoquinone derivative, has anti-inflammatory and neuroprotective activities in different models of HD and PD (Aguareles et al., *Transl Neurodegener.* 2019: 8:9; Garcia et al., *J Neuroinflammation.* 2018: 15(1):19). Herein we present the synthesis of two novel CBG acid (CBGA) quinone derivatives, CBGA-quinone (CBGA-Q) and the water soluble CBG quinone sodium salt (CBG-Q-Na Salt) and their efficacy in murine PD and HD models in comparison with VCE-003.2.

Oral CBGA-Q (10 mg/Kg in sesame oil) alleviated clinical symptomatology and striatal neuronal loss (Nissl staining) in a HD model induced by 3-nitropropionic acid (3-NP). In addition, CBGA-Q inhibited the mRNA expression of the proinflammatory cytokines TNFα and IL-6 that were induced by 3-NP. Oral CBG-Q-Na Salt (40 mg/Kg in saline) also showed neuroprotective and anti-inflammatory activity albeit to a lesser extent than CBGA-Q and VCE-003.2. Parkinsonism was induced by intracerebral stereotaxic injection of 6-hydroxydopamine (6-OHDA) in the mouse brain and we found that oral CBGA-Q as well as VCE-003.2 significantly improved the behavioral deficits measured by pole and cylinder rearing tests. Both compounds significantly prevented the loss of nigrostriatal neurons (TH staining) and reduced 6-OHDA-induced microgliosis (CD68 staining) and astrogliosis (GFAP staining). As in the HD model, CBG-Q-Na Salt was also less effective than CBGA-Q and VCE-003.2 in the 6-OHDA PD model.

Overall, our results confirm that oxidation and chemical modification of CBG and CBGA is a valid strategy to design novel chemical entities that have the potential to outperform CBG on specific biological activities.

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BIOCHEMICAL AND ANALYTICAL CHARACTERIZATION OF PEPTIDE ENDOCANNABINOIDS

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The cannabinoid receptors (CB) can be modulated by endogenous peptides named peptide endocannabinoids (pepcans) [1], [2]. The whole family of pepcans consists of N-terminal extended versions of pepcan-12 (RVD-hemopressin), the most biological active peptide with a dual function (CB1 negative allosteric modulator and CB2 positive allosteric modulator) [1], [3]. Pepcan-23 is the longest physiological pepcan and shows no biological activity at CB receptors. Localization experiments with ELISA and LC-MS/MS showed pepcans specifically present in noradrenergic neurons in the locus coeruleus of the CNS as well as in chromaffin cells of the adrenal medulla [4]. This was the first indication of a connection between pepcans and the sympathetic nervous system. The adrenal glands could be a site of production or storage of pepcan-12 since analytical quantification experiments indicated high amounts of pepcan-12 in mouse adrenal glands but a lack of pepcan-23. To address this hypothesis, quantification studies with LC-MS/MS on adrenalectomized mice were performed, showing an involvement of the adrenal glands in constitutive pepcan production. Pepcans are derived from the α-hemoglobin gene [1], [2]. however, the coding sequence between the α-hemoglobin 1 and 2 is identical. Our recent qPCR data suggests that the HBA-A2 gene has a prominent role in generating pepcans within the adrenal glands.

Based on quantification experiments in mouse tissues as well as *ex vivo* and *in vivo* experiment, pepcan-23 is the potential precursor of pepcan-12. It has an increased stability in serum, plasma and whole blood compared to pepcan-12 and shows metal binding properties. Already the amino acid sequence of pepcan-23 indicates the possibility to chelate metal ions. To investigate this, mass spectrometry, CD-Spectroscopy as well as *in vitro* assays were applied. First results show clear differences in the metal binding properties between pepcan-23 and its n-terminal shorter versions. Already Pepcan-9 and -12 have been shown to interact with Cu^{2+} and Ni^{2+} ions [5]. The biological relevance has still to be investigated in detail. Emendato et al. suggested an α-helical structure of pepcan-12 for CB1 receptor binding [6]. Metal ions could induce this structural change or even stabilize the peptide in order to support receptor binding. Likewise, enzymatic recognition for the processing and/or degradation of pepcans could be influenced upon metal binding.

[1] M. Bauer *et al.*, "Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB 1 receptors," *J. Biol. Chem.*, vol. 287, no. 44, pp. 36944–36967, 2012.

[2] A. S. Heimann *et al.*, "Hemopressin is an inverse agonist of CB1 cannabinoid receptors.," *Proc Natl Acad Sci U S A*, vol. 104, no. 51, pp. 20588–20593, 2007.

[3] V. Petrucci *et al.*, "Pepcan-12 (RVD-hemopressin) is a CB2 receptor positive allosteric modulator constitutively secreted by adrenals and in liver upon tissue damage," *Sci. Rep.*, vol. 7, no. 1, pp. 1–14, 2017.

[4] S. C. Hofer, W. T. Ralvenius, M. S. Gachet, J. M. Fritschy, H. U. Zeilhofer, and J. Gertsch, "Localization and production of peptide endocannabinoids in the rodent CNS and adrenal medulla," *Neuropharmacology*, vol. 98, pp. 78–89, 2015.

[5] M. Remelli *et al.*, "DOES hemopressin bind metal ions in vivo?," *Dalt. Trans.*, vol. 45, no. 45, pp. 18267–18280, 2016.

[6] A. Emendato *et al.*, "Disordered Peptides Looking for Their Native Environment: Structural Basis of CB1 Endocannabinoid Receptor Binding to Pepcans," *Front. Mol. Biosci.*, vol. 5, pp. 1–9, 2018.

ANTINOCICEPTIVE EFFICACY OF VAPORIZED CANNABIS EXTRACTS IN A RAT MODEL OF INFLAMMATORY PAIN

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The phytocannabinoids Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are considered the primary cannabis constituents with analgesic potential. However, animal studies rarely reflect the cannabinoid products or routes of administration that are most commonly used by humans. Typically, ultra-purified cannabinoids are administered via injection in contrast to the whole-plant extracts that are commonly inhaled or eaten by human users. The purpose of the present study is to characterize the antinociceptive efficacy of vaporized cannabis extracts that are either THC- or CBD-predominant. Adult male and female Sprague-Dawley rats were handled and habituated to equipment for three days prior to baseline testing (day 1). On day 1, baseline tests were conducted to determine mechanical threshold, hindpaw weight-bearing, and locomotor activity. Dorsal-ventral hindpaw thickness was also recorded. Immediately after baseline testing, Complete Freund's Adjuvant (CFA) was injected in the plantar right hindpaw to induce inflammation and pain. One hour post-CFA, rats were placed in acrylic glass chambers and exposed to vaporized cannabis extracts for 60 min (5-sec puff every 2 min). High THC:low CBD (24.6%:1.2%) or high CBD:low THC (59.3%:1.9%) extracts at concentrations of 200 mg/mL were suspended in grapeseed oil and vaporized. Rats were exposed to vaporized THC-predominant extract, CBD-predominant extract, or grapeseed oil vehicle twice-daily (morning and evening) on days 1-3. On the morning of day 4, rats that had been exposed to vaporized vehicle on days 1-3 were again exposed to vaporized vehicle, or to THC- or CBD-predominant extract. Rats that had been exposed to vaporized cannabis extract on days 1-3 were exposed to the same extract again on the morning of day 4. Rats were then tested for mechanical allodynia, biased weight-bearing, and locomotor activity at 15, 45, 90, and 120 min post vapor exposure. Dorsal-ventral paw thickness was measured after behavioral testing at the 120-min time point only. Rats that were exposed to vehicle vapor only on days 1-4 showed mechanical allodynia, biased weight-bearing (towards the non-CFA paw), depressed locomotor activity, and hindpaw edema, compared to pre-CFA baselines. In male rats, acute administration of THC-predominant vapor reversed biased weight-bearing, and repeated administration of CBD-predominant vapor attenuated both biased weight-bearing and pain-suppressed locomotion. In contrast, repeated exposure to the THC-predominant extract attenuated mechanical allodynia and pain-suppressed locomotor activity in females. We are currently examining higher concentrations of both cannabis extracts. Information obtained from these studies will be useful not only in expanding our current knowledge of the efficacy of cannabinoids to treat inflammatory pain, but also to address limitations in the current literature: specifically, to increase face validity by examining routes of administration and products commonly used by medical cannabis users.

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CONDITIONED GAPING PRODUCED BY DELAYED, BUT NOT IMMEDIATE, EXPOSURE TO COCAINE IN RATS

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Wheeler (2008) reported that following several daily pairings of multiple exposures to a saccharin cue with the delayed (30 min) opportunity to self-administer cocaine, rats eventually display conditioned gaping reactions (Grill & Norgren, 1978) during the waiting period, suggesting a conditioned withdrawal effect. In contrast, Parker (1993) demonstrated that following several spaced (72 hr apart) conditioning trials with a 2-min exposure to a saccharin cue immediately followed by a subcutaneous (sc) injection of cocaine, rats did not display conditioned gaping reactions. Here we determined if both effects could be reproduced under similar conditioning protocols (daily conditioning trials) that differed by short single exposure and delayed multiple exposures to saccharin. In Experiment 1, rats were given daily conditioning trials with a 2-min exposure to saccharin which was immediately followed by 5, 10 or 20 mg/kg cocaine sc (1a), cocaine ip (1b) or 50 mg/kg LiCl (1c). In Experiment 2, rats were given daily multiple brief (10 sec) exposures to saccharin over a 30-min period prior to cocaine (20 mg/kg, both sc and ip) or LiCl (50 mg/kg, ip) injections. Experiment 3 evaluated the potential of a context which signals delayed access to cocaine to produce aversive response. Experiment 4 evaluated the potential of another rewarding drug, morphine (10 mg/kg sc) to produce aversive reactions following pairings of delayed access (10 min). Experiment 5 evaluated the potential of a brief 2-min exposure saccharin prior to a 30 min delayed sc injection of cocaine to produce conditioned gaping. Experiment 6 is currently evaluating the potential of ondansetron, a D1 antagonist and possibly the endocannabinoid like compound oleoyl glycine to interfere with the gaping produced by delayed access to cocaine. Conditioned gaping reactions and chin rubbing reactions were elicited by saccharin (but not a context) paired with delayed cocaine (sc stronger than ip), but not by immediate exposure to cocaine; however, neither immediate nor delayed cocaine produced the aversive reactions paw treading. Both cocaine and LiCl produced the ingestion related effects of suppressed tongue protrusions and enhanced passive drips consistent with previous reports (Parker 1993). When injected sc, but not ip, cocaine also elicited the potential withdrawal reactions of yawning. A brief exposure to saccharin prior to delayed access to cocaine did not produce conditioned gaping. The results are consistent with Wheeler et al that when paired with delayed access to cocaine, the flavor triggers a negative affective state that is revealed by gaping.

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OLEIC ACID SUPPLEMENTATION IN FEAR EXTINCTION

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Exposure to stress and psychological trauma have been increasing in parallel to changes in lifestyle. While some people are resilient, some are highly susceptible and are at great risk for developing stress and anxiety-related disorders. Previous studies identified oleic acid, a monounsaturated fatty acid to be potent endogenous inhibitor of fatty acid amide hydrolase (FAAH), the catabolic enzyme degrading anandamide which is a key lipid mediator required for facilitating fear memory inhibition (extinction). Although the effects of different dietary fatty acid compositions have been studied in depression, a possible preventive role of an oleic acid-rich diet and mechanistic insight into the biosynthetic machinery of anxiety disorders are missing.

Here we hypothesized that oleic acid utilization is disrupted during traumatic events and it has a role in extinction, therefore supplementation could be effective in prophylaxis, slowing progression and treatment of stress and anxiety disorders. We used behavioral, biochemical and genomic approaches to test if increased dietary intake of oleic acid can be used in adjunct to extinction training to promote fear inhibition, and second, if the increased oleic acid levels are responsible for the augmented endocannabinoid anandamide levels in basolateral amygdala. Investigating the previously unexplored role of monounsaturated fatty acid oleic acid in fear inhibition may reveal preventive or therapeutic effects that justify the prophylactic use of dietary oleic acid in people who are at risk for stress and trauma.

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OBESITY-INDUCED CHRONIC KIDNEY DISEASE IS AMELIORATED BY DUAL INHIBITION OF CANNABINOID-1 RECEPTOR AND iNOS

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Obesity has become a worldwide epidemic and considered a major risk factor for the development of chronic kidney disease (CKD). The deleterious effects on the kidney caused by obesity are attributed to two main molecular signaling pathways: the endocannabinoid/CB_1R system, which has been shown to mediate obesity-induced renal inflammation, fibrosis, and injury; and the inducible nitric oxide synthase (iNOS), which generates reactive oxygen species (ROS) resulting in oxidative stress. Hence, a combined peripheral inhibitory molecule that targets both CB_1R and iNOS may serve as an efficacious therapeutic agent against obesity-induced CKD.

In this study, we assessed the effect of a novel peripherally restricted, orally bioavailable dual CB_1R/iNOS antagonist, MRI-1867 (3 mg/kg, for 28 days), in ameliorating CKD in high-fat diet (HFD)-induced obese mice. Its renal effect was compared to the peripherally restricted CB_1R antagonist, JD5037 (3 mg/kg) and the iNOS inhibitor, 1400W (10 mg/kg). HFD-vehicle, HFD-pair-fed, and standard diet-fed groups were served as controls.

Dual inhibition of CB_1R and iNOS ameliorated obesity-induced morphological and functional changes in the kidney via decreasing inflammation, fibrosis, oxidative stress, and renal injury. Whereas no such effects were demonstrated by the iNOS inhibitor 1400W, peripheral blockade of CB_1R alone was able to significantly attenuate obesity-induced CKD. On the other hand, comparing the effect of these drugs *in vitro* in human renal proximal tubule cells exposed to fatty acid flux, which acutely induces oxidative stress and ROS production, revealed differential effect in which only MRI-1867, but not JD5037, was able to attenuate the oxidative stress, indicating the iNOS inhibitory effect of this molecule. Molecularly, these beneficial effects on the kidney and proximal tubule cells were partially associated with modulating renal adiponectin signaling.

Collectively, our results highlight the therapeutic relevance of blocking CB_1R and iNOS in peripheral organs, and may further support the preclinical development and clinical use of MRI-1867 in ameliorating obesity-induced CKD.

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THIENOPYRIMIDINE DERIVATIVES AS GPR55 LIGANDS

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GPR55 is a G-protein coupled receptor that is attracting much attention as a putative cannabinoid receptor. This receptor is involved in pathophysiological conditions such as cancer, inflammatory and neuropathic pain, metabolic disorders, vascular functions, bone physiology, and motor coordination. Thus, GPR55 ligands might be interesting agents for the treatment of various diseases. Even though lysophosphatidylinositol (LPI) has been suggested as a GPR55 endogenous ligand, this receptor remains an orphan receptor. GPR55 has been proposed as a cannabinoid-like receptor since endogenous, plant-derived, and synthetic cannabinoid ligands have been described to act on this receptor. Nonetheless, pharmacological inconsistencies, and the lack of potent and selective GPR55 ligands are delaying the exploitation of such a promising therapeutic target.

The studies presented here are based on the thienopyrimidine ML192 (CID1434953) that was previously discovered as a GPR55 antagonist by high-throughput screening.[1] Initially, a GPR55 homology models allowed us to identify structural features of GPR55 ligands.[2,3] Then, further docking studies in the GPR55 inactive state model allowed us to design optimized ML192 derivatives. These compounds were synthesized and evaluated using a β-arrestin recruitment assay in CHO cells overexpressing human GPR55 and βarr2-GFP. Interestingly, several compounds revealed increased potency and efficacy as GPR55 antagonists when compared with the hit ML192.

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1. Heynen-Genel, S. *et al.* Probe Reports from the NIH Molecular Libraries Program; 2010; http://www.ncbi.nlm.nih. gov/books/NBK66153/.
2. Kotsikorou, E. *et al. Biochemistry* **52,** 9456–9469 (2013).
3. Lingerfelt, M. A. *et al Biochemistry* **56,** 473–486 (2017).

INVESTIGATING THE STATUS OF THE ENDOCANNABINOID/CB_1R SYSTEM IN CHRONIC ALCOHOL-INDUCED LUNG INFLAMMATION

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Chronic alcoholism predisposes lungs to the 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.

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, including alveolar macrophages (AM). This upregulation contributed to the pathology of lung fibrosis. Furthermore, activation of CB_1R in alveolar macrophages induced pro-inflammatory and profibrogenic phenotypes (*Cinar et al., JCI Insight 2017 2(8):92281*). However, the status of the endocannabinoid/CB_1R system has not yet been explored in the lung upon chronic alcohol consumption.

In order to address this question, 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 in mice. This diet consisted of chronic feeding of 5% ethanol in Lieber-DeCarli liquid diet for 14 days followed by a single acute alcohol binge at the end of the 14-day period.

We found that chronic alcohol drinking increased gene expression levels of inflammatory cytokines and chemokines, such as tumor necrosis factor (*TNFα*), interleukins 1β (IL1β), *Cxcl2,* and *Ccl2*. On the other hand, there was significantly decreased gene expression of certain inflammatory and anti-inflammatory cytokines and chemokines such as *Il17a, Il10 Il6, Cxcl9,* and *Cxcl10.* Furthermore, alcohol significantly increased gene expression of inducible nitric oxide synthase (iNOS) and arginase 1 (Arg1), suggesting activated and altered AM function. Importantly, chronic alcohol increased AEA but not 2AG levels in the lung. CB_1R gene and protein expressions were increased in alveolar macrophages in the lung as observed by RNAscope and immunohistochemisty assays.

In conclusion, chronic alcohol consumption with the NIAAA model induces lung injury in addition to liver injury. It also dysregulates lung immunity, likely altering AM function. Endocannabinoid/CB_1R is also overactivated in the lung upon chronic alcohol drinking. It is an intriguing possibility that CB_1R overactivity might contribute alcohol-induced lung inflammation, and further studies are warranted with testing the role of CB_1R in AM in this experimental context.

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RETRO ESTER AND AMIDE ENDOCANNABINOID ANALOGUES WITH RESISTANCE TO ENZYMATIC HYDROLYSIS

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CB1 and CB2 are two $G_{i/o}$-protein-coupled cannabinoid receptors (CBRs) that are currently being pursued as potential targets for an array of conditions including pain, inflammation, CNS disorders, and cancer. 2-Arachidonoylglycerol (2-AG) and *N*-arachidonoylethanol amine (AEA) are well-characterized endogenous ligands for CB1 and CB2. However, due to their low chemical and biochemical stability, it is relatively difficult to use these two endogenous ligands directly, to probe their biological role(s) and to explore the functions of cannabinoid receptors and enzymes. In most tissues, 2-AG and AEA are metabolized by monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) respectively. 2-AG is more labile than AEA and can also be deactivated by esterases such as the brain hydrolase ABHD6. Moreover, recent studies have demonstrated that oxidative enzymes including cyclooxygenase-2 (COX-2), cytochrome P450, and lipoxygenases (LOXs) can transform endocannabinoids into eicosanoid-related bioactive products. Here, we are reporting novel analogs with enhanced bio-activities at CB receptors and increased stabilities to the actions of hydrolytic and/or oxidative enzymes. Toward this end, currently, we are exploring the head group of 2-AG and AEA with special emphasis on the ester and amide moieties and the methylene linker. Our design focuses on the reverse ester/amide design approach and the incorporation of steric features at the methylene linker of the endogenous prototype.

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GPR55 DELETION CAUSES DECREASES IN CNS PROSTAGLANDINS AND INCREASES IN 2-AG AND RELATED LIPIDS

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G-protein-coupled receptor 55 (GPR55) has been identified as part of the endocannabinoid signaling system because of its binding affinity for both endogenous and synthetic cannabinoids. It is found in tissues ranging from the central nervous system to the periphery and has demonstrated therapeutic potential in that GPR55 antagonists decrease prostaglandin levels *in vitro*. However, the impact of GPR55 on lipid signaling more generally has yet to be explored. To determine how GPR55 may influence the regulation of lipid signaling molecules associated with the endogenous cannabinoid system in the central nervous system, both globally and in a regio-specific manner, 3 different brain regions were analyzed for levels of Anandamide and its structural analogs (fatty acids conjugated to amines referred to here as lipoamines), 2-arachidonyl glycerol and its structural analogs, free fatty acids including arachidonic acid, and prostaglandins (PGs) in WT and GPR55 knock out mice. Lipids were partially purified from methanolic extracts using C-18 solid extraction columns, and eluants screened through a large lipid library (~85 individual species) using HPLC/MS/MS. Analysis revealed changes in both endocannabinoids, lipoamines, and PGs. 2-arachidonoyl glycerol levels ($p=.040$) and N-stearoyl leucine ($p=.024$) were significantly increased in the hypothalamus of GPR55 KO mice, in the midbrain levels of the lipoamine, arachidonoyl tyrosine ($p=.019$) significantly increased; however, levels of PGE_2 significantly decreased ($p=.014$). Likewise, levels of PGE_2 in the striatum of the GPR55KO ($p=.058$) were significantly lower. Importantly, PG levels decreased in 2 of the 3 brain areas examined, which supports previous data that GPR55 antagonists decrease levels of PG *in vitro*. These data extend our understanding of the interconnected endocannabinoid lipidome and provide further evidence for the relationship between cannabinoid signaling and PG signaling.

CHRONIC (*E*)-B-CARYOPHYLLENE ADMINISTRATION IN RAT MODEL OF OSTEOARTHRITIS RESULTS IN CB_2 AND OPIOID RECEPTOR DEPENDENT ANALGESIC EFFECT

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Osteoarthritis (OA) is chronic musculoskeletal disease with complex etiology. Current pharmacological therapies are only symptomatic, yet they are insufficient and may cause unwanted side effects. *CB_1 and CB_2 receptors activation results in analgesic effect, which is limited by CB_1-mediated psychotropic side-effects.* (E)-β-caryophyllene (BCP), sesquiterpene majorly found in essential oils derived from *Cannabis sativa,* is a natural agonist of CB_2 with well-established anti-inflammatory properties. BCP may also engage endogenous opioid system. In the present study we aim to assess anti-nociceptive potential of BCP and its mechanism of action in the animal model of OA.

Intrarticular (i.a.) injection of monosodium iodoacetate (MIA, 1mg) has been used to induce OA in Wistar rats. Two treatment paradigms were studied: 1) therapy following early symptomatic detection of OA (every second day from D10 to D28 post MIA injection) and 2) treatment paradigm reflecting patient –doctor experience in advanced stage of OA (BCP treatment every second day from D20 to D28 post MIA injection). BCP was administered i.p. at dose of 25mg/kg in both treatment paradigms, however we also studied subthreshold dose of 10mg/kg in the early-treatment paradigm. To classify if analgesic effect is mediated by cannabinoid or opioid system we combined BCP (25mg/kg) with either selective CB_2 receptor antagonist AM630 (3mg/kg) or non-selective competitive opioid receptor antagonist naloxone (1mg/kg), both in late-treatment paradigm. Also, we tested AM630 (3mg/kg) and naloxone (1mg/kg) alone in a similar paradigm. All drugs were dissolved in vehicle composed of 5% DMSO, 5% Kolliphor® EL, 5% ethanol and 85% saline. Pain symptoms were assessed by behavioral test: kinetic weight bearing (KWB test) in D21 and D28 after OA induction.

We observed significant asymmetry in peak force parameter between left and right paw in in vehicle-treated OA rats at D21 and D28. This difference was not detected in any of BCP treated groups (both at D21 and D28), suggesting analgesic effect, which is mediated through CB_2 receptors since it was abolished by AM630 co-treatment. Administration of AM630 alone did not restore weight bearing in D21 and D28 however naloxone reversed peak force asymmetry. This "paradoxical" analgesic effect (reported before in the literature, e.g. D. Greeley et al. 1988) may occur due to relatively low naloxone dose administration. BCP and naloxone co-treatment leads to inhibition of BCP analgesic effect, suggesting engagement of opioid system in the analgesic action of BCP.

In summary, BCP exerts anti-nociceptive effect that involves endogenous cannabinoid and opioid systems. Considering safety profile of BCP, these results may lead to development of novel OA treatment.

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SLEEP DISTURBANCES IN MICE DURING CHRONIC THC ADMINISTRATION AND ABSTINENCE

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The diagnosis of cannabis withdrawal is contentious because reliable, objective measures of withdrawal from delta-9-tetrahydrocannabinol (THC), the major psychoactive ingredient in cannabis derivatives, have been difficult to observe. Typically, in laboratory animal studies withdrawal symptoms are elicited by treatment with a cannabinoid type 1 receptor antagonist, which does not mimic the normal course of withdrawal in human cannabis users. A known consequence of chronic cannabis usage in humans is altered sleep, particularly disputed non-rapid eye movement (NREM) sleep. We sought to determine if cannabis withdrawal-induced changes in sleep can be modeled in rodents. We tested this using electrocorticogram and electromyogram recordings from chronically implanted mice combined with our fully automated sleep analysis system to score sleep before, during and after chronic injection of either THC or vehicle control. Mice were maintained on a 12:12 light:dark cycle throughout the experiment. Baseline data (48 hr) were obtained prior to systemic administration of vehicle to all mice just before the onset of the dark photoperiod. The first THC injection augmented total time spent in non-rapid eye movement (NREM) sleep, but this effect was significantly attenuated following the last injection in the chronic treatment regimen. Measurements obtained over six days following cessation of THC treatment revealed that time spent in NREM sleep was reduced largely because of a decrease in NREM bout duration. Additionally, rapid eye movement (REM) sleep was reduced on the first day of acute THC administration and was enhanced 6 days following treatment. The augmentation in REM during the abstinence phase of the experiment could be due to an increase in the number of REM bouts, and this effect persisted throughout the 6 days of abstinence. None of these changes were observed in controls. Paradoxically, the power of delta oscillations (0-4 Hz) was no different between THC and controls during the first day of abstinence, but THC mice displayed markedly less delta power by the last day of abstinence. This suggests that impairment of processes contributing to slow oscillations in the cortex gradually begins to manifest over recovery from chronic THC exposure. To assess the effects of extended abstinence from chronic THC treatment, 48 hr recordings were obtained from these mice after 77 days of abstinence. We observed an augmentation of NREM sleep time following extended abstinence from chronic THC, and a reduction in delta power during NREM. These findings indicate a chronic THC regimen can produce overt withdrawal symptoms, and in addition, extended abstinence from chronic THC may reveal allostatic processes that emerge during the recovery from acute withdrawal. Currently we are performing studies to test the extent to which these observed sleep effects contribute to behavioral abnormalities.

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PERIPHERAL CANNABINOID 1 RECEPTOR BLOCKADE IMPROVES INSULIN SENSITIVITY BY SUPPRESSING ADIPOSE TISSUE INFLAMMATION VIA NLRP3 INFLAMMASOME IN MOUSE MODELS OF OBESITY

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The overactivity of cannabinoid 1 receptor (CB1R) is associated with obesity and type 2 diabetes. First-generation CB1R antagonists, such as rimonabant, offered therapeutic advantages for the control of obesity and related metabolic abnormalities, but their therapeutic potential was limited by undesirable neuropsychiatric side effects. Here we report that the peripherally restricted CB1R antagonist AJ5018 suppresses adipose tissue inflammation without eliciting the centrally mediated neurobehavioral effects. AJ5018 had a higher degree of selectivity for CB1R over CB2R and markedly reduced brain penetrance, as reflected by the lower brain/plasma concentration ratio and the attenuated centrally mediated neurobehavioral effects, compared with its brain-penetrant parent compound rimonabant. In mouse models with genetic or diet-induced obesity, AJ5018 exhibited comparable effects with rimonabant in improving metabolic abnormalities and suppressing macrophage infiltration into white adipose tissue, activation of the NLRP3 inflammasome, and production of proinflammatory cytokines. Our findings identified AJ5018 as a novel peripheral CB1R antagonist and suggest that peripheral CB1R blockade might break the links between insulin resistance and adipose tissue inflammation.

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NOVEL AND HIGHLY SELECTIVE CB2R FLUORESCENT PROBES FOR TRACING CB2R-POSITIVE CELL POPULATIONS

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The cannabinoid receptor 2 (CB2R) has been shown to be a promising target in several diseases including chronic and inflammatory pain, diabetic neuro- and nephropathy as well as liver cirrhosis and ischemic reperfusion injury. Controversies exist about the protein expression levels in *CNR2*-mRNA positive cells due to the lack of CB2R specific antibodies in human or animal models. To better identify CB2R-positive cells and understand the role of CB2R signalling in target cells of disease models, we developed novel chemical tools for CB2R detection by flow cytometry. Therefore, highly potent and selective CB2R agonists have been linked to different fluorophores by chemical synthesis. Validation of these fluorescent probes by FACS analysis of CHO cells overexpressing mouse and human CB2R or CB1R has identified probes which are highly specific for human as well as mouse CB2R. Moreover, flow cytometry-based displacement studies with cold CB2R agonists and inverse agonists could confirm the specific binding of the CB2R fluorescent probes to its receptor.

The identification of these novel and versatile CB2R-specific fluorescent probes will allow us to better understand CB2R-mediated signalling in disease and fine tune CB2R therapeutic targeting.

MONOACYLGLYCEROL LIPASE INHIBITOR MJN110 REDUCES HIV-1 TAT-INDUCED EXCITOTOXICITY *IN VITRO*

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The advent of combined antiretroviral therapy (cART) has greatly increased expected longevity in persons living with HIV-1 (PLWH), but therapeutics targeting residual viral proteins are largely unestablished. Transactivator of transcription (Tat) is among the first viral proteins to enter the central nervous system upon initial infection, and is poorly suppressed by cART. Previous work has demonstrated both the role of Tat in development of neurocognitive deficits which contribute to phenotypes of HIV-associated neurocognitive disorder, and the protective role of the endocannabinoid system in ameliorating neural dysfunction. Thus, the present study aimed to investigate the potential utility of blocking enzymatic breakdown of endocannabinoid ligand 2-arachidonoylglycerol (2-AG) with monoacylglycerol lipase inhibitor MJN110 in Tat-treated prefrontal cortex neuron cultures. Tat exposure significantly increased neuron excitability induced by 100 nM - 50 μM glutamate application in live-cell microscopic recordings of intracellular calcium ($[Ca^{2+}]_i$) activity, an effect which was significantly reduced in cultures treated with 500 nM and 1 μM MJN110. The observed effect was time- and concentration-dependent, such that longer MJN110 exposure at higher concentrations more effectively protected against excitotoxicity. Further investigations will examine behavioral outcomes of MJN110 treatment against Tat-induced deficits *in vivo* to ultimately identify therapeutic targets for mitigation of HAND symptoms in PLWH.

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THC AND FENTANYL INTERACTION IN RATS: EVALUATION OF RESPIRATORY EFFECTS AND ADVERSE HEALTH OBSERVATIONS

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Polysubstance abuse, whether engaged in voluntarily or consequent to adulteration of a primary substance of abuse, is an under-researched area. In the context of abuse of prescription or synthetic opioids, dual use of cannabis is of special concern. First, one of the proposed strategies for combating the opioid crisis is to decrease opioid use or dosage through co-administration (or substitution) of cannabis. Second, the number of states that have legalized recreational use of cannabis is growing, with (as yet) uncertain effects on rates of use. Wider use of cannabis in the population of substance abusers is likely to result in greater co-use with other substances, including opioids. Third, a body of previous research suggests that opioid-cannabinoid co-use/abuse may be affected by underlying interactions between the endocannabinoid and endogenous opioid neuromodulator systems. Given these considerations, the primary goal of this study was to characterize the effects of dual acute and chronic administration of Δ^9-tetrahydrocannabinol (THC) and fentanyl on physiological and behavioral measures in a rat model.

Adult male Sprague-Dawley rats were dosed acutely or chronically with THC or fentanyl alone or in combination and subsequently tested for analgesia using tail flick, respiratory effects using whole body plethysmography, and observational scoring for somatic signs of precipitated withdrawal. Acutely, both drugs alone dose-dependently increased analgesia, and decreased minute volume in the plethysmography assay. In acute combinations, administration of THC dose-dependently enhanced fentanyl-induced analgesia and respiratory suppression at doses that had negligible effects alone. Upon chronic fentanyl administration, rats exhibited tolerance to both analgesic and respiratory effects of acute fentanyl and robust naloxone-precipitated withdrawal. Repeated administration of THC alone further sensitized rats to fentanyl-induced respiratory suppression. In addition, the rats that received repeated treatment with THC showed signs of toxicity (e.g., shaking, excessive salivation, seizures) upon receiving a second 0.3 mg/kg dose of fentanyl six days after receiving this dose initially. These toxic effects were not observed after the initial dual injection and overt signs of toxicity were not seen during chronic THC alone administration. However, when both drugs were administered together repeatedly, the health of the rats rapidly deteriorated over the course of the 6-day dosing period. In contrast, rats that received fentanyl alone did not show this toxicity. Hence, repeated administration of THC appeared to be required for occurrence of the toxic effects. To the extent that these results are translatable to humans, this potentiation of fentanyl's effects in rats that received repeated THC has sobering implications, as it suggests that individuals who regularly consume THC may be more sensitive to fentanyl's toxic effects. Further, these results highlight the need for more research in this area, as the population of humans using both opioids and cannabis continues to increase.

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INTRAVITAL IMAGING OF INFLAMMATION AND MICROCIRCULATION FOR CANNABINOID RESEARCH

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Modern microscopic methods facilitate intravital imaging of the dynamic immune responses within the microcirculation of several organs and tissues in experimental animal models of inflammation. The endocannabinoid system (ECS) plays an important role in the modulation of the immune response in inflammation. We are presenting our most recent experimental data obtained by intravital microscopy of the brain, gut, pancreas and other organs in murine inflammatory disease models of stroke, sepsis and diabetes.

Brain: Using intravital imaging we are capable to visualize leukocyte adhesion and capillary perfusion within the pial microcirculation during CNS inflammation induced by experimental stroke. Administration of CB2R agonist, HU308, significantly reduced inflammatory parameters. Gut: We are using the intestinal microcirculation as general readout for peripheral immune response under conditions such as stroke or sepsis and are able to study the immune-modulatory impact of various ECS-related pharmacological strategies.

Pancreas: We established a model for intravital imaging of early inflammatory changes in the pancreas during development of Type 1 Diabetes (T1D). We observed significant less inflammation within the microcirculation of the pancreas when cannabidiol was administered early in the course of the disease.

Intravital imaging can provide important scientific information to prove novel concepts in cannabinoid research but requires sophisticated technologies in laboratory animals.

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ENDOCANNABINOID REGULATION OF CUE-INDUCED INCENTIVE MOTIVATION IN THE NUCLEUS ACCUMBENS AND VENTRAL TEGMENTAL AREA OF MALE RATS

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The endocannabinoids (eCBs) 2-arachidonoyl glycerol (2-AG) and anandamide (AEA) are metabolized by the enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively. Our previous work demonstrated that enhancement of 2-AG levels by a systemically administered MAGL inhibitor, MJN110, leads to a robust increase in operant responding to reward predictive incentive cues (ICs) in male rats, while rimonabant dose dependently decreases responding. In this current study we sought to determine the brain regions that mediate these effects by microinfusing the drugs directly into the ventral tegmental area (VTA) or nucleus accumbens (NAc) prior to testing. Initially, free fed rats are trained to nosepoke during discrete 8-sec audiovisual incentive cues (ICs) to obtain 64µl of 10% sucrose. Once stable (with average responding to 85-95% of the ICs presented) the rats are switched to a decreasing reward IC task in which the sucrose volume delivered is progressively decreased every 15 mins (from 64µl, 48µl, 32µl, to 16µl). During this task variant the overall response ratio decreases, allowing us to examine changes in responding in both directions. The choice and vigor to respond to the ICs and collect the reward decrease proportionately with the volume of sucrose delivered, thus rats respond more frequently and quickly when reinforced with 64µl and much less so to 16µl.

Intra-VTA MJN110 blocks the decrease in IC responding, leading to an overall enhancement in the choice (response ratio) and vigor of responding (decreased latency to nosepoke, and enter the reward cup) at ICs reinforced by lower volumes. However, while intra-VTA infusions of rimonabant and the FAAH inhibitor PF3845 did not alter operant responding, intra-NAc infusions of both affected responding to ICs. Preliminary data further suggests that intra-VTA infusions of the CB2 antagonist SR-144528 enhances the vigor of the response (e.g. decreases the latency to nosepoke) but does not affect the choice to respond to the IC. Our results suggest normal endocannabinoid tone in the NAc, but not the VTA, can be suppressed to attenuate cue-induced reward seeking behaviors. Increasing either 2-AG or AEA in the NAc, enhances cue-induced reward-seeking as does artificially increasing endocannabinoid signaling in the VTA through elevation of 2-AG levels. In addition, our results indicate a potential specific role of CB2 receptors in mediating the vigor of responding to the cue. Together our data demonstrates heterogeneous regulation of reward seeking by eCBs in mesoaccumbal circuits.

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BDNF-INDUCED ENDOCANNABINOID RELEASE REGULATES SYNAPTIC PLASTICITY

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Endocannabinoids and neurotrophins, particularly brain-derived neurotrophic factor (BDNF), are potent synaptic modulators that are highly expressed throughout the forebrain and play critical roles in many behavioral and physiological processes. Disruption of either BDNF or endocannabinoid signaling is associated with an overlapping set of neuropsychiatric diseases, including autism, epilepsy, multiple sclerosis, and neuropathic pain. Both BDNF and endocannabinoids are currently major targets for the development of novel therapeutics, particularly in relation to post-traumatic stress disorder, depression, and anxiety. Recent evidence from our laboratory and others suggests physiologically-relevant interactions between BDNF and cannabinoid signaling. In particular, we have shown that BDNF induces the postsynaptic release of the endocannabinoid 2-AG at inhibitory synapses in both cortical layer 2/3 and the hippocampal CA1 region, which acts retrogradely to reduce presynaptic GABA release. Furthermore, we found that BDNF also induces endocannabinoid release at excitatory synapses, where CB1 activation suppresses presynaptic glutamate release, opposing the direct enhancing effects of BDNF.

The goal of the present studies is to explore the functional impact of these synergistic and antagonistic interactions between BDNF and endocannabinoids in regulating synaptic plasticity. We find that theta-burst stimulation induces a form of long-term depression at inhibitory synapses (i-LTD) that requires endogenous BDNF and subsequent eCB mobilization. This form of i-LTD appears to be independent of metabotropic glutamate receptor (mGluR) activation. In addition to the effects of 2-AG, exogenous anandamide suppresses inhibitory transmission in layer 2/3 pyramidal cells via activation of CB1, but not TRPV1, receptors. We are exploring a potential contributing role for endogenous anandamide, which may have synergistic, overlapping, or distinct effects from 2-AG in regulating activity-dependent plasticity. We are also examining BDNF-eCB interactions at excitatory synapses. We find that pharmacologically-induced LTP in layer 5 pyramidal neurons produces a sustained increase in the frequency of spontaneous excitatory transmission and a parallel increase in the amplitude of evoked synaptic responses. This form of LTP requires endogenous BDNF/TrkB signaling. We are examining the potential mitigating role of BDNF-induced endocannabinoid release at these excitatory synapses, as well as endocannabinoid effects at GABAergic synapses that could enhance excitatory synaptic plasticity.

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ENHANCEMENT OF ENDOCANNABINOID TONE AS A NOVEL TARGET FOR TREATMENT OF MIGRAINE

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Migraine is one of the most common, yet under studied, neurological disorders, characterized by unilateral pulsating headaches accompanied by sensory disturbance. It affects more than 38 million people in the U.S., resulting a loss of between $5.6 and $17.2 billion in annual productivity. Despite the large prevalence and severe symptoms, understanding of migraine pathology is lacking, limiting discovery of novel therapeutics. It is clearly demonstrated that the endocannabinoid system (ECS) is centrally and peripherally engaged during pain signaling. Recent clinical findings raised the idea that Clinical Endocannabinoid Deficiency (CED) syndrome underlies the pathophysiology of migraine. Although the exact ECS-dependent mechanisms in migraine pain is not fully understood, the available results strongly suggest that activation of ECS, like pharmacological manipulation of endocannabinoid tone by blocking the degradative enzymes could represent a promising therapeutic tool for reducing migraine pain. The biochemical studies providing strong evidence for the potential efficacy of eCBs in migraine are limited. Moreover, to date, no study has investigated the efficacy of enhanced endocannabinoid tone by blocking of MAGL in migraine-like pain. Thus, we hypothesize that dysregulated endocannabinoid signaling contributes the pathophysiology of migraine, and that enhancing endocannabinoid tone, by targeting eCBs degradation, attenuates headache-like pain induced by cortical spreading depression (CSD). Our study focused on the validation of monoacylglycerol lipase (MAGL) as a target by using the selective inhibitor, MJN110 in the treatment of migraine along with the elucidation of the role of endocannabinoids in migraine pathophysiology. Using a cortical KCl-injection (0.5uL, 1mM) to induce cortical spreading depression and periorbital allodynia, phenomena associated with clinical migraine, we investigated the dynamic changes of each components of endocannabinoid system in nuclei of the craniofacial pain axis (cortex (Ct), trigeminal ganglia (TG), and periaqueductal grey (PAG) during migraine phases. Our data showed significantly elevated expression of MAGL in cortex and PAG at 90 min after KCl injection (1M, 0.5µL, $p<0.05$ vs. naïve), but no significant differences was detected in TG samples, suggesting overactivity of MAGL in pain centers engaged during CSD but not trigeminal primary afferents. We observed that the total expression of CB1 receptor and the phosphorylated CB1 receptor were significantly decreased 90 min (cortex) and 3 h (PAG), respectively after KCl manipulation; expression of CB2 receptor was not changed. Together these suggest the ratio of eCB signaling at CB1 versus CB2 receptors in the CNS may be shifted during KCl-induced CSD. In order to study the efficacy of MJN110 against migraine pain, we performed single-dose, time-course experiment with intraperitoneal injection of MJN110 (10 mg/kg, i.p.), which reversed KCl-induced mechanical allodynia with maximal effects occurring 2-3 h post-injection in female rats (n=5). This result suggests the positive, antiallodynic effect of MAGL inhibition, in support of our hypothesis. Moreover, the inhibition of MAGL induced alteration of AEA-2AG ratio in the cortex, enhancing 2AG levels, as we detected by LC-MS. Overall, our data strongly suggest the dysfunction of endocannabinoid system in KCl-induced CSD/migraine and indicate the anti-migraine potential of MAGL inhibition, which can serve as the foundation for initiation of novel migraine pain drug discovery program.

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A DUAL-TARGET PERIPHERALLY RESTRICTED CB_1R ANTAGONIST PROMOTES PERIRENAL FAT BROWNING IN OBESE MICE VIA AMPK SIGNALING

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Obesity occurs when excess energy accumulates in white adipose tissues (WAT) which can be converted to thermogenic brown-like cells (beige cells, browning) to dissipate energy and potently counteract obesity. Visceral obesity is associated with increased activity of the endocannabinoid/CB_1R system. CB_1R antagonists reduce body weight and improve the metabolic complications of obesity. Moreover, the enzyme adenosine monophosphate kinase (AMPK) is a crucial energy sensor that regulates energy metabolism and its activity is reduced in obesity. We have developed a novel bivalent compound (*S*)-MRI-1891 that selectively and potently inhibits peripheral CB_1R and additionally acts as a direct activator of AMPK.

(*S*)-MRI-1891 increased total energy expenditure in high-fat diet-induced obese (DIO) mice by increasing fat oxidation. This effect is in part due to the direct activation of AMPK as judged by its blunting by the AMPK inhibitor Compound C and by its continued presence in $CB_1R^{-/-}$ mice. Treatment of DIO mice with (*S*)-MRI-1891caused a selective increase in AMPK phosphorylation and UCP-1 expression (marker of beige fat) in perirenal, but not in epididymal, subcutaneous or brown adipose tissue. Correspondingly, UCP-1 expression was higher and perirenal fat mass was lower in high-fat-fed $CB_1R^{-/-}$ mice compared to wild-type DIO mice, which may contribute to the resistance of $CB_1R^{-/-}$ mice to DIO. Moreover, surgical removal of perirenal fat pads resulted in reduced basal rate of fat oxidation compared to sham-operated mice.

In conclusion, we have demonstrated that peripheral CB_1R inhibition or genetic deletion of CB_1R specifically promotes perirenal fat trans-differentiation into beige fat thus antagonizes obesity and we have developed a novel anti-obesity compound that targets CB_1R pathway.

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MICROGLIA AND DOPAMINE NEURON SPECIFIC CNR2 GENE KNOCKOUT MOUSE BRAINS SHOW CB2R BIASED INFLAMMATION SIGNALING PATHWAYS

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Neuronal and microglia cannabinoid receptor 2 (CB2R) expression is drastically elevated during brain injury. We used Cre-LoxP technology to generate Cx3cr1-*Cnr2* and DAT-*Cnr2* conditional knockout (cKO) mice with deletion of CB2Rs in microglia and dopamine (DA) neurons, respectively. RNAscope *in situ* hybridization using CB2R probe with triplex ISH of Cd11b and vGluT2 probes for CX3cr1-*Cnr2* cKO mice, and with duplex ISH of tyrosine hydroxylase (TH) probe for DAT-*Cnr2* cKO mice validated the deletion of CB2Rs in microglia and DA neurons, respectively.

Behavioral tests showed that DAT-*Cnr2* cKO mice exhibited a hyperactive phenotype compared with the Cx3Cr1-*Cnr2* cKO and WT mice. In the plus-maze test DAT-*Cnr2* cKO mice were less aversive to the open arms than the Cx3cr1-*Cnr2* cKO mice whereas in the tail-suspension test, depression-like behavior was provoked in both genotypes with Cx3cr1-*Cnr2* cKO mice more sensitive than that of DAT-*Cnr2* cKO mice. The mixed cannabinoid agonist WIN55212-2 reduced locomotor activities in all the genotypes. We found that neuroinflammation pathways of PI3K/AKT/mTOR, MAP/ERK, and NF-κB were differentially affected by cell-type specific deletion of CB2R in cerebral cortexes of Cx3Cr1-*Cn2*, DAT-*Cnr2* cKO, and WT mice. Our experiments indicated that CB2R neuro-immune cross-talk might modulate neuroinflammation and serve as a potential therapeutic target for neurodegeneration diseases.

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Withdrawn

INVESTIGATION OF THE EFFECTS OF THE PERIPHERALLY RESTRICTED FAAH INHIBITOR URB937 IN A RAT MODEL OF POST-OPERATIVE PAIN FOLLOWING INGUINAL HERNIA REPAIR SURGERY

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Hernia repair is a common surgical procedure associated with moderate to severe acute post-operative pain (POP) in approximately 40% of patients (McGrath et al., 2004, Canadian J. Anesthesia, 51(9) 886-891). Endocannabinoids have antinociceptive effects at peripheral, spinal and supraspinal levels (Starowicz K, Finn DP, 2017 Adv Pharmacol,80:437-475).

The aim of the present study was to investigate the effects of URB937, a peripherally restricted inhibitor of fatty acid amide hydrolase (FAAH), the enzyme that catabolises the endocannabinoid anandamide and related *N*-acylethanoamines, on POP-related behaviour in a rat model of inguinal hernia repair surgery.

Thirty-six adult male Lister-Hooded rats were used (n=9 per group) and home-cage locomotor activity, open-field activity and hindpaw and inguinal area mechanical hypersensitivity (von Frey testing) were assessed 24hrs pre-surgery and up to 4hrs post-surgery. Rats underwent either a sham surgery procedure or the inguinal hernia repair procedure under isoflurane anaesthesia and received a subcutaneous injection of either vehicle or URB937 (1 mg/kg) immediately post-surgery.

Surgery induced a reduction in home-cage and open-field locomotor activity which was not affected by URB937 administration. Von Frey testing revealed surgery-induced mechanical allodynia of both the ipsilateral inguinal area and the ipsilateral hind paw, with the former, but not the latter, partially attenuated by URB937. HPLC with tandem mass spectrometry showed that URB937 significantly increased levels of OEA, AEA, and PEA in the inguinal tissue and contralateral T13-L2 spinal cord of surgery rats, with similar trends in the ipsilateral T13-L2 spinal cord which did not reach significance. HPLC with tandem mass spectrometry also indicated that URB937 was not detectable in spinal cord samples. Real-time quantitative PCR showed that surgery had no effect on FAAH or CB_1 mRNA levels in the pre-frontal cortex, T13-L2 or L3-L6 dorsal spinal cord.

These results suggest that a peripherally restricted FAAH inhibitor can attenuate pain-related behaviour in a rat model of POP following inguinal hernia repair. The results also indicate development of mechanical allodynia at a secondary site suggestive of a 'pain spreading' phenomenon, and that this phenomenon may not be sensitive to modulation of peripheral FAAH activity.

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NEURONAL PROPERTIES POST TRAUMATIC BRAIN INJURY: QUANTITATIVE COMPARISON, CLASSIFICATION AND MODULATION BY CANNABINOID DEGRADATION ENZYME INHIBITORS

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Traumatic brain injury (TBI) commonly affects otherwise healthy individuals and currently lacks effective therapeutic intervention. Cannabinoids represent a promising therapeutic tool to treat and protect against the neurological and behavioral consequences of TBI. Recent work from various TBI models demonstrates synaptic dysfunction in the neocortex at the site of injury, specifically hyperactivity of superficial cortical layers resulting from an imbalance of synaptic excitation and inhibition. In layer 5 (L5) cortex, the picture is not as clear; while some evidence shows that sensory responses of L5 neurons are attenuated, spontaneous action potential firing rates of these neurons are elevated. Our previous work showed that focal TBI (fluid percussion model) over somatomotor cortex results in compromised neurological and neurobehavioral outcomes in rats as well as elevated markers of inflammation and increased synaptic excitation in deep layers of cortex. We also previously showed that systemic administration of the endocannabinoid degradation enzyme inhibitor (JZL184 – inhibits 2-AG degradation by MAGL) rescues behavior, inflammation and synaptic excitation. These findings suggested that cannabinoids may be promising therapeutic tool to treat and protect against the neurological and behavioral consequences of TBI.

The aim of this study was to fully characterize intrinsic neuronal properties in the area of injury (L5 somatomotor cortex pyramidal neurons) and to test and contrast the effects of endocannabinoid (2-AG and/or AEA) degradation enzyme inhibitors that inhibit MAGL and/or FAAH to varying degrees; specifically, we tested the effects of JZL184, JZL195, URB597 and MJN110 on TBI outcomes. The TBI injury model we use is fluid percussion over somatomotor cortex in adult male Wister rats. We recorded intrinsic currents and voltage responses to inputs to assess intrinsic neuronal properties, including resting membrane voltage, input resistance, spike threshold, spiking responses to current input, and voltage "SAG" (rebound response to hyperpolarization activated inward current). We also measured spontaneous excitatory postsynaptic currents (sEPSC) and calculated the frequency and unitary amplitude of these events. We then used the aggregate parameter sets (intrinsic + synaptic properties) to apply a machine learning classification algorithm to quantitatively compare neural population responses between different injury groups (Sham vs. TBI) and the different degradation inhibitor groups. Our electrophysiological and computational results collectively indicate that Sham neurons are the most distinguishable from TBI neurons, while L5 cortical neurons from TBI animals that received specific post-injury degradation inhibitors are more similar to L5 cortical neurons from Sham animals. These findings suggest that EC degradation inhibition effectively restores (rescues) neuronal phenotype post-TBI and this may be an underlying mechanism for improved neurobehavioral outcomes in treated animals.

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ENDOCANNABINOID MACHINERY REGULATES CEREBELLAR GRANULE CELL DEVELOPMENT

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The cerebellum plays a crucial role in learning and execution of complex automated behaviors. The majority of cerebellar growth and synaptogenesis continues to take place during a protracted postnatal period. The most numerous neurons in the cerebellum are the cerebellar granule cells (GCs), which are generated in a dedicated secondary proliferative zone, the external granule cell layer (EGL). The robust expansion of granule cells throughout development is responsible for the majority of cerebellar expansion.

Endocannabinoids (eCBs) have been identified as key players regulating neuron proliferation, differentiation, and migration, however, their role in cerebellar development has not yet been explored in detail. Our preliminary results show robust expression of cannabinoid receptor 1 (CB1) in the inner EGL GCs, concomitant with expression of diacylglycerol lipase α (DGLα), an enzyme contributing to the synthesis of eCB 2-arachidonoylglycerol (2-AG), in Purkinje cells (PCs). Furthermore, our results show that cerebellar size is reduced in CB1 KOs as well as in pups perinatally exposed to THC. Proliferation and differentiation analyses indicate that there are shifts in GC developmental dynamics in both CB1 KO and THC exposed animals indicating that eCB signaling machinery plays a critical role in cerebellar development

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ENDOCANNABINOIDS AND THEIR ACTION AT TRPV1

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Whether caused by inflammation or dysfunctional nerves, chronic pain affects nearly 10% of the world's population. Since there are few treatments that are effective while being non-invasive and non-addictive, new targets are being explored. Found in the peripheral nervous system, the transient receptor potential subfamily vanilloid type 1 (TRPV1) ion channel can be activated by a plethora of exogenous and endogenous stimuli including capsaicin, temperatures above 43°C and acidic conditions. In recent years, it has been discovered that TRP channels, including TRPV1, act as ionotropic cannabinoid receptors. The endocannabinoid anandamide has been shown to have a similar binding affinity to TRPV1 as capsaicin and can rapidly desensitize the channel giving an analgesic effect.[1] Another endocannabinoid, 2-arachidonoylglycerol, has also been shown to activate and desensitize TRPV, though at a lower potency than anandamide. [2]

Models of the open and closed structures of TRPV1 were constructed from the published cryo-EM structures (PDB: 5IRX, 5IRZ).[3] Prime (Schrodinger, Inc. Version 11.8.012) was used to complete unresolved regions of extracellular loops and ankyrin repeat domains were incorporated in the model using the available crystal structure (PDB: 2PNN). These models have been equilibrated (50 ns) in a fully hydrated POPC bilayer for use in molecular dynamics simulations (AMBER 16). Currently, simulations are focused on understanding the mechanism of anandamide entry and activation of the channel. Future work will include ligand docking and molecular dynamics simulations of other endogenous cannabinoids and acyl amides.[4] The results of these simulations and dockings to date will be presented. [Support: NIDA DA003934]

(1) Lowin, T.; Straub, R. H. *Arthritis Res. Ther.* **2015**, *17* (1), 226.
(2) Petrosino, S.; Schiano Moriello, A.; Cerrato, S.; Fusco, M.; Puigdemont, A.; De Petrocellis, L.; Di Marzo, V. *Br. J. Pharmacol.* **2016**, *173* (7), 1154–1162.
(3) Gao, Y.; Cao, E.; Julius, D.; Cheng, Y. *Nature* **2016**, *534* (7607), 347–351.
(4) Raboune, S.; Stuart, J. M.; Leishman, E.; Takacs, S. M.; Rhodes, B.; Basnet, A.; Jameyfield, E.; McHugh, D.; Widlanski, T.; Bradshaw, H. B. *Front. Cell. Neurosci.* **2014**, *8* (August), 195.

CHRONIC INJECTIONS OF WIN 55, 212-2 IN YOUNG RATS CAUSE SLEEP DISTURBANCES IN ADULTHOOD

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Endogenous (anandamide and 2-AG) and exogenous (Δ^9-tetrahydrocannabinol and WIN55 212-2) cannabinoids modulate diverse neurobiological processes via the engagement of the CB_1 and CB_2 cannabinoid receptors. Despite that significant advances in the comprehension of the mechanism of action of cannabinoids in neurobiology have been achieved, further complexity has been identified since the use of medicinal cannabis-derived products for managing pediatric disorders, such as epilepsy or autism, would increase the legal use of cannabinoids in young subjects. Thus, the use of cannabinoids in young population could induce unknown medical long-term effects. Here, we investigated the effects of chronic systemic treatments in adolescent rats with synthetic cannabinoid agonist WIN 55, 212-2 on the sleep-wake cycle in adulthood. From postnatal day 30 (PND30), rats received during 2 weeks a daily administration of either vehicle (control; 1mL, intraperitoneally [i.p.]), or WIN 55, 212-2 (0.1, 0.3 or 1.0 mg/Kg/1mL, i.p.). Once reaching adulthood (PND-80), electrodes for sleep recordings (EEG/EMG) were implanted in all rats. Data showed that pharmacological treatments with WIN 55, 212-2, as compared to respective control, decreased wakefulness (W) and increased slow wave sleep (SWS) and rapid eye movement sleep (REMS). However, sleep data during lights-on period (12h) showed that adult rats displayed a significant increase in W whereas SWS was decreased. In opposite direction, during the lights-off period (12h), WIN-treated animals showed a significant diminution in W and an enhancement in SWS and REMS. Our study shows that chronic stimulation of the CB_1 cannabinoid receptor during adolescence by WIN 55, 212-2 during provoked sleep disturbances in adulthood.

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EFFECTS OF EHP-101 ON INFLAMMATION AND REMYELINATION IN MURINE MODELS OF MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is characterized by a combination of inflammatory and neurodegenerative processes that are dominant in different stages of the disease. Thus, immunosuppression is the gold standard for addressing the inflammatory stage and novel remyelination therapies are being pursued to restore lost function. VCE-004-8 is a multitarget synthetic cannabinoid derivative acting as a dual PPARγ/CB_2 ligand agonist that also activates the HIF pathway. VCE-004.8 prevented neuroinflammation in two different models of MS: Experimental Autoimmune Encephalomyelitis (*EAE) and* Theiler's murine encephalitis virus-induced demyelinating disease (Navarrete et al., 2018. J Neuroinflammation, 2018; 15(1): 64). We also reported that oral EHP-101 (a lipidic formulation of VCE-004.8) showed a dose-dependent efficacy profile with prevention of neuroinflammation in the EAE model (Navarrete et al., ICRS2018). EHP-101 has entered into a Phase 1 clinical study (clinicaltrial.gov: NCT03745001) and we report here the preliminary results of studies focused on the potential effect of EHP-101 in preventing demyelination or enhancing remyelination.

In EAE, transcriptomic analysis by RNA-Seq and qPCR demonstrated that EHP-101 prevented the expression of a large number of genes closely associated with MS pathophysiology in the spinal cord. In addition, EHP-101 normalized the expression of several genes associated with oligodendrocyte function, such as Teneurin 4 (Tenm4) that was downregulated in EAE. Immunohistochemistry analysis confirmed the recovery of Tenm4 expression in the spinal cord. Confocal analysis revealed that EHP-101 treatment prevented microglia activation (Iba1 staining), and demyelination (MBP staining) in both the spinal cord and the brain. Moreover, EAE was associated with a loss in the expression of Olig2 in the corpus callosum, a marker for oligodendrocyte differentiation, which was restored by EHP-101 treatment. In addition, EHP-101 enhanced the expression of glutathione S-transferase pi (GSTpi), a cytosolic isoenzyme used as a marker for mature oligodendrocytes in the brain. These data are indicative of the potential of EHP-101 to prevent demyelination in an MS murine model.

To further evaluate the potential of EHP-101, we investigated its effect in a cuprizone model of demyelination. Mice were fed with a diet containing 0.2% cuprizone for 6 weeks and then the animals were switched to a normal diet and treated or not with EHP-101 (10 and 20 mg/Kg) for 2 weeks. Cuprizone induced a clear loss of myelin in the brain measured by cryomyelin staining and MPB expression. Spontaneous recovery from demyelination was negligible after 1 and 2 weeks but remyelination was significantly accelerated by EHP-101 treatment. Moreover, EHP-101 also prevented cuprizone-induced microglial activation and astrogliosis detected by Iba1 and GFAP staining, respectively.

In conclusion, we provide evidence that EHP-101 represents a promising drug candidate for the potential treatment of different forms of MS.

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MAGL REGULATION OF EXCITATORY SYNAPSES IN ASD PATIENT IPSC DERIVED CORTICAL ORGANOIDS

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Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder that is characterized by social deficits, behavioral abnormalities, and disrupted stimuli processing. At the cellular level, there is often an imbalance of excitatory and inhibitory neuronal synapses as well as defective synaptic pruning. Induced pluripotent stem cell (IPSC) derived neurons from ASD patient fibroblasts exhibit altered an increased ratio of excitatory to inhibitory synapses. Changes to the endocannabinoid system (ECS), a global regulator of synaptic plasticity, may play a role in producing ASD during early fetal development. The ECS decreases presynaptic neurotransmitter release thru the activation of Gi-coupled CB1 receptors by the endocannabinoids 2-AG and AEA among other cannabinoid agonists. Preliminary qRT-PCR results indicate that MAGL, the enzyme which metabolizes 2-AG, has increased mRNA levels in ASD patient-derived neurons relative to controls. We are thus investigating MAGL to determine if it is a mediator of excitatory dysregulation in ASD patients.

Preliminary mRNA evidence suggests comparable levels of CB1 receptor expression in control and ASD patient-derived neurons. Because of this, we believe that there is less 2-AG mediated agonist effect on CB1 and thus a decrease in the amount of Gi coupled inhibitory effect on the neuron. **Our overall hypothesis is that increased expression of MAGL in ASD patient derived cortical organoids is associated with the dysregulation of normal excitatory-inhibitory tone.** So far, we have seen that administration of CB1R antagonist SR141716 in control organoids produces a dose-dependent increase in CB1R-positive excitatory synapses. We expect to find an increase of MAGL expression in our ASD patient-derived organoids, mirroring our qRT-PCR results. Future work includes quantifying endocannabinoid levels and evaluating CB1R at inhibitory synapses in control and ASD patient-derived organoids.

SMALL INTESTINE METABOLIC AND ENDOCANNABINOIDE ADAPTATION IN RESPONSE TO AN OBESOGENIC DIET

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Several studies in human and rodent models have demonstrated the presence of metabolic alterations in the small intestine in a context of obesity and insulin resistance. Increased peripheral endocannabinoid system tone has also been involved in metabolic dysfunctions. However, the precise adaptation occurring in the small intestine during the establishment of obesity and metabolic complications remains unclear.

Aim: To characterise the chronology of small intestine metabolic and endocannabinoidome disturbances in response to an obesogenic high-fat, high-sucrose (HFHS) diet.

Methods: Six-week-old C57BL/6J male mice, susceptible to diet induced insulin resistance and obesity, were fed a low-fat, low sucrose diet (10% fat, 20% protein and 70% carbohydrate [7% sucrose]) and sacrificed at the baseline or at 3, 10, 21 and 56 days following the initiation of an HFHS diet (45% fat, 15% protein and 35% carbohydrate [17% sucrose]). Body weight, food intake, glucose tolerance (OGTT) and intestinal lipoprotein production (OLTT) were monitored at each timepoint. Expression profile of genes related to enteric functions and the endocannabinoidome was assessed by qPCR array (TaqMan). Endocannabinoidome mediators in the small intestine and in circulation were measured by LC-MS/MS.

Results: As expected, significant weight gain was observed in response to the HFHS diet which was associated with the development of an early glucose intolerance but delayed lipid disturbance. Transcriptomic analysis has revealed increased expression of thigh junction protein (e.g. Tjp1 and Ocln), lipid metabolism (e.g. Fabp2) and inflammatory (e.g. Tnfa) genes. Expression of endocannabinoidome converting enzymes (e.g FAAH) was increased, while the expression of some receptors (e.g. Cnr1) was decreased. Accordingly, tissue levels of some mediators (e.g. AEA) were rapidly increased in response to the HFHS diet. Interestingly, several genes show an early response to the HFHS diet, as early as days 3 and 10, which precedes weight gain and the development of most peripheral metabolic complications.

Conclusion: Development of an obesity state with systemic glucose intolerance and lipid disturbance is associated with alterations in small intestinal functions and local alterations in the endocannabinoidome. This study provides evidence of an early small intestine involvement in obesity-related metabolic dysfunction as well as on longitudinal adaptation of the endocannabinoidome in diet-induced obesity.

PERIPHERAL CANNABINOID CB_1 RECEPTORS CONTROL NUTRIENT-INDUCED INCRETIN SECRETION *IN VIVO*

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Enteroendocrine cells along the intestinal epithelium secrete incretins and related hormones (i.e. GIP, GLP-1, and CCK-8) following ingestion of nutrients. Incretins activate their respective receptors on cells located in – but not limited to – the endocrine pancreas, which enhances insulin secretion and maintains a tight window of circulating blood glucose. The ability to adequately regulate circulating glucose levels is lost in patients with prediabetes and type-2 diabetes. The endocannabinoid system controls food intake and glucose homeostasis, and its activity becomes upregulated in the proximal small-intestinal epithelium and plasma from Western-diet induced obese mice. Cannabinoid subtype-1 receptors (CB_1Rs) are present in various incretin-producing cells in the intestinal epithelium (e.g. K, L, I-cells), yet their function in these cell types are poorly characterized. In the current investigation, we examined the ability for CB_1Rs to control incretin secretion *in vivo* in male C57CL/6N mice. We administered the cannabinoid receptor agonist, WIN 55,212-2 (WIN, 3 mg per kg), alone and in combination with the peripherally-restricted neutral CB_1R antagonist, AM6545 (10 mg per kg). Incretin secretion was then stimulated via corn oil gavage (0.5 mL, 0.5 hr after drug treatment) and circulating incretins in plasma were quantified 0.5 hr later by sensitive enzyme-linked immunosorbent assays. Activating CB_1Rs with WIN blunted corn-oil induced secretion of GIP, bioactive GLP-1, and bioactive CCK-8. Co-administration of WIN with AM6545 completely reversed the inhibitory actions of WIN on incretin release. Collectively, our results suggest that CB_1Rs in the small-intestinal epithelium control nutrient-induced incretin release. The functional consequences of these findings on glucose homeostasis are currently under investigation.

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TONIC ENDOCANNABINOID SIGNALLING GATES STRESS-LIKE STEREOTYPIC BEHAVIORS

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Endocannabinoid (eCB) signalling is known to gate many aspects of the stress response; however, a wealth of studies have strongly implicated that eCB signalling can negatively regulate the hypothalamic-pituitary-adrenal (HPA) axis. A nexus of the HPA axis is a cluster of corticotropin releasing hormone (CRH) producing neurons in the paraventricular nucleus of the hypothalamus (PVN). Studies have shown that under both resting conditions, and in response to stress, disruption of eCB signalling can increase drive on the HPA axis. The site of action by which eCB signalling has the ability to dampen activation of the HPA axis is not entirely understood, but immunohistochemical studies have clearly demonstrated that blockade of CB1 receptor signalling can increase neuronal activation (using c-fos) in CRH neurons of the PVN. Recently, Fuzesi and colleagues (2016) demonstrated using optogenetic approaches, that activation of CRH neurons produces a characteristic behavioural sequelae consistent with what is seen following exposure to stress, with noted increases in stereotypic, self-directed behaviors such as grooming. Many reports over the years have noted that antagonism of CB1 receptors seems to influence many of these stereotypic behaviors, although rigorous analysis of this behavioural sequelae, and whether it is directly mediate by activation of CRH neurons in the PVN, remains to be determined. To this extent, we are examining the impact of CB1 receptor antagonism on self-directed behaviors (e.g., grooming and scratching), as well as activation of CRH neurons in the PVN using both immunohistochemical approaches and *in vivo* fiber photometry of CRH neurons, using a CRH-Cre mouse expressing GCaMp6 in a Cre-dependent manner. Our initial findings have focused on the behavioural aspect on this approach doing a careful, temporally specific analysis of behavioural sequelae which occur following antagonism of the CB1 receptor under non-stress conditions. To this extent, we examined the impact of CB1 receptor antagonism on self-directed behaviors. Consistent with our predictions, 3 mg/kg of the CB1 receptor antagonist AM251 resulted in a significant increase in self-directed behaviors, similar to what is seen following exposure to stress. Ongoing work is aiming to examine activation of CRH neurons in the PVN using both immunohistochemistry of defined CRH neurons as well as fiber photometry to be able to examine temporal changes in calcium dynamics of CRH neurons in the PVN following disruption of CB1 receptors. This work will help us to understand how eCB signalling regulates components of the HPA axis, but may also provide insight into how eCB signalling could regulate stereotypic behaviors associated with many psychiatric conditions, such as depression and autism.

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THE ROLE OF THE CANNABINOID RECEPTORS IN THE EFFECTS OF GABAPENTIN ON FORMALIN-INDUCED HYPERALGESIA

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Gabapentin is commonly used to treat pain, but little is known about how the drug interacts with the endocannabinoid system. A prior study has shown gabapentin use in ICU patients to reduce pain increases circulating 2-arachidonoylglycerol concentrations (Raff et al., 2017), suggesting the hypothesis that activation of CB1 or CB2 receptors could play a role in its analgesic effects. The purpose of this project was to determine if gabapentin's analgesic effects in mice require either the CB1 or CB2 receptors.

In separate studies, mice with either CB1 and CB2 receptor genetic knockouts and wildtype controls were given intraperitoneal injections of gabapentin (30 mg/kg) or saline 15 minutes prior to introduction of a pain stimulus. To induce pain, 0.925% formalin solution was injected subcutaneously into a hind paw, and pain behaviors, such as biting/licking and shaking the impacted paw, were observed for an hour immediately following the paw injection.

Formalin injection produced reliable behavioral signs of pain (paw licking, biting and shaking) that fell into 2 phases. The first phase (0-15 min following injection) was unaffected by gabapentin treatment in wild type mice. However, pain responses in the second phase were significantly reduced by gabapentin pretreatment in both wild type (WT) ICR, outbred male mice and WT C57 Bl6 male mice (Table). The CB1R knock out (KO) mice exhibited significantly greater pain responses compared to the WT after both saline and gabapentin pretreatment. The ability of gabapentin to reduce pain responding was intact in both CB1R and CB2R KO mice. Interestingly, CB2R KO mice exhibited a greater response to gabapentin than their WT littermates.

Strain and Genotype	Saline (AUC ± SEM)	Gabapentin (AUC ± SEM)
ICR WT*	258 ± 43#	134 ± 27#
ICR CB1R KO*	384 ± 23#	258 ± 37#
C57 Bl6 WT*	339 ±31	173 ± 33
C57 Bl6 CB2R KO***	336 ± 68	106 ± 17

WT compared to CB1R knock out is significantly different with $p<0.05$; * saline and gabapentin treated groups are significantly different with $p<0.05$ or *** $p<0.005$

These data show that gabapentin does not likely work through either CB1 or CB2 receptors, but may suggest that the absence or reduced CB2 receptors may potentiate the efficacy of gabapentin to reduce inflammatory pain.

Raff, H., Kramer, D.J. & Hillard, C.J. Endocrine (2017) 58: 203.
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FAAH-KO ASTROCYTES EXHIBIT A PRO-INFLAMMATORY PHENOTYPE

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The role and functions of the fatty acid amide hydrolase (FAAH) in the context of neuroinflammation are still controversial. We have recently reported that the genetic inactivation of FAAH is consequential, leading to a paradoxical, two-sided effect: i) on one hand, the absence of FAAH triggers a pro-inflammatory state, both *in vivo* and *in vitro*, in which glial cells seem to play a prominent role; and, ii) on the other hand, these effects are accompanied by significant improvements in, for instance, amyloid burden or memory impairment in a mouse model of Alzheimer's disease (AD) that exhibits an enhanced inflammatory milieu. These phenotypic modifications linked to the genetic deletion of FAAH have been also reported by other groups. Further, recent data suggest that the interleukin-1beta (IL1β) neurotransmission system may be instrumental in these FAAH-associated changes. For instance, the *in vivo* inhibition of IL1β production by exposure to the caspase-1 inhibitor minocycline results in the prevention of some of these changes, including behavioural performance and amyloid levels in the cortex and hippocampus of AD mice.

We now focused on the role that astrocytes may play in these effects. Our data indicate that neonatal astrocytes obtained from FAAH-KO mice exhibit a dysregulated IL1β system, with increased expression of tumor necrosis factor-alpha (TNFα), IL1β, IL1β-receptor adaptor protein, and of the IL1βr antagonist, and decreased expression of the scavenger receptor A. In addition, the activity of membrane hemichannels was also altered, with a significantly increased level of activation under basal conditions. Furthermore, the exposure to the pathogenic form of beta-amyloid led to significant changes in the production of anandamide. These data confirm that the genetic deletion of FAAH induces profound phenotypic alterations in brain astrocytes, exhibiting a pro-inflammatory phenotype that leads to functional changes in several processes directly involved in the inflammatory response.

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THERAPEUTIC EFFECT OF THE SUBSTRATE-SELECTIVE CYCLOOXYGENASE-2 INHIBITION IN A MOUSE MODEL OF REPETITIVE CLOSED HEAD INJURY

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Traumatic brain injury (TBI) is the predominant type of brain injury in young adults and is a risk factor for developing chronic traumatic encephalopathy and other neurodegenerative diseases. Although excitotoxicity, oxidative stress and inflammation are known to contribute to the pathogenesis of the secondary brain injury following the initial insult, the effective treatment are still not available. Besides being inactivated by MAGL and FAAH (hydrolytic enzymes), the endocannabinoids 2-arachidonylglycerol (2-AG) and anandamide (AEA) can be oxygenated by cyclooxygenase-2 (COX-2) to terminate the cannabinoid signaling. Although studies from our lab and others have shown that inhibition of AEA and 2-AG hydrolysis is protective in several animal models of TBI, it is unknown whether targeting the endocannabinoid oxygenation is also effective for the TBI treatment. LM-4131 is a recently characterized substrate-selective COX-2 inhibitor that selectively elevates the brain levels of AEA and 2-AG without affecting the non-cannabinoid N-acylethanolamines and monoacylglycerols, and it also does not affect the oxygenation of arachidonic acid to prostaglandins, and therefore can avoid the gastrointestinal and cardiac side effects caused by traditional COX-1/2 inhibitors. In a mouse model of repetitive closed head injury (rCHI) induced by three impacts with a 24-hour apart, we found that administration of LM-4131 at 10 mg/kg (i.p.) starting at 30 min after each impact and then once daily for 5 days significantly improved motor coordination in TBI animals demonstrated by the reduced foot faults in the beam-walk test at 4, 6 and 10 days after the first impact. The accumulation of microglia in ipsilateral cortex and hippocampal dentate gyrus was also diminished in the drug treated animals. Although the use of lower doses of LM-4131 (5 mg/kg) or MJN110 (0.25 mg/kg), a selective inhibitor of the major 2-AG hydrolytic enzyme monoacylglycerol lipase had no effect on locomotor function and working memory, these behavioral deficits associated with TBI were significantly blocked by co-administration of these two compounds. Furthermore, the reduced mRNA expression in cortex and protein level of the $GABA_A$ receptor subunits beta2/3 and gama2, the glutamate receptor subunits N2B and GluR1 in hippocampus were restored by the combined drug treatment. These results suggest that selective inhibition of endocannabinoid oxygenation, and the combined inhibition of 2-AG oxygenation and hydrolysis, can suppress neuroinflammation and improve neurological function in TBI animals. The therapeutic mechanisms of LM-4131 and its combination with MJN110 are likely due to the increased 2-AG signaling and the reduced production of proinflammatory prostaglandin glycerol esters.

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TRAUMATIC BRAIN INJURY ALTERS THE ENDOCANNABINOID SYSTEM IN THE BASOLATERAL AMYGDALA OF FEMALE RATS

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Traumatic brain injury (TBI) is a growing national health concern; nearly 2.5 million Americans sustain a TBI each year, and this number has increased since 2000 by nearly 60%. TBI can lead to cognitive impairment, affective disorders, and increased propensity for addictive behaviors. Previous work from our laboratory showed escalation in alcohol self-administration, alcohol-induced exacerbation of neuroinflammation, and delayed neurobehavioral recovery from TBI. Subsequent studies showed that inhibiting endocannabinoid degradation by post-TBI administration of JZL184 improved neurobehavioral outcomes, attenuated neuroinflammation, and blunted alcohol rewarding effects. The amygdala is a brain structure with a critical role in mediating negative affective states and addictive behavior. TBI and alcohol each alter amygdala function and brain endocannabinoid (eCB) signaling. Our working hypothesis is that basolateral amygdala (BLA) eCBs may modulate alcohol drinking and its impact on neurobehavioral outcomes post-TBI. This study's aims were to determine TBI effects on eCB system protein expression in BLA of female rats with and without an alcohol drinking history. We hypothesized that TBI would increase degradative eCB enzyme monoacylglycerol lipase (MAGL) expression and decrease 2-arachidonylglycerol (2-AG) signaling in BLA of alcohol drinking and alcohol naïve animals. Adult female Wistar rats were trained to self-administer alcohol using an operant task. Following stabilization of alcohol responding, all rats underwent a 5-mm left lateral craniotomy. Three days later, mild-to-moderate TBI (25ms, 28-32 PSI) or sham injury was delivered via fluid percussion over the somatomotor cortex. For animals used in Western blot analyses, operant alcohol self-administration was measured every two d post-injury for 10 d, and animals were sacrificed 11 d post-TBI. Animals used for eCB quantification did not self-administer alcohol post-TBI and were sacrificed 1 hour, 5 d, or 11 d following injury. Following sacrifice, brains were excised, BLA were bilaterally dissected, and micropunches were used for either Western blot analysis of eCB system protein expression (eCB synthetic and degradative enzymes and cannabinoid receptors) or quantification of eCB (i.e., 2-AG, anandamide) levels via isotope dilution, electrospray ionization liquid chromatography/mass spectrometry.

Western blot analysis revealed significant downregulation of the 2-AG synthetic enzyme, diacylglycerol lipase alpha, and degradative enzyme, MAGL, in BLA of female alcohol drinking rats 11 d post injury relative to alcohol-naïve animals. No changes in eCB-related enzymes were observed in alcohol-naïve rats. Mass spectrometry analysis of BLA tissue suggests TBI reduces eCB levels 11 d post injury. Collectively, our results suggest that TBI dysregulates BLA eCB system proteins in female rats, and that these effects may differ in individuals with and without chronic alcohol drinking history.

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IMPACT OF A PERIPHERALLY RESTRICTED CANNABINOID AGONIST ON INFLAMMATION-INDUCED NOCICEPTION AND TRPV1 SENSITIZATION IN DORSAL ROOT GANGLION NEURONS

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Activation of cannabinoid-type 1 (CB_1) receptors reduces nociception in preclinical models of inflammatory, neuropathic and visceral pain. However, direct activation of CB_1 receptors results in unwanted side-effects, including psychoactivity and antinociceptive tolerance. Thus, we need alternative approaches to utilize the antinociceptive action of cannabinoid receptor signaling in a manner that circumvents these unwanted side effects. The presence of CB_1 receptors have been confirmed on the cell bodies of DRG neurons, suggesting a potential site-of-action by which peripheral CB_1 receptors may exert antinociceptive effects. Indeed, peripheral CB_1 receptors have recently been described as a potential therapeutic target in models of neuropathic (Dziadulewicz et al. J Med Chem. 2007 Aug 9;50(16):3851-6) (Seltzman et al. J Med Chem. 2016 Aug 25;59(16):7525-43) and cancer-induced pain (Zhang et al. Pain. 2018 Sep;159(9):1814-1823). In the present study we sought to extend these findings and evaluate the effects of CB-13 (CRA13, SAB-378), a peripherally-restricted cannabinoid agonist, on inflammatory nociception induced by complete Freund's Adjuvant (CFA) *in vivo* and in a model of TRPV1 sensitization induced by the pro-inflammatory mediator prostaglandin E2 (PGE2) in primary DRG neurons *in vitro*.

To induce inflammatory nociception mice were administered CFA (10 µl) into the left hindpaw. A within-subjects dose-response of CB-13 in reducing mechanical allodynia induced by CFA was generated with a 1-2 day spacing between dosing beginning 24 hours following CFA administration. In a separate set of animals, the peripherally restricted CB_1 antagonist AM6545 (10 mg/kg i.p.) was administered 30 minutes prior to a maximally effective high dose (3 mg/kg i.p.) of CB-13. AM6545 blocked the anti-allodynic effects of CB-13 without altering mechanical responsivity on its own, suggesting the anti-allodynic effects of CB-13 were mediated by peripheral CB_1 receptors.

Calcium imaging experiments are currently ongoing to evaluate the potential impact of CB-13 on PGE2-induced TRPV1 sensitization. Lumbar DRG from mice will be isolated and cultured, and calcium imaging will commence on day 2 *in vitro*. On the day of testing, DRG neurons will be incubated with Fura-2AM and Tyrode's solution. Changes in fluorescence will be measured in response to two 20-second pulses of capsaicin (a TRPV1 agonist, 100nM) applied 7 minutes apart. Vehicle, PGE2 (1µM), or PGE2 (1µM)+CB-13 (1µM) will be applied between pulses to potentially alter TRPV1 sensitization. The change in fluorescence following capsaicin application between the 2 pulses will be calculated and compared across conditions. The results of these experiments will provide valuable insight into the function and potential utility of peripheral CB_1 receptors in treating pain states related to inflammatory insults.

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POTENTIAL DEVELOPMENT OF NOVEL ANALGESICS THROUGH ACTIONS OF MINOR CANNABINOIDS TARGETING TRANSIENT RECEPTOR (TRP) SUPERFAMILY MEMBERS TRPV1, TRPV2, TRPM8 AND TRPA1

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There is a need for pain medications that are mechanistically likely to be able to address chronic pain as well as acute presentations, by acting analogously to current methods for desensitization of nocioceptive neurons and pathways. Cannabinoid compounds are potential analgesics. Users of medicinal Cannabis report efficacy for pain control, clinical studies show that cannabis can be effective and opioid sparing in chronic pain, and some constituent cannabinoids have been shown to target nociceptive ion channels. Here, we explore and compare a suite of cannabinoids for their impact upon the physiology of TRPV1. The cannabinoids tested evoke differential responses in terms of kinetics of activation and inactivation. Cannabinoid activation of TRPV1 displays significant dependence on internal and external calcium levels. Cannabinoid activation of TRPV1 does not appear to induce the highly permeant, pore dilated channel state seen with Capsaicin, even at high current amplitudes.

Additionally, we analyzed cannabinoid responses at nocioceptive channels other than TRPV1 (TRPV2, TRPM8 and TRPA1), and report that cannabinoids differentially activate these channels. The distinct response profiles of the different cannabinoids that we observe also provides the possibility of fine-tuning or shaping desirable responses using cannabinoid mixtures. At the level of the sensory neuron bundles, the fact that cannabinoids appear to discriminate between TRP receptors and that the receptors in turn respond distinctively to the compounds, again offers the potential for rational design of therapeutic mixtures. In conclusion, on the basis of response activation and kinetics, state-selectivity and receptor selectivity, it may be possible to rationally design approaches to pain using single or multiple minor cannabinoids.

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TERPENE REGULATION OF TRPV1: POTENTIAL PAIN THERAPEUTICS

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Transient Receptor Superfamily ("TRP") channels have been identified as targets for treating pain disorders due to their roles in nocioception. Both antagonism and agonism of the TRP channel have been exploited for pain management. Since complex plant derived mixtures of cannabinoids and the *Cannabis* component myrcene have been regarded as pain therapeutics, we screened a suite of terpenes found in *Cannabis* for activity at TRPV1. The inducible expression of TRPV1 in a non-TRPV1 containing cell type confers capsaicin-sensitive calcium flux responses. Terpenes contribute significantly and differentially to calcium fluxes via TRPV1 induced by *Cannabis*-derived mixtures of cannabinoids and terpenoids.

Myrcene contributes significantly to TRPV1-mediated calcium responses seen with terpenoid mixtures, and this signal depends on the presence of the TRPV1 ion channel. Myrcene-induced calcium influx responses are inhibited by a specific pharmacological inhibitor of the TRPV1 ion channel. Electrophysiological measurements of current development and current-voltage relationships for TRPV1 were carried out in a whole cell patch clamp configuration. Myrcene-induced TRPV1 currents are highly sensitive to the internal calcium environment, and while Myrcene can elicit large TRPV1 currents under buffered conditions, under free calcium conditions Myrcene currents are not detected. When Myrcene currents are evoked, they are distinct from responses to capsaicin on the basis of Imax and their lack of shift to a pore-dilated state. These data establish TRPV1 as a target of myrcene and suggest the therapeutic potential of analgesic formulations containing myrcene. We also identify several non-*Cannabis* plant-derived sources of myrcene and other compounds targeting nocioceptive TRPs using a data mining approach focused on analgesics suggested by non-Western Traditional Medical Systems.

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MECHANISM OF ACTIVATION OF GPR18 BY ITSELF

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The orphan G-Protein Coupled Receptor (GPCR) GPR18 has been recently proposed as a potential member of the cannabinoid family. This is due to the modulatory effects exhibited by several endogenous, phytogenic and synthetic cannabinoids. Recent studies suggest that N-arachidonoyl glycine may be the endogenous agonist for GPR18.[1] However, GPR18 at present remains classified as an orphan Class A GPCR. Potential therapeutic applications for GPR18 include intraocular pressure, metabolic disorders and cancer. No highly potent GPR18 ligands have been developed to date. Our long-term goal is to develop both nanomolar IC50 antagonists and nanomolar EC50 agonists at GPR18. The first step towards this goal is the development of models of the GPR18 inactive and activated states.

We report here such development. As an initial test of these models, we then examine the molecular level changes that occur in a A3.39N GPR18 mutation and compare these with the known pharmacological effects of this mutation: reduced constitutive activity and increased surface expression. This report not only provides insights into the biological role of this receptor, but also guidance for future drug design by identifying the key residues involved in activation of the GPR18. Since this experimental data cannot provide a comprehensive insight into its structural mechanism, a computational dynamic approach was pursued using our GPR18 bundle. The inactive state of this model was embedded in a 66 POPC lipid bilayer environment and allowed to produce more than 700 ns of Molecular dynamics (MD) simulations.

Our MD simulations show that there is a strong relation between Q6.43 and D7.49 (Hydrogen bond) and R3.50 and S6.33 ("ionic lock" hydrogen bond). We showed that in the A3.39N mutant receptor the Q6.43/D7.49 interaction causes a counter-clockwise rotation (from the top view) of the cytoplasmic region of the receptor, which in turn helps S6.33 to get close enough to R3.50 to form the "ionic lock". These results are in agreement with the experimental data that shows a high constitutive activity for GPR18.[2]

1. Kohno, M.. *et al.* Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. *Biochemical and biophysical research communications* **4,** e1835 **3**, 827 (2006).
2. Finlay, D. B. *et al.* GPR18 undergoes a high degree of constitutive trafficking but is unresponsive to N-Arachidonoyl Glycine. . *Peer J* **4,** e1835 (2016).

ANTI-ARTHRITIC EFFECTS OF ENDOCANNABINOID ENZYME INHIBITION IN A MOUSE MODEL OF INFLAMMATORY ARTHRITIS

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Rheumatoid arthritis (RA) is a debilitating autoimmune disease characterized by inflammation at the synovial joints and is associated with swelling, cartilage destruction, and chronic pain. Current treatments for RA have negative side effects and lack prolonged efficacy. The present experiments profiled the efficacy of the MAGL inhibitor JZL184, the DAGLβ inhibitor KT109, and the synthetic glucocorticoid agonist dexamethasone to attenuate the progression of inflammatory arthritis in the mouse model of collagen-induced arthritis (CIA). Male DBA1/J mice were subjected to CIA, and paw thickness and clinical scores were assessed daily. Novel applications of functional assays (e.g., grip strength, digging, and balance beam tests) were used to determine arthritis severity, treatment efficacy, and drug side effects. Paw cytokine and chemokine levels were quantified by ELISA. Male DBA1/J mice subjected to CIA exhibited robust paw inflammation in paw thickness and clinical scores. CIA also decreased paw function, reducing latency to fall from a wire grid and digging time, while increasing time to traverse a balance beam. JZL184 (40 mg/kg, s.c. x 16 days) significantly attenuated CIA-induced paw swelling, clinical severity, and functional deficits. Somewhat surprisingly, JZL184 (40 mg/kg) suppression of digging (but not balance beam performance) persisted, despite JZL184 being administered for 16 days. The effects of JZL184 were attenuated by the selective CB_2 receptor antagonist SR144528 (3 mg/kg), indicating that a CB_2 receptor mechanism mediates the effects of JZL184. The effects of JZL184 on paw swelling and clinical scores were found to be less profound than the effects of repeated low dose dexamethasone. Dexamethasone (0.0625-0.5 mg/kg) significantly blocked paw swelling and clinical severity. These data suggest that inhibition of enzymes important for the metabolism of 2-AG have anti-inflammatory efficacy to reduce the morphological and behavioral effects of inflammatory arthritis.

EXPRESSION LEVEL INDEPENDENT INHIBITION OF LEPTIN RECEPTOR EXPRESSION BY THE CANNABINOID TYPE-1 RECEPTOR (CB_1R)

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The anorexigenic hormone leptin and the orexigenic endocannabinoids (ECs) work in an antagonistic manner under several conditions as leptin decreases endocannabinoid levels in the hypothalamus (Di Marzo *et al*., Nature 410 (2001) 822-825) whereas CB_1Rs contribute to leptin resistance in obesity (Tam *et al*., Mol. Metab. 10 (2017) 1113-1125). Although of utmost clinical significance, the exact molecular nature of the CB_1R-leptin interplay is not fully elucidated. We hypothesized that CB_1R-leptin antagonism emerges, at least partially, from inhibitory interactions between CB_1R and leptin receptor (LR) signaling within target cells.

In order to identify interactions between the signal transduction of LRs and CB_1Rs, we planned to co-express these receptors and aimed to test the sensitivity of CB_1R or LR signalling to leptin or CB_1R agonists, respectively. Selective transfection of HEK293 cells with plasmids coding for either LR or CB_1R resulted in high-level expression of functional receptors. However, when cells were transfected with both plasmids simultaneously, LR expression became virtually absent whereas high expression of CB_1R was preserved. We theorized that the expression of CB_1Rs in supraphysiological amounts overloads the cell's transcription-translation machinery thus curtailing the expression of the large LR construct. In order to reduce CB_1R levels, we replaced the strong cytomegalovirus (CMV) promoter with human herpes virus thymidine kinase (TK) promoter within the vector. TK-CB_1R expression level was comparable to that of endogenous receptors in mouse neuroblasts and was reduced by approx. 2 orders of magnitude in HEK293 cells when compared to the CMV-CB_1R expression. Despite the drastic differences in receptor amount, ERK phosphorylation evoked with the stable anandamide analogue arachidonyl-2-chloroethylamide was identical between the two receptor variants. Finally, TK-CB_1Rs inhibited LR expression to the same extent as CMV-CB_1Rs in HEK cells.

Our data suggest that the antagonistic interplay between leptin and the endocannabinoid system may be the result of, among others, CB_1R-mediated inhibition of leptin receptor expression. Also, smaller amount of recombinant receptors may already saturate endogenous signal transduction pathways and attenuated expression should be considered when studying receptor function.

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ANTI-INFLAMMATORY EFFECTS BY PHARMACOLOGICAL INHIBITION OR KNOCKDOWN OF FATTY ACID AMIDE HYDROLASE IN BV2 MICROGLIAL CELLS

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Fatty acid amide hydrolase (FAAH) has been recognized as a therapeutic target for several neurological diseases, since its inhibition can exert neuroprotective and anti-inflammatory effects by boosting the endogenous levels of N-acylethanolamines. However**,** several recent studies report that genetic deletion of FAAH exaggerates microglia activation and inflammatory response. In this study we selected two commonly used pharmacological inhibitors of FAAH, PF3845 and URB597, together with its siRNA knockdown to further characterize the impact of targeting FAAH in BV2 microglial cells. Treatment with PF3845 suppressed lipopolysaccharide (LPS)-induced prostaglandin E_2 (PGE_2) production along with downregulation of cyclooxygenase-2 and microsomal PGE synthase expression. PF3845 dose-dependently reduced the expression of pro-inflammatory cytokines, but had no effect on the anti-inflammatory cytokines. Notably, the anti-inflammatory effects of URB597 was not as potent as that of PF3845. Consistently, knockdown of FAAH gene with siRNA also suppressed PGE_2 production and the pro-inflammatory gene expression. However, distinct from the pharmacological inhibitors, downregulation of FAAH enhanced the expression of several anti-inflammatory markers with or without LPS treatment. The anti-inflammatory effects of FAAH inhibitor or knockdown were not affected by the cannabinoid receptor or the peroxisome proliferator-activated receptor (PPAR) antagonists. Despite inhibition or targeted knockdown of FAAH has potent anti-inflammatory effects and possibly leads to the dynamic change of M1 to M2 microglia phenotypes, the underlying mechanisms remain to be elucidated.

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ANALYSIS OF THE ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF CANNABIDIOL IN ARTHRITIS

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The anti-inflammatory effects of cannabidiol (CBD) have been long recognised but the distinct changes in cellular function and phenotype that occur have not been well characterised, particularly in the context of inflammatory arthritis. In the current study, we have investigated the anti-arthritic capacity of synthetic CBD and determined its effects on cytokine expression *in vitro* and cell migration *in vivo.*

Using the zymosan-induced arthritis model, CBD was shown to exert a potent analgesic effect, as measured by dynamic weight bearing analysis. However, the effect on joint swelling was less pronounced and we are further analysing the knee joints microscopically to assess the effect of CBD on immune cell infiltrate and joint damage. Investigations using IVIS optical imaging and inflammation-sensitive fluorescent reporters are also underway to quantify the anti-inflammatory capacity of CBD, which should enable us to determine whether the analgesic effects observed *in vivo* may be attributed to decreased inflammation in the knee.

In vitro studies performed in human primary cells supports an anti-inflammatory effect for CBD. We demonstrate that CBD treatment in results in a dose-dependent reduction in TNFα release by rheumatoid arthritis fibroblast-like synoviocytes, $CD14^+$ monocytes and $CD4^+$ T lymphocytes. Surprisingly, treatment with CBD *in vivo* increased granulocyte infiltration in the thioglycollate-induced model of peritonitis after 4 hours, but dose-dependently reduced TNFα and CXCL1 levels in the peritoneal lavage supernatant. This was accompanied by reductions in IL-6 and CXCL1 levels in the plasma of treated mice.

These results confirm previous findings reporting the anti-inflammatory potential of CBD and shed new light on its mechanisms of action. Future work will focus on the identification of the molecular targets of CBD *in vivo* and on the synthesis of CBD analogues that my offer improved PK and bioavailability for improved drug delivery in a clinical setting.

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DEVELOPMENT OF BENZOIMIDAZOLIC STRUCTURE INHIBITORS OF HUMAN FATTY ACID AMIDE HYDROLASE ENZYME (h-FAAH)

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During the last few years, the endocannabinoid system has emerged as a highly relevant topic in the scientific community, due to the fact that many different regulatory actions have been attributed to it and it is involved in several pathophysiological conditions [1]. The relevance of the system is further strengthened by the notion that drugs interfering with the activity of the endocannabinoid system are considered as promising candidates for the treatment of various diseases, including obesity, Parkinson's disease, Alzheimer's and others [1,2].

Based on this background, our research group has designed series of compounds aimed at the direct modulation of CB_1 and CB_2 receptors [3, 4, 5]. The modulation strategy has been extended to indirect agonist action through the design of FAAH inhibitors. The interest in designing this last type of molecules is due to the fact that FAAH inhibitors generate a specific increase of anandamide in tissues where endocannabinoids are produced by physiological protection mechanisms, giving a finer response in terms of site-selectivity. This characteristic makes it possible to suppose that the pharmacological effects associated with the inhibition of FAAH would be less adverse than those commonly associated with the direct activation of the CB_1 receptor. In addition, FAAH inhibitors have already shown anti-inflammatory effects by suppressing the release of inflammatory chemical mediators by stimulating CB_2 receptors in immune cells [5].

Based on the same work line and having the in-silico model of the FAAH enzyme, we designed 2 series of new benzoimidazole central core molecules with inhibitory capacity on FAAH.

The work carried out so far, has consisted mainly in the synthesis of 2 families of compounds, containing the basic structure of a ring of benzoimidazole associated through a connector to various substituted arylpiperazines. In addition, the docking studies of the compounds with the protein is reported and their subsequent evaluation as inhibitors of the fatty acid amide hydrolase enzyme.

Acknowledgements: Funded by CONICYT Fund (Grant 21160678)
FONDECYT (Grant 1150121)

1. Pagotto et al. *Endocr Rev* 2006; 27 (1): 73-100.
2. Manzanares et al. *Curr Neuropharmacol.* 2006 Jul; 4 (3): 239–257.
3. Romero-Parra et al *Eur J Med Chem* 2016 Nov 29; 124: 17-35.
4. Mella-Raipán et al. *Molecules* 2013; 18 (4): 3972-4001.
5. Pessoa-Mahana *Rev. Farmacol. Chile* 2016; 9 (2): 7-15

THE CORRELATION OF ANANDAMIDE AND CORTISOL IN DOGS FOLLOWING AN ACUTE STRESSOR

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The endocannabinoid system (ECS) exists in the brain and periphery of humans and animals, and has been shown to be both a regulator and effector of the stress response (Hill et al., Psychoneuroendocrinology 34 (2009) 1257-1262; Dlugos et al., Neuropsychopharmacology 37 (2012) 2416-2427). Endocannabinoids, endogenous lipid-based retrograde neurotransmitters which target the ECS, could thus be potential therapeutic targets of or biomarkers for disorders associated with anxiety (Micale et al., Pharmacology & Therapeutics 138 (2012) 18-37). The objective of this study was to evaluate biomarkers of anxiety in healthy Beagle dogs (n=16; 8 males, 8 females; 3.8 to 9.6 years) following a 10-minute car ride, a model of situational anxiety. Post-hoc analyses were conducted to ascertain the potential relationship between anandamide (a plasma endocannabinoid) and serum cortisol (a biomarker of stress).

Subjects were dosed with one of three cannabinoid formulations or placebo approximately 4 hours (± 10 minutes) prior to the car ride. A four-arm Williams design was used (n=4 dogs/group), balanced for sex and serum cortisol (as determined from a preliminary car ride). The car ride route and environment were identical for each subject, with speed, noise, and temperature kept as consistent as possible and car rides occurring at the same time of day for each animal. There was a seven-day washout between treatments. Blood collected one day prior to and 7 (± 2) minutes after each car ride was analyzed for cortisol and anandamide.

Subjects showed a robust change in blood cortisol levels when exposed to the car ride, with post-car ride cortisol levels showing two to three-fold increases over pre-car ride levels ($p<0.001$), validating the car ride as a model of situational anxiety. Post-car ride plasma levels of anandamide were also found to be significantly increased over pre-car ride levels (32 to 62%; $p<0.001$) across the four treatments. Post-car ride cortisol and anandamide levels were positively correlated for all treatment groups ($r=0.33$ to 0.70) and the correlation achieved statistical significance for two of the three cannabinoid formulations ($r=0.56$ and 0.70). Hill et al. (2013) similarly found anandamide levels to positively and significantly correlate with circulating cortisol in humans following exposure to a stressor ($r=0.35$; $p=0.02$) (Hill et al., Psychoneuroendocrinology 38 (2013) 2952-2961). While our data demonstrate the responsiveness of the ECS to a stressful stimulus in dogs, both the cortisol and anandamide responses decreased over repeated testing, indicating a partial adaption effect. This study also showed potential sex-dependent ECS-mediated responses to our anxiety model. We observed sex-related differences in cortisol response following acute cannabinoid treatment and a greater anandamide response to stress in males, although additional studies are necessary to confirm these observations.

INVESTIGATING THE RELATIONSHIP BETWEEN INFLAMMATION AND ENDOCANNABINOIDS IN THE AMYGDALA DURING COLITIS

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There is a well-established link between chronic inflammatory diseases (e.g. arthritis, inflammatory bowel disease) and stress-associated psychiatric disorders (e.g. depression, anxiety disorders). To date, the mechanisms underlying this interplay are not fully elucidated. In order to investigate these mechanisms, we utilized a rodent model of inflammatory bowel disease (colitis), induced with intracolonic administration of 2,4,6-trinitrobenzene sulfonic acid, which resembles Crohn's Disease, in adult male, Sprague Dawley rats. We have previously shown that there is a reduction in the levels of one of the endogenous cannabinoids, anandamide (AEA), driven by a corticotropin releasing factor receptor 1 (CRF-R1)-induced increase in its metabolic enzyme, fatty acid amide hydrolase (FAAH), seven days following the onset of colitis. Furthermore, we have shown that the anxiety-like behaviour comorbid with colitis at seven days can be reversed by acutely, centrally augmenting AEA levels.

In this study we examined whether cytokines and chemokines in the amygdala are potential mediators of these effects. We investigated the expression of a number of inflammatory mediators and saw a significant ($p<0.05$) increase in the expression of COX-1, COX-2, PPARα, a trending ($p<0.1$) increase in IL-1RA and IL-6 expression levels, and a trending reduction in the expression C reactive protein and IL-12, but no changes in CX3CL1, G-CSF, GM-CSF, IFNα, IFNγ, IL-1α, IL-1β, IL-2, IL-17A, iNOS, MIP-1a, MIP-2, MCP-1, NFκB, PPARγ, SOCS3, TLR4 and TNFα. We also observed an increase in the protein levels of key proinflammatory mediators (i.e. IL-1β, IL-6, and MCP-1) at three days following the onset of colitis, with no changes in these cytokines at seven days following colitis onset. Interestingly, this increase occurs prior to the reduction of AEA levels in the amygdala, which we had previously demonstrated occurs at seven days following colitis onset, but not at three days. These data suggest the possibility of the increase in inflammatory mediators may contribute to the alterations in endocannabinoid levels seen during colitis.

We are currently investigating the relevance of this increase in inflammatory mediator production to the increase in FAAH activity and reduction of AEA levels necessary for colitis-induced anxiety. Furthermore, we are looking into whether boosting endocannabinoid levels for the duration of colitis will both reverse the inflammation, as well as the increase in anxiety-like behaviour. Our work will contribute to an understanding of the relationship between changes in inflammatory mediators and the endocannabinoid cascade leading to the increase in anxiety-like behaviour comorbid with colitis. This work adds to our understanding of the mechanism behind anxiety behavior alterations during chronic inflammatory conditions, providing a potential two-pronged strategy of using cannabinoids as an approach for treating inflammatory conditions, as well as the ensuing changes in mental health.

CANNABIS AS A COMPLEMENTARY PALLIATIVE CARE TREATMENT FOR CANCER PATIENTS: EXPLORATORY ANALYSIS OF DATA FROM THE CANNABIS PILOT PROJECT OF THE MCGILL UNIVERSITY HEALTH CENTRE

[1]MariaLuisa Vigano*, [2]Rihab Gamaoun, [2]Popi Kasvis and [2]Antonio Vigano

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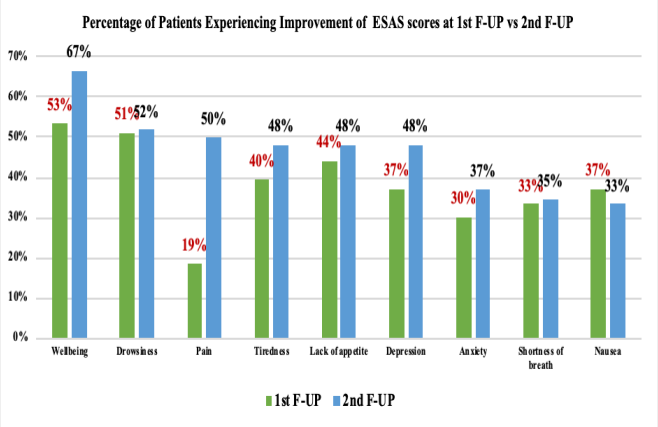
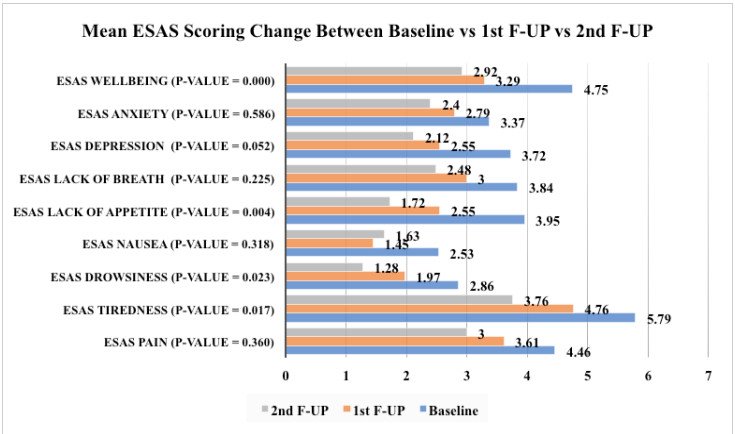
Access to cannabinoids-based medicine (CBM) is a common request by patients and caregivers in oncology, palliative care and chronic pain clinics worldwide. However, its integration in most clinical settings has been challenged by social stigma, poor academic training and significant research gaps. To address the above challenges, the Cannabis Pilot Project (CPP) was co-funded by Cedars Cancer Foundation and the Rossy Cancer Network and implemented between January and August 2018 at the Cancer Mission of the McGill University Health Centre. The goal of the CPP was to pilot access to medical cannabis as a complementary tool for symptom control in cancer patients. Referral to the CPP was reserved for patients who were already receiving supportive and palliative care but did not achieve adequate symptom relief. The CPP was based on a model at the private medical cannabis clinic, Sante Cannabis, in Montreal and included involvement of three roles of healthcare professionals: physician, nurse educator and research coordinator. We determined safety and efficacy of CBM for symptom relief in cancer patients who were part of the Cannabis Pilot Project (CPP) of the McGill University Centre, between January and September 2018.

Cannabis treatment seemed to improve all ESAS (Edmonton Symptom Assessment System) indicators over time, and in a significant way, for well-being ($p < 0.001$), tiredness ($p<0.05$), drowsiness ($p<0.05$), and lack of appetite ($p<0.005$). During follow-ups, over two thirds of patients reported improvement by at least 1 point on a 0-10 scale in well-being, whereas around half of the sample reported improvement in drowsiness, pain, tiredness, depression and appetite. Patients reported better results in the second follow-up as compared to the first one. Around 15% of patients reported mild adverse events at both follow-ups (i.e. light headedness). This study provides some initial evidence on the role of medical cannabis for improving symptom burden in cancer patients. Future study should confirm the relevance of CBM as a complementary therapeutic option in cancer care through bigger sample sizes as well as through more detailed accounts of cannabis doses and impact on the concomitant use of medications.





POTENTIAL IMPACT OF MEDICAL CANNABIS TREATMENT ON PAIN CONTROL AMONG CANCER PATIENTS IN QUEBEC – CANADA: THE CANNABIS PILOT PROJECT AT THE MCGILL UNIVERSITY HEALTH CENTRE

Rihab Gamaoun[123], Popi Kasvis[12], Maria-Fernanda Arboleda[3], Gligorka Raskovic[4], Filareti Patronidis[2] and Antonio Vigano*[12]

[1] Supportive and Palliative Care Division, McGill University Health Centre
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Therapeutic applications of medical cannabis within the cancer population, particularly for chemotherapy induced peripheral neuropathy, and how this complementary approach can impact patients' quality of life are still under-investigated.

The Cannabis Pilot Project (CPP) accepted McGill University Health Centre cancer patients already receiving supportive care but referred to the CPP because they did not achieve adequate symptom relief. This study examines the efficacy of cannabis treatment for pain relief using the Brief Pain Inventory (BPI) scale. An interdisciplinary team was established to systematically assess patients, prescribe and monitor cannabis treatments.

Sixty-five patients have been enrolled (mean age 61 years; 52% female) in the CPP over seven months. At baseline, more than a third of participants with at least one follow-up had reported neuropathic pain, 62% had reported a previous cannabis experience, 36% were prescribed (natural) medical cannabis and 64% pharmaceutical (Nabilone) along with medical cannabis.

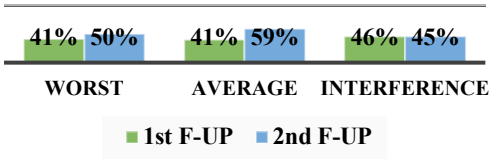
Worst pain, average pain and pain interference improved by at least 1 point on the BPI between the baseline, first (n=45) and second (n= 27) follow-up in 50%, 59% and 46% of patients respectively.

Across baseline and follow-ups, a significant difference ($p<0.05$) was found for worst pain means, whereas a strong statistical trend was found for differences in pain interference mean values ($p=0.06$).

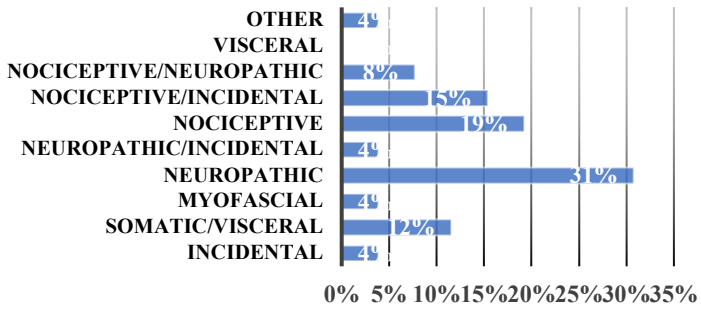
Approximately 15% of patients reported mild adverse events at both follow-ups (i.e. light headedness in the morning).

Cannabis treatment seems to be safe and effective for cancer pain improvement. Studies with larger sample size will allow for examination of associations between the type and dose of cannabis (CBD / THC ratio) and pain relief.

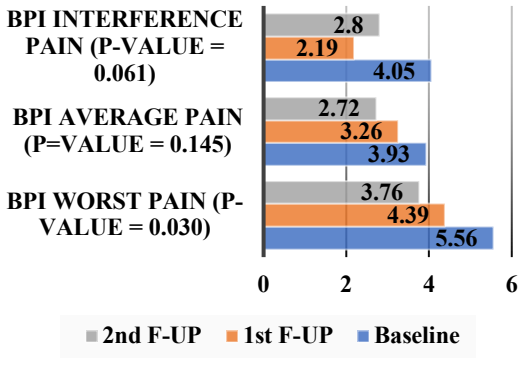
Percentage of Patients Experiencing Improvement of BPI scores at 1st F-UP vs 2nd F-UP



Type of Pain Among Patients with at Least 1 F-UP



Mean BPI Scoring Change Between Baseline vs 1st F-UP vs 2nd F-UP



THE EFFECT OF MEDICAL CANNABIS ON APPETITE IN CANCER ANOREXIA: POTENTIAL MECHANISM OF ACTION AND PRELIMINARY RESULTS

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Cancer anorexia (CA) affects between 40-60% of all cancer patients, and 50-80% advanced cancer patients. Among the most promising therapeutic agents for CA are cannabinoids.

Cannabis, particularly delta-9-tetrahydrocannabinol, acts on the CB1 cannabinoid receptor to stimulate appetite, partly through the modulation of ghrelin production. Ghrelin levels increase and Peptide YY (PYY) levels, an anorexigenic peptide released by gastrointestinal mucosa, decrease after smoking cannabis in HIV patients. Cannabis-related changes in these hormones have a magnitude similar to what is observed with food intake over the course of a day in healthy volunteers, suggesting physiological relevance.

We have recently demonstrated positive results in appetite improvement with medical cannabis use from the Cannabis Pilot Project (CPP) of the McGill University Health Centre. CPP patients have already received supportive care however have not achieved adequate symptom relief with conventional treatments. The Edmonton Symptom Assessment System (ESAS) questionnaire was completed at baseline (BL), visit 1 (>30-75 days after BL) and visit 2 (>75-120 days after BL) to determine improvement in appetite. Weight was available at each visit in a subset of patients. Of the 37 patients (mean age 61±11 y, 51% female) who were assessed at BL, 43% reported anorexia as a symptom. Synthetic cannabis was prescribed to 62% of patients. The majority of patients (81%) were prescribed oral cannabis (oil), with 51% receiving Cannabidiol-rich products. There was a significant improvement in appetite over the 3 visits (BL: 3.5±3.0; visit 1: 2.2±2.4; visit 2: 1.5±2.2, p=0.033) (Figure 1). Of patients who reported anorexia as a symptom, 75% reported improvement at visit 1, and 80% at visit 2. Weight remained unchanged over time (BL: 70.7±19.3 kg; visit 1: 67.4±21.5 kg; visit 2: 66.1±23.0 kg, p=0.509) (Figure 2).

Medical cannabis, in addition to standard supportive care, seems to improve appetite and stabilize weight over time in cancer patients.

Figure 1:

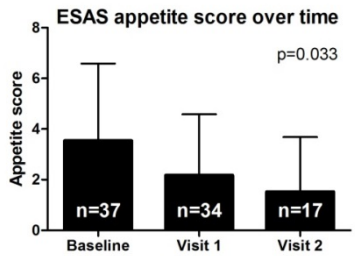
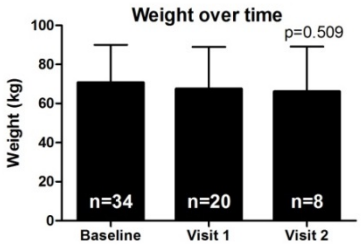


Figure 2:



ENDOCANNABINOID SIGNALING AFFECTS PINCEAU STRUCTURE

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Endocannabinoids (eCB's) have been implicated as key regulators of excitatory synaptic plasticity in the cerebellum, however their role in the regulation of development and remodeling of inhibitory cerebellar synapses has not yet been studied in detail.

Cerebellar Purkinje cells (PCs) are the primary cerebellar co-incidence detectors, integrating all excitatory inputs and generating the pattern of cerebellar output. The basket cells (BCs), inhibitory cerebellar interneurons, form synapses around the somata (i.e. the pericellular basket) and the axon initial segment (i.e. the pinceau synapse) of PCs, in key positions to control the pattern of PC activity, and consequently cerebellar output. However, very little is known about molecular mechanisms regulating activity and plasticity of BC/PC synapses.

Some excitatory synapses exhibit robust structural changes in response to changing patterns of activity both during development and in the adult. For example, hippocampal mossy fibers can sprout new boutons and additional active zones in response to learning. Much less is known about structural plasticity at inhibitory synapses. In this study, we set out to investigate whether eCB signaling is involved in pinceau development or structural plasticity.

First, we observed correlation between the size of the pinceau synapses and performance of cerebellar-regulated motor behaviors. Furthermore, average pinceau size is increased in mice following enriching life experiences, suggesting that the pinceau may undergo activity-dependent structural plasticity. Second, our results demonstrate that rCB1 is prominently expressed in the mature cerebellum in BC axons and in the pericellular and pinceau presynaptic compartments, while DAGLa, an enzyme involved in the synthesis of 2-AG, is expressed in the postsynaptic compartment – PC dendrites, somata, and the axon initial segment, suggesting that eCBs are likely to act as a retrograde signal in those synapses. Third, we show structural alterations of the pinceau synapses in CB1 knockout mice, suggesting that eCB signaling may be involved in the regulation of BC/PC synaptic development or structural plasticity. Finally, we observed structural changes at the pinceau in adult mice following developmental exposure to cannabis.

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ENDOCANNABINOID SIGNALING REGULATES CEREBELLAR BEHAVIORS

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The cerebellum plays an important role in automation and sequencing of motor, cognitive and emotional behaviors including language, attention, anxiety, and reward. Cerebellar dysfunction is implicated in a broad range of clinical deficits including motor disorders, autism spectrum, and schizophrenia. Phytocannabinoid exposure can lead to shifts in perception, cognition, and motor and coordination. Interestingly, some of the behaviors that phytocannabinoid exposure alters include cerebellum-mediated behaviors such as time perception, gait and emotional regulation. In rodents, exposure to phytocannabinoids has been shown to produce anxiogenic effects and defects in learning and memory. Cannabinoid receptor 1 (CB1) is expressed at high levels in human and rodent cerebella, however its role in cerebellar behavioral regulation is not well understood.

In this study, we set out to investigate the effects of brief developmental exposure to phytocannabinoids on cerebellar function in the adult mice. We show that brief exposure to low doses of delta-9-tetrahydrocannabinol (THC) during the second postnatal week in mice leads to alterations in cerebellar-controlled behaviors in a sex-dependent manner. Additionally, we explored the interactions between developmental and adult intermittent exposure to THC, and observed cumulative effects on gait.

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THE ROLE OF CANNABINOID RECEPTOR MEDIATION OF OPIOID INDUCED RESPIRATORY DEPRESSION IN THE PREBÖTZINGER COMPLEX

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The scope of the opioid epidemic remains a massive problem in the United States and North America with accidental overdose fatalities at an all-time high despite decades of research on alternative means to address opioid use disorder (OUD). Overdose fatalities occur from activation of mu opioid receptors (MOR) within the preBötzinger complex of the medulla causing respiratory depression and resulting in death. Considering that opioids are prescribed for pain at alarmingly high rates, and 80% of global opioids are consumed by Americans, it is imperative that research is done on novel medications for pain relief that do not induce respiratory depression. Recent studies have shown that cannabis and cannabinoids may hold the key as a viable analgesic, on their own or in combination with opioids. As more states legalize cannabis for medicinal and recreational use, it is important to understand the mechanisms of action of cannabinoids on respiratory depression. In this study we sought to understand the impact of cannabinoid 1 receptor (CB1R) and cannabinoid 2 receptor (CB2R) activation on respiratory depression using both peripherally restricted or brain-penetrant CB1/CB2 agonists alone and in combination with morphine. Results suggest that CB1 and CB2 activation do not induce respiratory depression on their own. In addition, CB2 activation can mitigate opioid induced respiration depression. Importantly, these findings suggest that prevention of morphine induced respiratory depression with a cannabinoid requires a brain penetrant compound. These data support further research of cannabinoids as potential therapeutic analgesics/adjuncts in the face of the opioid epidemic.

THE ROLE OF CANNABINOID RECEPTOR 1 (CB_1R) IN ALVEOLAR TYPE-2 EPITHELIAL AND MYELOID CELLS IN EXPERIMENTAL LUNG FIBROSIS DEVELOPMENT

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Pulmonary fibrosis (PF) is a progressive and life-threatening disease characterized by scarring of lung tissue. While its pathophysiology involves complex and multicellular interactions, the contribution of distinct cell populations to disease progression is not well understood. Recent studies have reported upregulation of CB_1R activity in PF (*Cinar et al., JCI Insight 2017 2(8):92281*). Significant increases in anandamide (AEA) levels are a feature of human Idiopathic-PF (IPF) as well as experimental mouse models of PF, and type II alveolar (AT2) cells and alveolar macrophages (AM), both of which express CB_1R, may be AEA targets pertinent to PF pathogenesis.

The present study explored the relationship between CB_1R expression in AT2 and myeloid cells, and lung function and survival in PF. This was accomplished using mice with cell-type-specific, tamoxifen-induced knockout of CB_1R in AT2 cells, or germline knockout of myeloid cells, respectively generated though breeding Cnr1 *flox/flox* mice with Sftpc-$CreER^{t2}$ and LysM-Cre mice. Mice were of a C57BL/6J background. PF was induced in cell-specific KO mice via oropharyngeal instillation of bleomycin (1U/kg), and likewise induced in WT and global Cnr1 knockout mice for purpose of comparison. Lung function and respiratory system mechanics were monitored using the flexiVent FX system.

Twenty-eight days after bleomycin instillation, WT mice had a mortality rate of 60%, while both global Cnr1 KO ($Cnr1^{-/-}$) as well as myeloid-specific Cnr1 KO (My-$Cnr1^{-/-}$) mice had 0% mortality. The mortality rate in AT2-specific Cnr1 KO (AT2-$Cnr1^{-/-}$) mice was 40%. At the same time point, bleomycin-exposed WT mice experienced significant decline in lung function as reflected by pressure-volume (PV) curve, lung tissue elastance (Stiffness index) and tissue damping (peripheral airway resistance). In $Cnr1^{-/-}$ and My-$Cnr1^{-/-}$ mice, bleomycin failed to induce a decline in lung tissue elastance and tissue damping. The PV curve was also normal in $Cnr1^{-/-}$ mice, whereas a partial decline was observed in My-$Cnr1^{-/-}$ mice. Pulmonary function parameters in AT2-$Cnr1^{-/-}$ mice were similar to those observed in WT mice.

These findings indicate a critical involvement of myeloid CB_1Rs in the fibroproliferative process of PF, and a lesser but significant involvement of AT2 CB_1Rs. These results bolster confidence in the therapeutic potential of peripheral CB_1R antagonists for PF and warrant further exploration of the role of CB_1Rs in different cell types in PF pathology.

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BIODISTRIBUTIONS AND FUNCTIONS OF ENDOCANNABINOIDS ACROSS THE BLOOD BRAIN BARRIER UNDER NORMAL AND ISCHEMIC CONDITIONS

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The cannabinoid receptors and endocannabinoid system (ECS) was originally discovered in the brain, and then in other organs through the human body. To systematically investigate the ECS of the blood brain barrier (BBB), we confirmed the presence of CB1 and CB2 receptors in a human brain microvascular endothelia cell line (HBMEC), and quantified the basal level of endocannabinoids (eCBs), anandamide (AEA) and 2-arachidonoylglycerol (2AG), under normal and pathological conditions. We hypothesized that endocannabinoids are partially permeable across the BBB, although they are subjects of dynamic metabolism at the same time. Furthermore, eCBs may protect the BBB physical integrity against pathological stress such as ischemia, by activating the CB1 and CB2 receptors. Thus, the eCB levels in the brain and the blood circulation may both regulate through the ECS and impact the BBB integrity and functions.

To study the biodistribution of eCBs, isotope-labeled AEA and 2AG were added to the BBB model on transwells, and their diffusion across the HBMEC monolayer was quantified by LCMS. Significant amount of AEA and 2AG were found permeated through the BBB model in 6 hours. The impact of AEA and 2AG on endothelial membrane integrity and cellular functions were assessed by trans-endothelial electronic resistant (TEER) and other cellular assays, under normal and ischemic conditions. Increased TEER was found within 6 hours, and was concentration dependent. The expression of CB1 and CB2 of human brain microvascular endothelia cell line (HBMEC) were quantified and monitored by RT-PCR, which found CB1 expressed in higher level than CB2 in the cell line. Under ischemic stresses, the expression of CB1 and CB2 generally decreased, but recovered within 24 hours. Our preliminary results confirmed the presence of the ECS on the BBB, and endocannabinoids can be partially permeable through the BBB. The distribution of eCB across the BBB may be critical to maintain the integrity and functions of the BBB, including its permeability and barrier function. Upon these preliminary findings, further studies on the distribution of eCB components, their protective role on BBB integrity, and their mechanism of action through the CB receptors are underway.