28TH ANNUAL

SYMPOSIUM

OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

LEIDEN

NETHERLANDS

JUNE 30 – JULY 5, 2018

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Symposium Programming by Cortical Systematics LLC

International Cannabinoid Research Society
Research Triangle Park, NC
USA

ISBN: 978-604-0-00008-8

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

Author(s), Abstract Title (2018) 28th Annual Symposium on the Cannabinoids, International Cannabinoid Research Society, Research Triangle Park, NC, USA, Page #.

Funding for this conference was made possible in part by grant 2R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

2018 Symposium on the Cannabinoids

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Registration: June 30th, 2018 (16.00 – 18.00)
Leidse Schouwburg Stadsgehoorzaal

Welcome Reception

Day 1
Sunday, July 1st

8.45	Welcome and Opening Remarks		
Oral Session 1. Cannabis Evolution and Chemovars Chairs: John McPartland and Jordyn Stuart			
9.00	John M. McPartland* and Geoffrey W. Guy	THE NATIVE RANGE OF *CANNABIS SATIVA* AND ITS CENTER OF ORIGIN IN ASIA, PRIMARILY BASED ON FOSSIL POLLEN DATA	1
9.15	Michel McElroy, Hugo Maassen, Arno Hazekamp and Sean Myles*	CANNABIS RECLASSIFIED: REDEFINING 'INDICA' AND 'SATIVA' BY GENETICS AND CHEMISTRY	2
9.30	Mallory Loflin*, Philippe Lucas, Joshua Eades, Graham Eglit, Ryan Vandrey and Marcel O. Bonn-Miller	CANNABIS TERPENE PROFILES PARTIALLY ASSOCIATED WITH C. SATIVA/C. INDICA/HYBRID CLASSIFICATION	3
9.45	Kayla M. Hardwick and Alisha K. Holloway*	EVOLUTION OF CANNABINOID SYNTHASE PATHWAY GENES IN LANDRACE VARIETIES AND DRUG CULTIVARS	4
10.00	Malcolm Kavarana*, Richard Peet, Yuri Khmelnitsky and Peter Michels	CHARACTERIZATION OF BIOCATALYTICALLY PRODUCED CANNABINOIDS AND CANNABINOID PRODRUGS	5

10.15	**COFFEE BREAK**		
ORAL SESSION 2. SYNTHETIC CANNABINOIDS CHAIRS: CHRIS BREIVOGEL AND DAN MORGAN			
10.45	Samuel D. Banister*, Richard C. Kevin, Christa MacDonald, Rochelle Boyd, Michelle Glass, Mark Connor, Roy R. Gerona and Iain S. McGregor	STRUCTURE-ACTIVITY RELATIONSHIPS OF EMERGENT SYNTHETIC CANNABINOID NEW PSYCHOACTIVE SUBSTANCES AND THEIR PROPHETIC ANALOGUES	6
11.00	Igor Spigelman*, Yatendra Mulpuri, Brian. L. Schmidt, Todd W. Vanderah, Hong Zhang, Tally M. Largent-Milnes and Herbert H. Seltzman	SYNTHETIC CANNABINOID SUPPRESSES CANCER AND CISPLATIN-INDUCED NEUROPATHIC PAIN SYMPTOMS BY PERIPHERAL CB1 RECEPTOR ACTIVATION	7
11.15	Zoltan V. Varga*, Katalin Erdelyi, Resat Cinar, Raphael Mechoulam, George Kunos and Pal Pacher	THE SELECTIVE CB2-R AGONIST, HU-910 ATTENUATES HEPATORENAL SYNDROME DEVELOPMENT IN A MOUSE MODEL OF LIVER FAILURE	8
11.30	U. Grether*, S. M. Ametamey, K. Atz, E. M. Carreira, C. Davies, J. Fingerle, T. Gazzi, L. Gobbi, W. Guba, A. Haider, T. Hartung, L. Heitman, M. Honer, A. Ijzerman, A. Kimbara, J. Kretz, A. Martella, R. E. Martin, A. Mason, M. Nazare, M. Nettekoven, P. Pacher, A. de Paepe, A. Pedrina-McCarthy, M. Rogers-Evans, E. Roome, S. Röver, A. Rufer, R. Sarott, M. Soethoudt, C. Ullmer, M. van der Stelt, Z. Varga, D. B. Veprintsev and M. Westphal	RECENT PROGRESS TOWARD ENHANCED CHEMICAL PROBES FOR STUDYING THE CANNABINOID RECEPTOR 2	9

11.45	Marjolein Soethoudt*, Sara C. Stolze, Matthias V. Westphal, Luuk van Stralen, Andrea Martella, Eva J. van Rooden, Wolfgang Guba, Zoltan V. Varga, Hui Deng, Sander I. van Kasteren, Uwe Grether, Adriaan P. IJzerman, Pal Pacher, Erick M. Carreira, Herman S. Overkleeft, Andreea Ioan-Facsinay, Laura H. Heitman and Mario van der Stelt	A SELECTIVE PHOTOAFFINITY PROBE ENABLES ASSESSMENT OF CANNABINOID CB2 RECEPTOR EXPRESSION AND LIGAND ENGAGEMENT IN HUMAN CELLS	10
12.00	**LUNCH**		
12.00	**NIDA NETWORKING SESSION**		
13.30 - 14.30	**ICRS LIFETIME ACHIEVEMENT AWARD** CB2 RECEPTORS IN THE CNS **CECILIA J. HILLARD, PH.D.** **Professor, Director of the Neuroscience Research Center** Medical College of Wisconsin Milwaukee, WI USA		
ORAL SESSION 3. CANNABIDIOL CHAIRS: HEATHER BRADSHAW AND NETA RIMMERMAN			
14.30	Daniel Couch*, Catherine Ortori, David Barrett, Jon Lund and Saoirse O'Sullivan	THE SWEETPEA STUDY: A RANDOMISED DOUBLE BLIND CONTROLLED TRIAL EXAMINING THE EFFECT OF PEA AND CBD ON THE PERMEABILITY OF THE HUMAN GUT *IN VIVO*	11

14.45	Moshe Yeshurun, Oren Pasvolsk, Pia Raanani, Sari Prutchi-Sagi* and Liat Shargian	CANNABIDIOL – AN INNOVATIVE STRATEGY FOR THE TREATMENT OF GRAFT VERSUS HOST DISEASE (GVHD)	12
15.00	Ashleigh L. Osborne*, Nadia Solowij, Jeremy S. Lum, Ilijana Babic, Kelly A. Newell, Xu-Feng Huang and Katrina Weston-Green	MECHANISMS UNDERLYING THE PRO-COGNITIVE EFFECTS OF CANNABIDIOL: INSIGHTS FROM THE RAT BRAIN AND SEX-SPECIFIC IMPLICATIONS	13
15.15	Emma Leishman*, Sally Miller, Ken Mackie and Heather B Bradshaw	WIDESPREAD EFFECTS OF ACUTE CBD ON THE ENDOGENOUS CANNABINOID RELATED LIPIDOME IN 8 BRAIN REGIONS OF WT AND NAPE-PLD KO MICE	14
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16.00 – 18.00	**POSTER SESSION 1** RECEPTION		P1

Notes:

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Day 2
Monday, July 2nd

8.45	Opening Remarks		
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9.00	David J. Marcus, Gaurav Bedse, Andrew Gaulden, Andre Haymer and Sachin Patel*	PHYSIOLOGY AND FUNCTION OF AMYGDALO-CORTICAL ENDOCANNABINOID SIGNALING	15
9.15	Roger Hudson*, Walter Rushlow and Steven R. Laviolette	DELTA-9-TETRAHYDROCANNABINOL AND CANNABIDIOL PRODUCE DISTINCT EFFECTS ON EMOTIONAL MEMORY FORMATION AND SALIENCE ATTRIBUTION THROUGH ACTIONS IN THE VENTRAL HIPPOCAMPUS	16
9.30	Elizabeth Hay*, Philip Cowie, Roy Gray, Ruth Ross, Perry Barrett, Roger Pertwee and Alasdair MacKenzie	EXPLORING THE EFFECTS OF GENETIC AND EPIGENETIC VARIATION ON CANNABINOID RECEPTOR-1 GENE EXPRESSION; IMPLICATIONS FOR PERSONALISED AND STRATIFIED MEDICINE	17
9.45	Mariam Alaverdashvili*, Quentin Greba, Michael Anderson, Andrew J. Roebuck, Wendie N. Marks, Sumanta Garai, Terrance P. Snutch, Ganesh A. Thakur, John G. Howland and Robert B. Laprairie	EFFECTS OF THE TYPE 1 CANNABINOID RECEPTOR POSITIVE ALLOSTERIC MODULATOR GAT211 ON ABSENCE SEIZURES AND THE ANXIETY-LIKE PHENOTYPE OF GENETIC ABSENCE EPILEPSY RATS FROM STRASBOURG	18
10.00	Nageeb Hassan*, Victoria Elizabeth Mackey, Courtney Clarke, Jordan Osmond, Sarah Bugden, Shannon Waye, Joshua Dean Conway and Francis Rodriguez Bambico	PREFRONTOCORTICAL ENDOCANNABINOID-CB1 TRANSMISSION MEDIATES THE ANTIDEPRESSANT-LIKE BEHAVIOURAL AND NEUROPLASTIC EFFECTS OF STRESS CONTROLLABILITY	19

10.15	**COFFEE BREAK**		
ORAL SESSION 5. DEVELOPMENT AND DEVELOPMENTAL DISORDERS CHAIRS: SAOIRSE O'SULLIVAN AND STEVE ALEXANDER			
10.45	Collin R. Warrick, Hayden R. Wright, Janelle M. Lugo and Ryan J. McLaughlin*	MATERNAL CANNABIS VAPOR EXPOSURE DOSE-DEPENDENTLY IMPAIRS BEHAVIORAL FLEXIBILITY IN ADULT OFFSPRING	20
11.00	Vanessa Montoya-Uribe*, Cun Li, Stacy Martinez, Peter Nathanielsz and Natalia Schlabritz-Loutsevitch	FETAL HEPATIC AND PLACENTAL CANNABINOID RECEPTOR 1 (CB1a AND CB1b) TRANSCRIPT VARIANTS IN MATERNAL UNDERNUTRITION	21
11.15	S. Beggiato*, R. Schwarcz and L. Ferraro	GESTATIONAL CANNABINOID EXPOSURE INFLUENCES EXTRACELLULAR KYNURENIC ACID AND GLUTAMATE LEVELS IN THE MEDIAL PREFRONTAL CORTEX OF ADOLESCENT OFFSPRING	22
11.30	Dilara Bahceci*, Nicole A. Hawkins, Lyndsey L. Anderson, Jordyn M. Stuart, Iain S. McGregor, Jennifer A. Kearney and Jonathon C. Arnold	ENDOCANNABINOID SYSTEM-RELATED GENETIC MODIFIERS IN A MOUSE MODEL OF DRAVET SYNDROME	23
11.45	Lyndsey L. Anderson*, Ivan K. Low, Iain S. McGregor and Jonathon C. Arnold	ANTICONVULSANT EFFICACY OF PHYTOCANNABINOIDS IN A MOUSE MODEL OF DRAVET SYNDROME	24
12.00	**LUNCH**		

12.30	INDUSTRY BREAKOUTS		
13.30 - 14.00	**ICRS YOUNG INVESTIGATOR AWARD** CHEMICAL TOOLS TO STUDY ENDOCANNABINOID BIOSYNTHESIS AND METABOLISM **MARIO VAN DER STELT, PH.D.** **Professor of Molecular Physiology** Leiden University Leiden, Netherlands		
ORAL SESSION 6. ENDOCANNABINOID REGULATION AND SIGNALING CHAIRS: JACKIE BLANKMAN AND EMMA LEISHMAN			
14.00	Vasudev Kantae*, Annelot C. M. van Esbroeck, Floor Stevens, Rob C. van Wijk, Amy Harms, Piet H. Van der Graaf, Mario van der Stelt and Thomas Hankemeier	SYSTEMS PHARMACOLOGY OF AN ENDOCANNABINOID SYSTEM MODULATOR IN ZEBRAFISH LARVAE	25
14.15	Kata Kenesei, Benjámin Barti, Marco Ledri, Barna Dudok, Judit Glavinics, Vivien Miczán and István Katona*	QUALITATIVE AND QUANTITATIVE PROPERTIES OF TONIC CANNABINOID SIGNALING AT HIPPOCAMPAL GABAERGIC SYNAPSES	26
14.30	Saja Baraghithy*, Reem Smoum, Adi Drori, Rivka Hadar, Asaad Gammal, Shira Hirsch, Malka Attar-Namdar, Alina Nemirovski, Yankel Gabet, Yshaia Langer, Yehuda Pollak, Megan E Rech, Christian Patrick Schaaf, Varda Gross-Tsur, Itai Bab, Raphael Mechoulam and Joseph Tam	PRADER-WILLI SYNDROME-INDUCED OSTEOPOROSIS BY MAGEL2 LOSS IS REVERSED BY A NOVEL DERIVATIVE OF OLEOYL SERINE	27
14.45	Christina Kroos, Ahmed Sharaf, Timur A. Yorgan, Leonore Mensching, Sebastian Rading, Marina Scheffold, Lysann Palkowitsch, Nevena Djogo, Barbara Möpps, Thorsten Schinke and Meliha Karsak*	IDENTIFICATION OF P62 AS A NEW PROTEIN INTERACTION PARTNER OF THE CANNABINOID RECEPTOR 2	28

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15.15	Jacqueline L Blankman*, Jason R Clapper, Cassandra Henry, Anna Knize, Aundrea R Coppola, Justin Cisar, Olivia Weber, Micah Niphakis, Evan Friedman, Danielle Bocchino, Nicole White, Vinh Ngo, Forrest Hull, Julianne Hunt, Cheryl Grice, Iain Fraser, Chan Beals, Gary O'Neill and Alan Ezekowitz	PRECLINICAL AND EARLY CLINICAL CHARACTERIZATION OF THE MONOACYGLYCEROL LIPASE INHIBITOR ABX-1431 FOR THE TREATMENT OF NEUROLOGICAL DISORDERS	30
15.30 – 16.00	**FLASH TALKS 2**		F10 – F17
16.00 – 18.00	**POSTER SESSION 2** RECEPTION		P2
18.00	**BUSINESS MEETING**		

Notes:

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Day 3
Tuesday, July 3rd

8.45	Opening Remarks		
Oral Session 7. Immune System Chairs: Sharon Anavi-Goffer and Alex Straiker			
9.00	Petteri Rinne, Raquel Guillamat-Prats, Martina Rami, Larisa Ring, Laura Bindila, Leo-Pekka Lyytikäinen, Emma Raitoharju, Niku Oksala, Terho Lehtimäki, Emiel P.C. van der Vorst and Sabine Steffens*	PALMITOYL-ETHANOLAMIDE PROMOTES A PRO-RESOLVING MACROPHAGE PHENOTYPE AND ATTENUATES ATHEROSCLEROTIC PLAQUE FORMATION IN MICE	31
9.15	Hava Karsenty Avraham*, Othman Benchama, Michael Malamas and Alexandros Makriyannis	FUNCTION OF THE BIOACTIVE LIPID PALMITOYL-ETHANOLAMIDE (PEA) AND ITS HYDROLYZING ENZYME N-ACYLETHANOLAMINE ACID AMIDASE IN TNBC PROGRESSION, INFLAMMATION AND METASTASIS	32
9.30	Carmen Navarrete*, Adela García, Belén Palomares, Leyre Mestre, Miriam Mecha, Ana Feliú, Martin Garrido, Marco A Calzado, M. Luz Bellido, Carmen Guaza and Eduardo Muñoz	EFFECT OF ORAL VCE-004.8, A CANNABIDIOL QUINOL DERIVATIVE, ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS	33
9.45	Alex Straiker, Natalia Murataeva*, Matthew Lashmet and Sally Miller	ENDOCANNABINOIDS AS CHEMOTAXIC REGULATORS OF EARLY STAGE CORNEAL WOUND HEALING	34

10.00	Resat Cinar*, Nathan J. Coffey, Joshua K. Park, Tony Jourdan, Tadafumi Yokoyama, William A. Gahl, May Christine V. Malicdan and George Kunos	OVERACTIVITY OF CB1 RECEPTORS (CB1R) ON MYELOID CELLS PROPAGATE FIBROGENIC MICROENVIRONMENT IN LUNG FIBROSIS	35
10.15	**COFFEE BREAK**		
ORAL SESSION 8. PAIN CHAIRS: JOSÉE GUINDON AND SARA JANE WARD			
10.45	Laura Daily, Michaela Dvorakova, Sally Miller, Anne Gibson, Anaelle Zimmowitch, Emma Leishmann, Heather Bradshaw, Ken Mackie and Alex Straiker*	ACETAMINOPHEN ANTAGONIZES CB1 CANNABINOID SIGNALING AND EXERTS DUAL OPPOSING YET CB1-DEPENDENT EFFECTS ON MURINE INTRAOCULAR PRESSURE	36
11.00	Louise Corcoran*, Darragh Mattimoe, Michelle Roche and David P. Finn	REGULATION OF NOCICEPTIVE BEHAVIOUR AND FEAR-CONDITIONED ANALGESIA BY 2-AG IN THE RAT MEDIAL PREFRONTAL CORTEX	37
11.15	S. Boccella, C. Cristiano, L. Luongo, R. Romano, C. Belardo, M. Iannotta, M. Mazzitelli, D. De Gregorio, F. Guida, I. Marabese, E. Palazzo, V. de Novellis, Luigia Cristino, Vincenzo Di Marzo, A. Calignano and S. Maione*	PALMITOYL-ETHANOLAMIDE, VIA PPAR-ALPHA RECEPTOR, RESTORES THE ALTERED PLASTICITY AND AMELIORATES THE COMPROMISED PAIN-RELATED BEHAVIORS IN THE HIPPOCAMPUS OF NEUROPATHIC MICE	38

11.30	Divya Ramesh*, Emma Leishman, Katherine Bernier, Angela R. Starkweather and Heather Bradshaw	AN EXPLORATORY ANALYSIS OF CIRCULATING ENDOCANNABINOID-RELATED LIPIDOME ASSOCIATED WITH THE TRANSITION FROM ACUTE TO CHRONIC LOW BACK PAIN	39
11.45	Josée Guindon, Henry Blanton, Seth Brauman, Kelsey Donckels and Khalid Benamar*	HORMONALLY DRIVEN SEX-DIFFERENCES AND SPINAL CHANGES IN ENDOCANNABINOID LEVELS IN HIV- 1 GP120-INDUCED NEUROPATHIC PAIN	40
12.00 – 12.30	**ICRS Young Investigator Award** TARGETING FABP5 TO TREAT PAIN, INFLAMMATION, AND CANCER **Martin Kaczocha, Ph.D.** **Assistant Professor** Department of Anesthesiology Stony Brook University, NY USA		
12.30	For Outing Participants – **Box Lunch** For All Other Delegates – **Buffet Lunch**		
14.00 – 17.00	**OUTING**		

Notes:

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Day 4
Wednesday, July 4th

8.45	Opening Remarks		
Oral Session 9. Cannabinoid Interactions with Drugs of Abuse (Ethanol and Opioids) Chairs: Mary Abood and Matt Hill			
9.00	Grzegorz Godlewski*, Resat Cinar, Tony Jourdan, Nathan Coffey, Bani Mukhopadhyay, Jie Liu, Ziyi Liu, Joshua Park, Douglas Osei-Hyiaman, Malliga R. Iyer and George Kunos	PERIPHERAL CANNABINOID CB1 RECEPTOR BLOCKADE REDUCES ETHANOL DRINKING IN MICE VIA THE GUT-BRAIN AXIS, BY LIMITING GHRELIN ACYLATION IN THE STOMACH	41
9.15	Christopher Norris*, Hanna J. Szkudlarek, Brian Pereira and Steven R. Laviolette	Δ^9-TETRAHYDROCANNABINOL (THC) PRODUCES BI-PHASIC REWARDING AND AVERSIVE EFFECTS IN THE ANTERIOR VS. POSTERIOR NUCLEUS ACCUMBENS SHELL THROUGH DISSOCIABLE MU VS. KAPPA OPIATE RECEPTOR MECHANISMS AND DIFFERENTIAL MODULATION OF MEDIUM SPINY NEURON ACTIVITY STATE	42
9.30	Shanna Babalonis,* Michelle R. Lofwall, Paul A. Sloan, Paul A. Nuzzo, Laura C. Fanucchi and Sharon L. Walsh	CANNABINOID MODULATION OF THE ANALGESIC EFFECTS OF OPIOIDS IN HUMANS	43
9.45	Joel E. Schlosburg*, Anush R. Karnati, Leandro Vendruscolo, Benjamin F. Cravatt and George F. Koob	RATS LACKING FATTY ACID AMIDE HYDROLASE (FAAH) ACTIVITY SHOW REDUCED INTAKE AND MOTIVATION IN MODELS OF OPIOID ADDICTION	44

10.00	Sara Jane Ward*, Zachary W Reichenbach, Pattricia Reggio, Dow Hurst, Thomas J. Rogers, William G. Cornwell, Lee-Juan.Liu Chen and Ronald F Tuma	MODULATION OF MORPHINE ANTINOCICEPTIVE EFFECTS AND MU OPIOID RECEPTOR BINDING BY A SELECTIVE CB2 RECEPTOR AGONIST IN MICE	45
10.15	**COFFEE BREAK**		
ORAL SESSION 10. CANNABIS CLINICAL STUDIES CHAIRS: DIVYA RAMESH AND MARK WARE			
10.45	Natasha L. Mason*, Eef L. Theunissen, Nadia R.P.W. Hutten, Desmond Tse and Johannes G. Ramaekers	BRAIN KINETICS OF NEUROTRANSMISSION DURING THC INTOXICATION	46
11.00	Ryan Vandrey*, Marcel O. Bonn-Miller, William E. Fantegrossi, Staci A. Gruber, Nalin Payakachat, Nicolas J. Schlienz, Rosemary T. Smith and Mark A. Ware	DEVELOPMENT OF A CORE ASSESSMENT BATTERY FOR OBSERVATIONAL CANNABIS RESEARCH STUDIES	47
11.15	Angela D. Bryan*, Sophie YorkWilliams, Arielle S. Gillman, Charleen J. Gust, Gregory Giordano, Timothy B. Helmuth, L. Cinnamon Bidwell and Kent E. Hutchison	DOES CANNABIS USE FACILITATE EXERCISE BEHAVIOR AMONG OLDER ADULTS? RESULTS FROM A SUPERVISED EXERCISE INTERVENTION	48
11.30	Elisa Pabon* and Harriet de Wit	FIELD SOBRIETY TEST FOR CANNABIS	49

11.45	Maria-Fernanda Arboleda*, Erin Prosk, Guy Chamberland and Antonio Vigano	THE TRUTH IS IN TITRATION: IMPROVING SAFETY OUTCOMES OF THE WORLD'S FIRST INHALED CANNABIS PRESCRIPTION DRUG	50
12.00	**LUNCH**		
12.30	INDUSTRY BREAKOUTS		
ORAL SESSION 11. METABOLIC REGULATION CHAIRS: PAL PACHER AND HALEY VECCHIARELLI			
13.30	Balsevich G*, Petrie G, Singh A, Sticht M, Chelikani PK and Hill MN	A GENETIC VARIANT OF FATTY ACID AMIDE HYDROLASE (FAAH) EXACERBATES GLUCOCORTICOID-MEDIATED METABOLIC OUTCOMES	51
13.45	Francesca Guida*, Carmela Belardo, Francesca De Filippis, Serena Boccella, Monica Iannotta, Livio Luongo, Fabiana Piscitelli, Ida Marabese, Danilo Ercolini, Vincenzo Di Marzo and Sabatino Maione	ALTERED GUT MICROBIOTA AND ENDOCANNABINOID SYSTEM TONE IN VITAMIN D DEFICIENCY-MEDIATED CHRONIC PAIN	52
14.00	Ya Wang, Diederik Esser, Michiel Balvers, Yong-hao Chen, Sophie Schutte, Jean-Paul Vincken, Harry Gruppen, Lydia A. Afman, Renger F. Witkamp and Jocelijn Meijerink*	POSTPRANDIAL EFFECTS ON ENDOCANNABINOID PLASMA PROFILES AND FAT TISSUE CB1 EXPRESSION WITH A HIGH CALORIE MIXED-MEAL TEST IN OBESE HUMANS UPON A 12 WEEKS RANDOMISED CONTROLLED TRIAL WITH 2 DIFFERENT ENERGY-RESTRICTED DIETS	53
14.15	Isabel González-Mariscal*, Rodrigo A. Montoro and Josephine M. Egan	MUSCLE CANNABINOID 1 RECEPTOR GOVERNS PHYSICAL PERFORMANCE AND WHOLE-BODY METABOLISM	54

14.30 – 15.30	**KANG TSOU MEMORIAL LECTURE** STEM CELL-BASED ORGANOIDS AS AVATARS IN HUMAN DISEASE **HANS CLEVERS, M.D., PH.D.** University Medical Center Utrecht, Utrecht, The Netherlands University of Utrecht, The Netherlands Hubrecht Institute of the Royal Netherlands Academy of Arts, Utrecht, The Netherlands Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands	
15.30 – 16.00	**FLASH TALKS 3**	F18 - F27
16.00 – 18.00	**POSTER SESSION 3**	P3
18.00	RECEPTION **Pieterskerk**	
19.00	**AWARDS CEREMONY** AND **ICRS BANQUET** **Pieterskerk**	

DEPARTURE: **THURSDAY, JULY 5TH**

FLASH TALKS 1

SUNDAY, JULY 1ST: 15:30 - 16:00

Chloe Jordan*, Guo-Hua Bi, Peng Yang, Xiang-Qun Xie and Zheng-Xiong Xi	XIE2-64, A NOVEL CB2 RECEPTOR INVERSE AGONIST, INHIBITS COCAINE SELF-ADMINISTRATION AND OPTOGENETIC BRAIN-STIMULATION REWARD IN RATS AND MICE	F1 [P1-70]
Alì Mokhtar Mahmoud*, Viviana Marolda, Aniello Schiano Moriello, Luciano De Petrocellis, Vincenzo Di Marzo, Roberto Ronca and Alessia Ligresti	CANNABINOIDS AS METABOLIC REPROGRAMING AGENTS IN PROSTATE CANCER CELLS	F2 [P1-71]
Kent E. Hutchison*, L. Cinnamon Bidwell, Sophie YorkWilliams, Reaghan Mueller and Angela D. Bryan	HIGH POTENCY CANNABIS PRODUCTS: PUBLIC HEALTH THREAT OR RED HERRING?	F3 [P1-72]
Georgia Watt, Kani Shang, Magdalena Przybyla, Arne Ittner, Lars Ittner, Hongyun Li, Brett Garner and Tim Karl*	THE THERAPEUTIC POTENTIAL OF CANNABIDIOL (CBD) FOR ALZHEIMER'S DISEASE – A PRECLINICAL PERSPECTIVE	F4 [P1-73]
Philippe Lucas*	CANNABIS SIGNIFICANTLY REDUCES THE USE OF OPIOIDS AND IMPROVES QUALITY OF LIFE IN PATIENTS; PRELIMINARY RESULTS OF A LARGE PROSPECTIVE STUDY	F5 [P1-74]
Stevie C. Britch*, Jenny, L. Wiley and Rebecca M. Craft	CANNABIDIOL AND Δ^9-TETRAHYDROCANNABINOL TREATMENT OF HINDPAW PAIN AND INFLAMMATION IN MALE VS FEMALE RATS	F6 [P1-75]

Dow P. Hurst*, David B. Finlay, Diane L. Lynch, Michelle Glass and Patricia H Reggio	CB1 MUTATIONS TO TEST THE RELEVANCE OF N-TERMINAL RESIDUES F102 AND M103 CONTACT WITH TARANABANT	F7 [P1-76]
Chris Bladen*, Marina Santiago, Mitchell Longworth, Michael Kassiou, Sam Banister and Mark Connor	CHARACTERIZATION AND MODULATION OF HUMAN CAV3.2 CHANNELS BY SYNTHETIC CANNABINOIDS *IN VITRO*	F8 [P1-77]
Chris Breivogel* and Jacob Wells	THE SEIZURE-INDUCING ACTIVITY OF VARIOUS CLASSES OF CANNABINOIDS	F9 [P1-78]

Notes:

Presenting Author*

FLASH TALKS 2

MONDAY, JULY 2ND: 15:30 - 16:00

Hilary A. Marusak*, Craig Peters, Farrah Elrahal, Kyle J. Burghardt and Christine A. Rabinak	GENETIC VARIATION IN ENDOCANNABINOID SIGNALING AND BRAIN AND BEHAVIORAL MECHANISMS UNDERLYING FEAR EXTINCTION IN CHILDREN AND ADOLESCENTS	F10 [P2-75]
Andras Bilkei-Gorzo*, Onder Albayram, Kerstin Michel, Anastasia Piyanova, Mona Dvir-Ginzberg, Ildiko Rácz and Andreas Zimmer	A CHRONIC LOW DOSE OF Δ^9-THC RESTORES FAILING CANNABINOID SIGNALLING AND THUS BRAIN AGEING IN OLD MICE	F11 [P2-76]
Caitlin M. Nealon, Angela N. Henderson-Redmond, David E. Hale, Diana E. Sepulveda, Henry Blanton, Josée Guindon and Daniel J. Morgan*	EVIDENCE FOR AGONIST-SPECIFIC MECHANISMS OF CANNABINOID TOLERANCE IN PATHOLOGICAL PAIN	F12 [P2-77]
Zahir Hussain*, Toru Uyama, Katsuhisa Kawai, Smriti Sultana Binte Mustafiz, Kazuhito Tsuboi, Nobukazu Araki and Natsuo Ueda	CHARACTERIZATION OF CYTOSOLIC PHOSPHOLIPASE A2ε: PHOSPHATIDYLSERINE-STIMULATED PRODUCTION OF N-ACYL-PHOSPHATIDYLETHANOLAMINE	F13 [P2-78]
Lesley D. Schurman*, Terry L. Smith, Linda L. Phillips, Thomas M. Reeves and Aron H. Lichtman	DIACYLGLYCEROL LIPASE-β KNOCKOUT MICE DISPLAY A SURVIVAL PROTECTIVE PHENOTYPE FOLLOWING TRAUMATIC BRAIN INJURY	F14 [P2-79]
Xiaoyan Lin*, Lawrence M. Carey, Julian Romero, Alexandros Makriyannis, Cecilia J. Hillard, Ken Mackie, Philip J. Albrecht, Frank L. Rice and Andrea G. Hohmann	ELUCIDATION OF THE ROLE OF CANNABINOID CB2 RECEPTORS IN MODULATING NEUROPATHIC PAIN USING A CB2 REPORTER MOUSE	F15 [P2-80]
Matthew W. Elmes*, Dale G. Deutsch and Martin Kaczocha	FATTY ACID BINDING PROTEINS REGULATE CANNABINOID METABOLISM	F16 [P2-81]
Szabolcs Dvorácskó*, Attila Keresztes, Adriano Mollica, Ferenc Zádor, Gyöngyi Horváth and Csaba Tömböly	TARGETING THE MU OPIOID AND CANNABINOID RECEPTORS WITH HETEROBIFUNCTIONAL LIGANDS	F17 [P2-82]

FLASH TALKS 3

WEDNESDAY, JULY 4TH: 15:30 - 16:00

Bogna Ignatowska-Jankowska*, Douglas J. Hermes, Changqing Xu, Ian R. Jacobs, Rick B. Meeker, Micah J. Niphakis, Benjamin F. Cravatt, Ken Mackie, Aron H. Lichtman and Sylvia Fitting	INHIBITION OF FAAH PROTECTS AGAINST MICROGLIAL ACTIVATION AND NEUROTOXICITY INDUCED BY HIV-1 TAT PROTEIN IN VITRO	F18 [P3-66]
Baptiste Buisseret*, Owein Guillemot-Legris, Mireille Alhouayek and Giulio G. Muccioli	THE 2-AG METABOLITE PGD2-G DECREASES INFLAMMATORY PAIN IN MICE	F19 [P3-67]
Stephanie Lake*, Thomas Kerr, Daniel Werb, Rebecca Haines-Saah, Benedikt Fischer, Gerald Thomas, Zach Walsh, Mark A. Ware, Evan Wood and M-J Milloy	GUIDELINES FOR PUBLIC HEALTH AND SAFETY METRICS TO EVALUATE THE POTENTIAL HARMS AND BENEFITS OF CANNABIS REGULATION IN CANADA	F20 [P3-68]
Thomas K. Henthorn*, Cristina Sempio and Susan K. Mikulich-Gilbertson	ESTIMATING PLASMA PHARMACOKINETICS OF THC, THC-OH, TCH-COOH AND THC-COOH-GLUCURONIDE	F21 [P3-69]
Diane Bogdan, Jerome Falcone, Martha P. Kanjiya, Sang Hoon Park, Gregory Carbonetti, Keith Studholme, Yong Lu, Matthew W. Elmes, Su Yan, Iwao Ojima, Michelino Puopolo and Martin Kaczocha*	FATTY ACID BINDING PROTEIN 5 INHIBITION SUPPRESSES MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 INDUCTION AND PROSTAGLANDIN E2 BIOSYNTHESIS DURING INFLAMMATION	F22 [P3-70]
Nicholas Lintzeris*, David Allsop, Adrian Dunlop, Anjali Bhardwaj, Llew Mills, Iain McGregor, Jan Copeland and Mark Montebello	FINDINGS FROM A RANDOMISED PLACEBO CONTROLLED TRIAL OF NABIXIMOLS IN THE TREATMENT OF CANNABIS DEPENDENCE	F23 [P3-71]

Adi Drori*, Anna Permyakova and Joseph Tam	CANNABINOID-1 RECEPTOR REGULATES MITOCHONDRIAL DYNAMICS AND FUNCTION IN RENAL PROXIMAL TUBULAR CELLS	F24 [P3-72]
Belén Palomares*, Francisco Ruiz-Pino, Miguel A. Sanchez-Garrido, Martin Garrido, María E. Prados, Xavier Nadal, Carlos Ferreiro-Vera, Gaetano Morello, Giovanni Appendino, Marco A Calzado, Manuel Tena-Sempere and Eduardo Muñoz	TETRAHYDROCANNABINOLIC ACID TARGETS PPARγ AND PREVENTS DIET-INDUCED OBESITY	F25 [P3-73]
Aarón del Pozo Sanz*, María Villa Cruz, Guillermo Pagés Hernando, Carlos Vargas Coronado, María Ceprián Costoso, Wild Hind, José Martínez Orgado and Ana Gutiérrez Rodríguez	CANNABIDIOL REDUCES INTRAVENTRICULAR HAEMORRHAGE EXTENSION AND SECONDARY BRAIN DAMAGE IN NEWBORN RATS	F26 [P3-74]
María Ceprián*, María Villa, Guillermo Pagés, Carlos Vargas, Aarón Del Pozo, Adrián Olmos-Alonso, Will Hind, Rapahel Mechoulam, Javier Fernández-Ruíz, Ana Gutiérrez-Rodríguez, María Ruth Pazos and José Martínez-Orgado	ROLE OF CB2 RECEPTOR IN THE NEUROPROTECTIVE EFFECT OF CBD IN A NEONATAL RAT MODEL OF HYPOXIA ISCHEMIA	F27 [P3-75]

Notes:

Presenting Author*

POSTER SESSION 1

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

Chandni Hindocha*, Tom P Freeman, Meryem Grabski, Jack B Stroud, Holly Crudgington, Alan C Davies, Ravi K Das, Will Lawn, Celia JA Morgan and H Valerie Curran	CANNABIDIOL REVERSES ATTENTIONAL BIAS TO CIGARETTE CUES IN A HUMAN EXPERIMENTAL MODEL OF TOBACCO WITHDRAWAL	P1-1
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Carrie Cuttler*, Alexander Spradlin and Ryan J. McLaughlin	A NATURALISTIC EXAMINATION OF THE PERCEIVED ACUTE EFFECTS OF CANNABIS ON NEGATIVE AFFECT	P1-4
Iman Khuja, Reuven Or and Osnat Almogi-Hazan*	THE ANTI-INFLAMMATORY EFFECT OF CANNABINOIDS ADMINISTRATION IN GRAFT VERSUS HOST DISEASE MAY BE HAMPERED BY SUPPRESSIVE EFFECT ON LYMPHOCYTE RECONSTITUTION – COMPARISON OF Δ^9 -TETRAHYDROCANNABINOL (THC), CANNABIDIOL (CBD) AND CANNABIS EXTRACTS TREATMENT IN BONE MARROW TRANSPLANTATION MURINE MODELS	P1-5
Eef L. Theunissen*, Nadia R. P. W. Hutten, Natasha L. Mason, Stefan W. Toennes, Kim P. C. Kuypers and Johannes G. Ramaekers	NEUROCOGNITION AND SUBJECTIVE EXPERIENCE FOLLOWING ACUTE DOSES OF THE SYNTHETIC CANNABINOID JWH-018: RESPONDERS VERSUS NON-RESPONDERS	P1-6
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Marcus R. Goetz*, Javier Fernández-Ruiz, Bernd L. Fiebich, Laura Garcia-Toscano, María Gómez-Cañas, Oskar Koch, Eduardo Muñoz, Maria R. Pazos and Ulrike Holzgrabe	*IN VITRO* AND *EX VIVO* EFFECTS OF NOVEL SEMI-SYNTHETIC CANNABINOID DERIVATIVES	P1-8
Torsten Lowin* and Georg Pongratz	ACTIVATION OF TRPM3 DECREASES CANNABIDIOL-INDUCED LARGE CATION UPTAKE VIA INTRACELLULAR TRPA1 IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS	P1-9
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Sytze W. Elzinga*, Kevin F. Nolan, Raquel Keledjian and Jeffrey C. Raber	A HIGH-RESOLUTION LC-MS/MS METHOD FOR PESTICIDES IN CANNABIS PRODUCTS	P1-12
Sergiy Tyukhtenko*, Xiaoyu Ma, Kiran Vemuri and Alexandros Makriyannis	RATIONAL MODULATION OF DRUG-TARGET RESIDENCE TIME FOR MONOACYLGLYCEROL LIPASE INHIBITORS	P1-13
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Kushal Gandhi*, Maira Carrillo, Stacy Martinez, Iram P. Rodriguez-Sanchez, Cathy Perez, Edward Dick and Natalia Schlabritz-Loutsevitch	HEPATIC ENDOCANNABINOID SYSTEM (ECS) IN COMMON MARMOSETS (Callithrix jacchus)	P1-15
Erica Wymore*, Claire Palmer, George Sam Wang, Torri Metz, David Bourne, Cristina Sempio, Jost Klawitter and Maya Bunik	EXCRETION AND PERSISTENCE OF MARIJUANA IN HUMAN BREAST MILK	P1-16
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Khalil Eldeeb*, Kassim Traore, Sandra Leone-Kabler and Allyn C. Howlett	THE CANNABINOID 1 RECEPTOR ANTAGONIST O2050 POTENTIAL CARDIOPROTECTIVE EFFECTS IN CARDIOMYOBLAST CELLS	P1-18
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Wajd AlKabbani*, Silvia Alessi-Severini, Shawn Bugden, Ruth Ann Marrie, Paul Daeninck and Christine Leong	PRESCRIPTION CANNABINOID USE IN A CANADIAN PROVINCE: A POPULATION-BASED STUDY (2004-2014)	P1-23

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Qing-Rong Liu*, Nicholas S. Huang, Hong Qu, Jennifer F. O'Connell, Isabel Gonzalez-Mariscal, Sara Santa-Cruz Calvo, Maire E. Doyle, Zheng-Xiong Xi, Yun Wang and Emmanuel S. Onaivi	IDENTIFICATION OF NOVEL MOUSE AND RAT CB1R ISOFORMS AND *IN SILICO* MODELING OF HUMAN CB1R FOR PERIPHERAL CANNABINOID THERAPEUTICS	P1-25
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Daniel J. Farkas*, Jeffrey D. Foss, Joe J. Meissler, Toby K. Eisenstein, Ronald F. Tuma and Sara J. Ward	SINGLE AND COMBINATIVE EFFECTS OF CANNABIDIOL AND β-CARYOPHYLLENE ON THE LIEBER-DECARLI DIET	P1-32

Tiah Lee*, David P. Overy and Cory S. Harris	COMPARISON OF VAPORIZED AND NON-VAPORIZED *CANNABIS SATIVA* L. CHEMICAL COMPLEXITY USING HPLC-DAD AND MS-BASED METABOLOMICS	P1-33
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Richard C. Kevin*, Samuel D. Banister, Rochelle Boyd, Alexander L. Kovach, Brian F. Thomas, Michelle Glass, Mark Connor and Iain S. McGregor	PHARMACOLOGY, TOXICOLOGY, AND THERMAL STABILITY OF EMERGENT SYNTHETIC CANNABINOID 4-CYANO CUMYL-BUTINACA	P1-37
Christopher Stuart Tasker*, Jonathan Lund and Saoirse E. O'Sullivan	THE EFFECTS OF CANNABIDIOLIC ACID (CBDA) COMPARED WITH CANNABIDIOL (CBD) ON HUMAN COLON CANCER CELL PROLIFERATION	P1-38
Marta Bryk*, Jakub Mlost, Alessia Ligresti, Federico Corelli and Katarzyna Starowicz	ANALGESIC EFFECTS OF NEW PYRAZOLYL-PYRIDINE BASED SCAFFOLDS TARGATING CB2 RECEPTORS IN OSTEOATHRITIS-RELATED PAIN	P1-39
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Matthias Winkler*, Bernd L. Fiebich and Marcus R. Goetz	EFFECTS OF NOVEL SYNTHETIC (+)-ENANTIOMERS OF NATURAL OCCURING CANNABINOIDS AND THEIR DERIVATIVES ON CB1 AND CB2 SIGNALLING	P1-45
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Kristi Sadler* and Susan Snycerski	EDUCATIONAL AND EMPLOYMENT DEMOGRAPHICS OF MEDICAL CANNABIS PATIENTS IN THE CALIFORNIA BAY AREA	P1-48
Olga Karpińska*, Marta Baranowska-Kuczko, Monika Kloza, Mirosław Kozłowski, Hanna Kozłowska and Barbara Malinowska	HYPERTENSION MODIFIES THE CANNABIDIOL-MEDIATED VASCULAR RESPONSE IN ISOLATED HUMAN PULMONARY AND RAT SMALL MESENTERIC ARTERIES	P1-49
Franciele F Scarante, Rafael P Aguiar, Eduardo J Fusse, Francisco S Guimaraes and Alline C Campos*	CANNABIDIOL, BUT NOT URB597, ENHANCES BEHAVIORAL EFFECTIVENESS OF ESCITALOPRAM IN MICE SUBMITTED TO CHRONIC STRESS	P1-50
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Heather B Bradshaw* and Emma Leishman	Δ^9-TETRAHYDROCANNABINOL (THC), CANNABIDIOL (CBD) OR A THC/CBD COMBINATION DRIVE DIFFERENTIAL CHANGES IN ENDOCANNABINOIDS, PROSTAGLANDINS, AND RELATED LIPIDS IN MICROGLIA, ASTROCYTES, AND NEURONS	P1-52
Matthijs Geert Bossong*, Hendrika Heiltje van Hell, Chris Schubart, Wesley van Saane, Tabitha Iseger, Gerry Jager, Martijn Jansma, René Kahn, Marco Boks and Nick Ramsey	ACUTE EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON RESTING STATE BRAIN FUNCTION AND THEIR MODULATION BY COMT GENOTYPE	P1-53
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Linda E. Klumpers* and David L. Thacker	DEVELOPMENT OF A DATA-DRIVEN TOOL TO EDUCATE PATIENTS ON EFFICACY AND TOLERABILITY OF CANNABIS-BASED PRODUCTS	P1-55
Rupali Vyavahare*, Melissa M. Lewis, Yi Yang, Ewa Wasilewski, John Hanlon, Hance A.Clarke and Lakshmi P. Kotra	CB1 AND CB2 RESPONSES TO MEDICAL CANNABIS EXTRACTS	P1-56
Jonathon C. Arnold*, Sarah V. Abelev, Iain S. McGregor and Lyndsey L. Anderson	CBD DOES NOT POTENTIATE THE ANTICONVULSANT ACTION OF CLOBAZAM ON HYPERTHERMIA-INDUCED SEIZURES DESPITE INCREASING PLASMA CLOBAZAM CONCENTRATIONS IN A MOUSE MODEL OF DRAVET SYNDROME	P1-57
Antony Mersiades*, Annette Tognela, Paul Haber, Martin Stockler, Nicholas Lintzeris, John Simes, Iain McGregor, Ian Olver, David J Allsop, Craig Gedye, Adrienne Kirby, Rachael L Morton, Anh D Tran, Karen Briscoe, Peter Fox, Nicole Wong, Anna Walsh, Anjali Bhardwaj, Carmel Hahn and Peter Grimison	PILOT AND DEFINITIVE RANDOMISED DOUBLE-BLIND PLACEBO CONTROLLED TRIALS OF EVALUATING AN ORAL CANNABINOID-RICH THC/CBD CANNABIS EXTRACT FOR SECONDARY PREVENTION OF CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING	P1-58

Darryl J. Bornhop*, Amanda Kussrow, Mingfeng Bai, Michael Kammer and Michelle Sexton	FREE-SOLUTION, INTERFEROMETRIC ASSAYS ENABLE HIGH-SENSITIVITY, AFFINITY QUANTIFICATION OF MEMBRANE ASSOCIATED CB1 AND CB2 LABEL-FREE	P1-59
Jessica Reedy* and Jeremy Riggle	CONSISTENCY IN THIRD PARTY QUALITY CONTROL TESTING IN THE UNITED STATES	P1-60
Sarah Daniels*, Zach Walsh, Michelle S. Thiessen and Tatiana A. Sanchez	THERAPEUTIC USE OF CANNABIS AND SUBSTITUTION FOR PHARMACEUTICAL MEDICATIONS IN A PRENATAL SAMPLE	P1-61
Mohamed M. Radwan, Chandrani G. Majumdar*, Amira S. Wanas and Mahmoud A. ElSohly	GMP PRODUCTION OF HIGH CBD CANNABIS EXTRACT FORMULATION: STABILITY STUDY	P1-62
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Robert Walsh*, Rik Kline, Steven Gust, Brian Thomas and Mahmoud ElSohly	MARIJUANA AND CANNABINOID RESEARCH PRODUCTS AVAILABLE FROM THE NATIONAL INSTITUTE ON DRUG ABUSE	P1-66
Heather Jackson*, Nicolas J. Schlienz, Marcel O. Bonn-Miller, Joel Munson, Erin Martin and Ryan Vandrey	HEALTH OUTCOME COMPARISONS BETWEEN EPILEPSY PATIENTS WHO USE VERSUS DON'T USE CANNABINOID PRODUCTS	P1-67

Zuzanna Zajkowska*, Alice Russell, Nilay Hepgul, Alessandra Borsini, Daniel Forton, Kosh Agarwal, Valeria Mondelli, Patricia A. Zunszain and Carmine M. Pariante	DIFFERENTIAL EFFECT OF AEA AND 2-AG IN INFLAMMATION	P1-68
Alex Mabou Tagne*, Massimiliano Legnaro, Gioela Ferrario, Franca Marino, Barbara Pacchetti and Marco Cosentino	CANNABIDIOL MODULATES MIGRATION AND OXIDATIVE METABOLISM IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES	P1-69

Notes:

Presenting Author*

POSTER SESSION 2

MONDAY, JULY 2ND: 16:00 - 18:00

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Presenting Author*

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ICRS Lifetime Achievement Award

Sunday, July 1, 2018
13:30 – 14:30

CB2 Receptors in the CNS

Cecilia J. Hillard, Ph.D.

Professor, Director, Neuroscience Research Center
Department of Pharmacology and Neuroscience Research Center
Medical College of Wisconsin
Milwaukee, WI
USA

My laboratory has been interested in the enigmatic CB2 receptors since the early 1990s. CB2 receptors can be expressed by microglia, although at very low levels in the healthy brain. Microglial CB2 expression is up-regulated in a variety of pathological conditions, particularly when neuroinflammation is involved.

I will review our previous studies, and discuss our recent data regarding microglial CB2 receptor involvement in responses to cocaine

Young Investigator Award Presentation

Monday, July 2, 2018
13:30 – 14:00

Chemical tools to study endocannabinoid Biosynthesis and Metabolism

Mario van der Stelt, Ph.D.

Department of Molecular Physiology
Leiden University
Leiden, Netherlands

The success of new drugs depends on our ability to understand their molecular and cellular mechanism of action. Modulation of cannabinoid CB1 and CB2 receptors activity by the endocannabinoids is associated with therapeutic benefits in humans. Poor understanding of the physiological role of endocannabinoids and lack of detailed selectivity profiling of experimental drugs, however, led to the market withdrawal of Acomplia®, a CB1 receptor antagonist, and the death of one volunteer in a phase 1 clinical trial exposed to the FAAH inhibitor BIA 10-2474. To assess the therapeutic potential of the endocannabinoid system and its drug candidates, we have developed and characterized β-lactone-based activity-based probes (MB064) and a photoaffinity probe (LEI121) that targets brain lipases and the cannabinoid CB2 receptor, respectively. In a competitive activity-based protein profiling and chemical proteomics format these tailor-made probes were employed to discover the first brain active inhibitors of endocannabinoid biosynthesis [1-3], reported the off-target landscape of BIA 10-2474 [4] and to profile CB2 receptor expression and its ligands in human cells [5-6]. Finally, in this lecture I will also discuss the discovery of in vivo active NAPE-PLD inhibitors.

1. Baggelaar et al. *J. Am. Chem. Soc*, **2015**, 137, 8851
2. Ogasawara et al., *Proc. Natl. Acad. Sci*., **2016**, 113, 26
3. Van Rooden en al., *Nature Prot*. **2018**, 13, 752
4. Van Esbroeck et al., *Science*, **2017**, 356, 1084
5. Soethoudt et al., *J. Am. Chem. Soc*, **2018**, 140, 6067
6. Soethoudt et al. *Nature Comm*., **2017**, Jan 3, 13958

Young Investigator Award Presentation

Tuesday, July 3, 2018
12:00 – 12:30

TARGETING FABP5 TO TREAT PAIN, INFLAMMATION, AND CANCER

Martin Kaczocha, Ph.D.

Department of Anesthesiology
Department of Biochemistry and Cell Biology
Institute of Chemical Biology and Drug Discovery
Stony Brook University, Stony Brook, NY, USA

Fatty acid binding proteins (FABPs) are a family of intracellular lipid carriers that modulate diverse biological functions. In addition to their canonical roles in fatty acid metabolism and nuclear receptor signaling, accumulating evidence has pointed to FABPs as critical modulators of endocannabinoid signaling and inactivation. Since the discovery that FABP5 serves as a carrier for endocannabinoids nearly a decade ago, my laboratory has sought to elucidate the functions of FABP5 in endocannabinoid signaling in the brain and peripheral tissues and has embarked on a program to develop small molecule inhibitors targeting FABP5 to modulate the endocannabinoid tone. Here, I will first discuss our current understanding of the mechanistically distinct functions of FABP5 in endocannabinoid signaling in the brain and peripheral tissues. I will then discuss our efforts to develop small molecule FABP5 inhibitors for the treatment of pain and inflammation. Lastly, I will discuss our recent foray into the area of prostate cancer and our discovery that FABP5 plays an obligate role in controlling pro-tumorigenic lipid signaling and will highlight the therapeutic potential of targeting FABP5 to treat metastatic prostate cancer. Future directions and gaps in the current knowledge of FABP function will also be discussed.

Acknowledgements: This work was supported by the National Institutes of Health (DA035949 and DA035923) and the Stony Brook University Department of Anesthesiology.

KANG TSOU MEMORIAL SPEAKER

WEDNESDAY, JULY 4, 2018
14:30 – 15:30

STEM CELL-BASED ORGANOIDS AS AVATARS IN HUMAN DISEASE

Hans C. Clevers, M.D., Ph.D.

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University of Utrecht, The Netherlands
Hubrecht Institute of the Royal Netherlands Academy of Arts, Utrecht, The Netherlands
Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands

Stem cells are the foundation of all mammalian life. Stem cells build and maintain our bodies throughout life.

Every organ in our body is believed to harbor its own dedicated stem cells. These adult stem cells replace tissue that is lost due to wear and tear, trauma and disease. Adult stem cells are highly specialized and can only produce the tissue in which they reside; they ae 'multipotent'. Examples are bone marrow stem cells that make all blood cells, skin stem cells and gut stem cells. Even the brain is now known to harbor its specialized stem cells. The adult stem cells allow us to live 80-90 years, but this comes at a cost: they are the cells that most easily transform into cancer cells.

We have identified a gene (lgr5) that marks a series of known and novel adult stem cells, in organs such as the gut, the liver, the lung and the pancreas. We have learned to grow these stem cells in a dish into mini-versions of the human organs from which they derive. This so called organoid technology opens a range of avenues for the study of development, physiology and disease, and for personalized medicine. In the long run, cultured mini-organs may replace transplant organs from donors and hold promise in gene therapy.

THE NATIVE RANGE OF *CANNABIS SATIVA* AND ITS CENTER OF ORIGIN IN ASIA, PRIMARILY BASED ON FOSSIL POLLEN DATA

John M. McPartland* and Geoffrey W. Guy

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INTRODUCTION: Every species occupies an indigenous geographical area, known as its native range (NR). Within its NR is a species's center of origin (COO), from whence it dispersed. Botanists determine the NR of a cultivated plant by locating its wild relatives. COO is determined by NR, patterns of genetic diversity, and the fossil record. Resolving the COO of *C. sativa* is hampered by a lack of print fossils (rock fossils). Many fossil pollen studies (FPSs) report *Cannabis* microfossils (fossil pollen), but unfortunately they report lumped data, "*Cannabis/Humulus*" (*C/H*), because the morphology of *Cannabis* and *Humulus* pollen is nearly identical.

METHODS: We collated literature reports of wild-type *Cannabis,* and mapped their locations, as a first-order estimate of its NR. *C/H* pollen in FPSs was differentiated by applying ecological proxies (instead of morphology): Many ecological studies document wild-type *Cannabis* cohabitating with steppe plants—*Chenopodiaceae, Artemisia, Poaceae* (*CAP*). We applied this ecological evidence to FPSs: *C/H* in a pollen assemblage dominated by *CAP* pollen was identified as *Cannabis*. In contrast, *C/H* in a pollen assemblage dominated by tree species (*Alnus, Salix, Populus*) was identified as *Humulus*.

RESULTS: Reports of wild-type *Cannabis* span all of Eurasia, but their locations form a density ellipse in Central Asia—the NR of *Cannabis*. Out of 91 Asian FPSs with *C/H* pollen, the application of ecological proxies identified 26 FPSs with *Cannabis* pollen that predated the Neolithic period. The oldest *Cannabis* pollen dated to 19.6 million years ago (mya), located near Gùyuán, China. This is much younger than 27.8 mya—the estimated evolutionary divergence of *Cannabis* and *Humulus* (McPartland & Guy 2010).

The gap between 27.8 and 19.6 is spanned by an *aridification event* that occurred during the Oligocene Epoch (33.9-23.0 mya), when forests in Central Asia were replaced by a new ecosystem—steppe. Bosbom *et al.* (2011) mapped the *aridification zone*, defined by geological data (tectonic uplift, rain shadow, sea retreat). The oldest *Cannabis* pollen falls within this zone. This zone also correlates in time and space with separate studies regarding the geographical origins of two newly-evolved steppe plants: *Chenopodiaceae* (Cao *et al.* 2013) and *Artemisia* (Miao *et al.* 2011).

DISCUSSION: If we assume the ecological niches of modern plants can be extrapolated to past populations—the "coexistence" model (Mosbrugger & Utescher 1997), then we can deduce that *Cannabis*, along with her fellow travelers *Chenopodiaceae* and *Artemisia,* evolved in Bosbom's aridification zone, in northwest China and southwest Mongolia.

CANNABIS RECLASSIFIED: REDEFINING 'INDICA' AND 'SATIVA' BY GENETICS AND CHEMISTRY

Michel McElroy[1], Hugo Maassen[2], Arno Hazekamp[3] and Sean Myles*[1]

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[3] Hazekamp Herbal Consulting

The taxonomical classification of the genus Cannabis is contentious. Drug Cannabis cultivators and consumers tend to classify varieties using the terms 'Indica' or 'Sativa' as a way of informing consumers about their effects, aromas or purported pedigree. The degree to which these labels correspond to any meaningful biology remains largely unexplored. To investigate the genetic basis of reported ancestry labels, we genotyped over 25,000 genetic markers and assessed 35 metabolites in 149 cannabis varieties with varying degrees of reported Indica-Sativa ancestry from five sources within the Netherlands. We observed no substantial genetic differences between samples labeled 'Indica' and 'Sativa' that would suggest that there exist two distinct lineages within the species. However, we revealed a strong relationship between genetic relatedness and specific terpenes that are popularly associated with the ancestry labels: Sativa ancestry was associated with farnesene and bergamotene, while Indica ancestry was associated with myrcene, elemene, and sesquiterpene alcohols. Our results suggest that, rather than reflecting two independent ancestral populations converging through hybridization, the Indica-Sativa spectrum in Cannabis may instead be largely the result of two ideotypes having been selected for during breeding from a relatively unstructured gene pool. As Cannabis cultivation experiences a resurgence in many parts of the world and Cannabis breeding thus becomes the target of significant scientific investment, additional studies into the genetic identity of Cannabis germplasm are essential so that evidence-based legal and regulatory frameworks can be adopted that enable the tremendous potential of this plant to be realized.

Acknowledgements: The authors wish to thank the Natural Sciences and Engineering Research Council of Canada (NSERC), Bedrocan International and Bedrocan Canada for their support of this study and Eurofins Proxy labs (Leiden, The Netherlands) for providing facilities for DNA extraction.

CANNABIS TERPENE PROFILES PARTIALLY ASSOCIATED WITH C. SATIVA/C. INDICA/HYBRID CLASSIFICATION

Mallory Loflin[1,2], Philippe Lucas[3], Joshua Eades[3], Graham Eglit[2], Ryan Vandrey[4] and Marcel O. Bonn-Miller[5]

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[3]Tilray, Nanaimo, BC
[4]Johns Hopkins University School of Medicine, Baltimore, MD
[5]University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Background: There is an ongoing debate regarding the utility and accuracy of the Cannabis sativa/Cannabis indica distinction. Use of this "species" label is common nomenclature within the cannabis industry, with cannabinoid products labeled according to species and strain determinations. Moreover, C. sativa/C. indica labeling is often used within the cannabis industry to refer to drug effects (Pearce et al., 2014). Specifically, C. sativa is used to denote forms of cannabis with exhilarating/upper effects, and C. indica to refer to forms of cannabis with relaxing/sedating effects. If a true species distinction exists that is driving differences in pharmacodynamic effects between cannabis types, we would expect to see differences in chemical profiles between those forms of cannabis designated C. indica, C. sativa, and cross-breeds of the two (i.e., hybrid). Previous research has examined patterns of phytocannabinoid concentration within those designated C. sativa and C. indica. While relative ratio of cannabinoids do tend to differ on average between C. sativa and C. indica (Hillig & Mahlberg, 2004), within category variability is often greater than between category variability, calling into question the utility of use of the C. sativa/C. indica distinction (Piomelli & Russo, 2016). To date, little work has examined this "species" label distinction in regard to other chemical components of cannabis thought to drive psychoactive effects. Terpenes, which give individual cannabis plants their distinctive smell and taste, may contribute to cannabis' subjective effects (Russo, 2011). Terpenes are noteworthy candidates for examining the chemical composition of the C. sativa/C. indica distinction because specific terpenes may be responsible for effects that are similar to those described by the C. sativa/C. indica label (e.g., alerting effects of limonene, sedating effects of myrcene; Russo, 2011). The current study was interested in determining whether terpene content could be clustered into common terpene profiles across cannabis "strains," and whether those profiles would predict classification as C. sativa, C. indica, or hybrid.

Methods: Data were obtained from a convenience sample of one-hundred and six cannabis samples designated into 29 "strains" and labeled by the manufacturer as C. sativa, C. indica, or hybrid. The samples were analyzed by the manufacturer for their chemical profile constituents, including concentration of twenty-one terpenes. Principle components analysis (PCA) with varimax rotation was conducted to reduce terpene variables to a smaller number of principal components. PCA-derived component scores were then submitted to a hierarchical cluster analysis using ward's method in order to classify each cannabis sample based on its terpene profile. Cluster membership was tested for contingency with category label code (i.e., C. sativa, C. indica, hybrid) using Pearson Chi-square tests.

Results: Of the twenty-one terpenes analyzed, four did not show sufficient variability across samples for classification analysis (i.e., cymene, camphene, alpha terpinene, eucalyptol). Therefore, seventeen terpenes were included in the final analysis (alpha-pinene-a, pinene, myrcene, carene, terpinene-a, d-limonene, ocimene, linalool, isopulegol, geraniol, alpha-carophyllene, humulene, transnerolidol, carophyllene-oxide, guaiol, bisabolool). PCA indicated a three-component solution, with 59.20% of total variance explained and low cross-loading. Hierarchical cluster analysis produced three distinct clusters of these component loadings. Results of the Pearson Chi-square found significant dependence between cluster group membership and category label code ($X^2(4, N = 106) = 18.64$, $p < .001$). Follow-up analysis found that only one cluster group, which was defined by high levels of alpha-pinene-a, alpha-pinene, and myrcene, drove the significant association between cluster group and category label. This group consisted of seven cannabis samples, all of which were labeled C. sativa. After removal of this cluster group, the remaining two cluster groups did not predict category label.

Conclusions: The current study found evidence of three distinct profiles of terpenes within the cannabis samples. These terpene profiles were partially, though modestly, dependent on C. sativa/C. indica/hybrid labeling. However, elevations in only three terpenes were responsible for the significant association between terpene profiles and category label. High levels of these terpenes were only present in samples categorized as C. sativa, and present within a minority of samples labeled within that category. Moreover, myrcene, one of the three terpenes whose elevation defined this unique terpene profile, is associated with sedative effects (do Vale et al., 2002), rather than the "upper" effects commonly indicated by the C. sativa label. Controlled studies are needed to determine whether plants labeled C. sativa, C. indica, and hybrid are indeed pharmacodynamically distinct.

EVOLUTION OF CANNABINOID SYNTHASE PATHWAY GENES IN LANDRACE VARIETIES AND DRUG CULTIVARS

Kayla M. Hardwick[1] and Alisha K. Holloway*[1,2]

Phylos Bioscience, Portland, Oregon, USA[1]
Department of Epidemiology and Biostatistics,
University of California, San Francisco, USA[2]

Chemical profiling and genetic studies of drug cultivars indicate that cannabinoid synthase pathway genes have a complex pattern of duplication and functional divergence. However, little is known about cannabinoid synthase pathway genes in landrace varieties. Humans have carried cannabis seeds with them for millennia and the plant thrives on every continent except Antarctica. Landrace varieties are quite genetically diverse with levels of variation similar to maize and higher than many other crop species, including grape. The maintenance of diversity may be due to early distribution around the globe and limited controlled breeding. Primarily, selection has been for long fibers, healthy seed oils and protein in hemp-type varieties, and increased production of cannabinoids in drug cultivars.

In this work, we use population genomic data to reconstruct the timing and extent of duplication and divergence of cannabinoid synthase pathway genes. We have sequenced the genomes of 42 landrace varieties from 24 countries and 13 hemp varieties from 11 countries, as well as 44 modern drug cultivars. Signatures of directional selection will shed new light on amino acid and regulatory substitutions that are relevant for determining enzyme activity and transcription factor binding efficiency, respectively. Breeding efforts can leverage this information to develop new cannabis varieties that have more precise combinations and quantities of cannabinoids. We also identify landrace varieties that are likely to produce significant quantities of minor cannabinoids that are becoming important as we begin to understand their medical value.

CHARACTERIZATION OF BIOCATALYTICALLY PRODUCED CANNABINOIDS AND CANNABINOID PRODRUGS

Malcolm Kavarana*; Richard Peet*; Yuri Khmelnitsky[#] and Peter Michels[#]

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Bio-catalysis using the cannabinoid synthase enzymes THCA synthase and CBDA synthase offers a convenient method for manufacturing commercial quantities of pure cannabinoids including the rarer Varin compounds. The bio-catalytically produced cannabinoids including Varins have the same structure and stereo-configuration as their corresponding phytocannabinoids. Bi-directional permeability studies and Efflux studies using a cultured monolayer of Caco-2 cells indicates that bio-catalytically produced cannabinoids including Varins should exhibit high permeability across the intestinal epithelium. The bio-catalytically produced cannabinoids including Varins are stable in PBS, simulated gastric fluid (SGF) with and without the enzyme pepsin, and simulated intestinal fluid (SIF) in the presence or absence of the enzyme pancreatin. An exemplary prodrug of bio-catalytically produced cannabidiol (CBD) was synthesized chemically and the stability of this compound evaluated *in-vitro* using PBS, SGF, and SIF. The CBD prodrug is more stable than CBD indicating that the prodrugs may be more suitable candidates for the development of CBD-based therapeutics.

STRUCTURE-ACTIVITY RELATIONSHIPS OF EMERGENT SYNTHETIC CANNABINOID NEW PSYCHOACTIVE SUBSTANCES AND THEIR PROPHETIC ANALOGUES

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Synthetic cannabinoid receptor agonists (SCRAs) are the largest and most structurally heterogeneous class of new psychoactive substances (NPS). According to the United Nations Office on Drug and Crime, more than 240 SCRA NPS have been identified across the globe since the first examples were detected a decade ago. As individual SCRAs are prohibited, clandestine manufacturers design new putative SCRAs using medicinal chemistry techniques such as molecular hybridization, bioisosteric replacement, and core hopping, to circumvent the law. Many emergent SCRAs have no precedent in the scientific literature, and clusters of SCRA intoxication are increasingly associated with severe illness and death. The exponential increase in the number of SCRAs, and the inevitable latency in availability of commercial standards, impedes the pharmacological and toxicological profiling of this class of NPS.

Inspired by Markush structures and combinatorial chemistry, we have used cheminformatics, medicinal chemistry, and a divergent synthetic approach to rapidly and proactively develop a library of current and "prophetic" SCRAs and metabolites based on structural trends in recently identified SCRA NPS. Members of the SCRA library were screened *in vitro* at human cannabinoid type 1 and type 2 receptors (CB_1 and CB_2, respectively) using radioligand binding assays and fluorescence-based plate reader membrane potential assays, revealing differences in affinity and activity.

Several recently detected SCRAs and their prophetic analogues showed nanomolar affinity and subnanomolar activity at CB_1. Many compounds exhibited a preference for CB_2 receptors, and in some cases metabolites were more potent at CB_2 than the parent drugs. Strikingly, several compounds exhibited significantly higher efficacy at CB_1 than reference cannabinoids CP 55,940 and WIN 55-212. Selected SCRAs were evaluated in mice using biotelemetry and showed dramatic differences in magnitude and duration of hypothermia via CB_1-dependent mechanisms. Curiously, several SCRAs that have been recently identified on the NPS market were not CB_1 agonists at all (K_i >>10 μM, EC_{50} >>10 μM), and showed no overt bradycardic, hypothermic, or hypolocomotive effects at relatively high doses (up to 30 mg/kg).

This presentation will describe the key structure-activity relationships for emergent SCRAs and their prophetic analogues, and the utility of such libraries for proactive forensic toxicology. The implications of these findings for the toxicity of SCRAs remain to be elucidated, but unprecedented potency and efficacy at CB_1 receptors is an obvious potential cause of toxicity *in vivo*.

SYNTHETIC CANNABINOID SUPPRESSES CANCER AND CISPLATIN-INDUCED NEUROPATHIC PAIN SYMPTOMS BY PERIPHERAL CB1 RECEPTOR ACTIVATION

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Chronic cancer pain is difficult to manage as the therapeutic window of current medications is limited by their undesirable side-effects. We developed a novel class of cannabinoid based analgesics with a high peripheral selectivity and demonstrated that one of the compounds, PrNMI (4-{2-[-(1E)-1[(4-propylnaphthalen-1-yl) methylidene]-1H-inden-3-yl] ethyl} morpholine) suppressed neuropathic pain symptoms induced by sciatic nerve injury without central side effects (Seltzman et.al., J.Med.Chem. 59:7525-43, 2016). Here we examined PrNMI for effectiveness in alleviating pain symptoms in murine oral and bone cancer models and in a rat model of chemotherapy-induced neuropathy (CIPN), a dose-limiting side effect in the successful treatment of many cancers.

Tongue cancer-induced mechanical allodynia was suppressed after systemic PrNMI administration. Paw cancer (inoculated with human oral carcinoma cells) mechanical allodynia was also dose-dependently suppressed by systemic PrNMI. In the syngeneic bone cancer model, PrNMI significantly alleviated spontaneous pain behaviors. This analgesic effect of PrNMI was reversed by a systemic but not intrathecal administration of SR141716, a selective CB1 receptor antagonist. In addition, the cancer-induced bone loss was not exacerbated by repeated administration of PrNMI, and the in vitro cancer cell viability was unaffected by PrNMI treatment. Furthermore, PrNMI did not impair motor function in naïve animals, while sedation, catalepsy, and hypothermia, the common side effects induced by cannabinoids, were only observed when PrNMI was administered at a high dose. In rats, oral administration of PrNMI dose-dependently suppressed cisplatin (3 mg/kg, 1/week for 4 wks)-induced peripheral neuropathy symptoms of mechanical and cold allodynia with complete symptom suppression at 3 mg/kg. Daily oral administration at 1 mg/kg consecutively for two weeks resulted in similar daily suppression of mechanical and cold allodynia implicating minimal development of tolerance. Intraplantar PrNMI (0.25 mg/kg) injection completely suppressed CIPN symptoms, suggesting peripheral sensory nerve terminals as the main sites of PrNMI's anti-allodynic action. PrNMI co-administration with selective CB1 or CB2 receptor blockers revealed mainly CB1R contribution to its analgesic effects. The potency, peripheral selectivity, in vivo efficacy, and minimal CNS side effects of this novel class of CBR agonists point to their potential as a viable treatment for cancer and CIPN pain symptoms.

Acknowledgments: NIH grant CA196263 (B.L.S., I.S.) and a University of California Center for Accelerated Innovation grant U54HL119893 (I.S.).

THE SELECTIVE CB2-R AGONIST, HU-910 ATTENUATES HEPATORENAL SYNDROME DEVELOPMENT IN A MOUSE MODEL OF LIVER FAILURE

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Tubular dysfunction is an important feature of renal injury in hepatorenal syndrome (HRS) in end-stage liver disease patients. However, detailed understanding of the pathogenesis of kidney injury in HRS is lacking. Furthermore, there are no clinically relevant rodent models of HRS. We investigated the renal consequences of bile-duct ligation (BDL)-induced hepatic and renal injury in mice, and explored the therapeutic potential of the selective CB2-R agonist, HU-910 by using biochemical assays, real-time PCR, Western blot, mass spectrometry, histology and electron microscopic analyses.

BDL resulted in massive hepatic injury, which was paralleled by tubular dilation and tubulointerstitial nephritis. Renal injury was associated with dramatically impaired microvascular flow. Gene expression analyses signified proximal tubular epithelial injury, tissue hypoxia, inflammation, and activation of the fibrotic gene program. Daily HU-910 treatment reduced tissue injury both in the liver and the kidney, markedly reduced the expression of markers of hepatic and renal inflammation and fibrosis and restored microvascular flow in both organs.

Our findings suggest that liver failure induces a marked deterioration in renal function as a result of impaired microcirculation, increased inflammation (tubulointerstitial nephritis), accentuated vascular adhesion molecule expression, leading to tubular cell death and fibrosis. Pharmacological activation of CB2-R by the selective agonist HU-910 restores microcirculation, attenuates both the vascular and stromal inflammatory reactions and thereby alleviates tubular injury. We also demonstrate that BDL in mice is an excellent, clinically relevant model of HRS to study the renal consequences of end-stage liver diseases.

Acknowledgments: This work was supported by the Intramural Research Program of NIAAA/NIH (to G.Kunos, and P. Pacher).

RECENT PROGRESS TOWARD ENHANCED CHEMICAL PROBES FOR STUDYING THE CANNABINOID RECEPTOR 2

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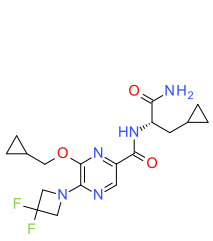
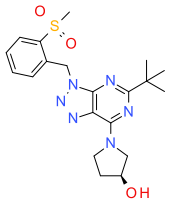
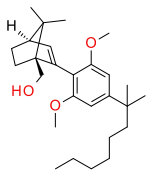
The type 2 cannabinoid receptor (CB_2R) plays an important role in cell migration and immunosuppression and is therefore a promising GPCR drug target for the treatment of tissue injury and inflammatory diseases. Highly selective CB_2R agonists show robust efficacy in various animal models of chronic and inflammatory pain, diabetic neuro- and nephropathy, liver cirrhosis, and ischemic-reperfusion injury. The successful development of new drugs strongly depends on the understanding of their underlying molecular and cellular mechanism of action as well as on knowledge about their *in vivo* target engagement. The lack of specific anti CB_2R antibodies and of suitable biomarkers for target occupancy hampers the clinical development of CB_2R agonists. 2,5,6-Trisubstituted pyridine/pyrazine[1] (**I**), triazolopyrimidine[2] (**II**) and novel cannabinoid derived ligands[3] (**III**) were found to be highly potent and selective CB_2 drug candidates.



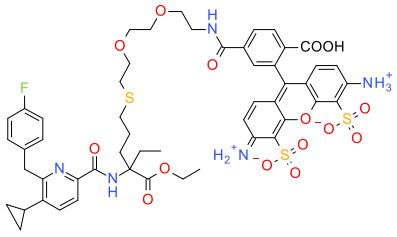
(**I**) RO6839828
CB_2 cAMP EC_{50} 9 nM 100% eff.



(**II**) RO6866959
CB_2 cAMP EC_{50} 0.1 nM 104% eff.



(**III**) HU-910
CB_2 cAMP EC_{50} 6 nM 98% eff.



(**IV**) RO7253104
CB_2 cAMP EC_{50} 12 nM 95% eff.

These compound classes furthermore offer excellent starting points for generating improved chemical probes to help answering biological questions and have been exploited for generating enhanced CB_2 specific radioisotope and fluorescence labels, Raman probes as well as bifunctional probes which will be the subject of this communication. We will report results on CB_2 and CB_1 binding and functional activity which guided the probe optimization. Early absorption, distribution, metabolism and excretion properties of advanced probes including e.g. solubility, permeation, lipophilicity and selectivity data will be disclosed. First applications of novel probes such as the CB_2 fluorescence label **IV** will be shown.

[1] R. Slavik et al., *J. Med. Chem.* **2015**, *58*, 4266.
[2] M. Nettekoven et al., *ChemMedChem* **2016**, *11*, 179.
[3] M. Soethoudt et al., *Nat Commun.* **2017**, *8*, 13958.

A SELECTIVE PHOTOAFFINITY PROBE ENABLES ASSESSMENT OF CANNABINOID CB_2 RECEPTOR EXPRESSION AND LIGAND ENGAGEMENT IN HUMAN CELLS

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The cannabinoid CB_2 receptor (CB_2R) is a promising therapeutic target for tissue injury and inflammatory diseases. Determination of endogenous CB_2R expression levels and occupancy in cells is imperative to understand its biological role and to develop therapeutics. Reverse transcription polymerase chain reaction and immunohistology have been widely applied to detect the CB_2R, but these methods cannot report on target engagement and CB_2R-antibodies are not specific. Here, we describe a complementary chemical biology approach in which CB_2R expression and occupancy is visualized in human cells by photoaffinity-based protein profiling and fluorescent-activated cell sorting (FACS) analysis. We designed and synthesized chemical probe LEI121 and incorporated a diazirine as photoreactive molecular tag to covalently capture the CB_2 receptor and an alkyne as ligation handle to enable visualization of the protein by conjugation to fluorophores. LEI121 was able to covalently label CB_2R in membrane fractions of CB_2R-overexpressing CHO cells upon UV-irradiation and conjugation to a Cy5-fluorophore. This labeling could be prevented by various CB_2R ligands with different chemotypes, was dependent on UV-irradiation, copper(II)-catalyzed conjugation of the fluorophore and was absent in non-transfected cells. Using FACS analysis, endogenous CB_2Rs was studied in the human monocytic cell line HL60 and human PBMCs. Target engagement was confirmed by two different CB_2R ligands. Finally, the highest CB_2R expression was detected in $CD19^+$ B-cells, whereas it could not be detected in $CD4^+$ or $CD8^+$ T-cells identified from human peripheral blood mononuclear cells. These findings indicate that fully functionalized chemical probes can detect endogenous cannabinoid CB_2 receptor on human cells and hold promise as pharmacological tools to determine target engagement to aid drug discovery.

THE SWEETPEA STUDY: A RANDOMISED DOUBLE BLIND CONTROLLED TRIAL EXAMINING THE EFFECT OF PEA AND CBD ON THE PERMEABILITY OF THE HUMAN GUT *IN VIVO*

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Introduction: Palmitoylethanolamide (PEA) and cannabidiol (CBD), have been shown to prevent inflammation-induced falls in trans-epithelial electrical resistance in Caco-2 cells, suggesting that they prevent increases in gut permeability in humans. The permeability of the human gut can be estimated by comparing the ratio of absorption of D-mannitol, which is absorbed and excreted in the urine at a steady state, and lactulose, which is only absorbed and excreted during periods of inflammation. This ratio, the lactulose-mannitol ratio (LMR) is a frequently used marker of intestinal permeability. We examined for the first time the effect of CBD and PEA on the permeability of the human gastrointestinal tract in vivo using a randomised, double blind, placebo-controlled trial.

Methods: In fasted healthy volunteer males between 18 and 50 years we induced a state of intestinal inflammation using aspirin (600mg orally). Permeability of the gut was measured by giving participants oral lactulose (1g) and D-mannitol (1g), collecting multiple regular urine samples over a 6 hour period. The urinary concentration of these sugars were then determined using targeted (multiple reaction monitoring) liquid chromatography-mass spectrometry. At the time of sugar and aspirin administration, participants were also given CBD (600mg orally, a gift from Artelo Biosciences), PEA (600mg orally, from Russel Science) or placebo (600mg base cellulose capsules, Artelo Biosciences). Differences between groups were analysed using two-way ANOVA, differences within groups were measured with repeated measures ANOVA, significance was set at <0.05.

Results: 30 participants completed the study. In participants taking placebo, aspirin caused an increase in the LMR, becoming significant at 1 hour until the end of the study period ($p<0.001$, figure 1). Both PEA and CBD reduced the LMR by 45% and 67% respectively, becoming significant at 1 hour ($p<0.01$) until the end of the study period. There were no adverse events.

Conclusion: We have shown for the first time in humans that an acute dose of CBD or PEA reduce the increased permeability induced by aspirin. This is further evidence that CBD and PEA may be used clinically in inflammatory bowel disease.

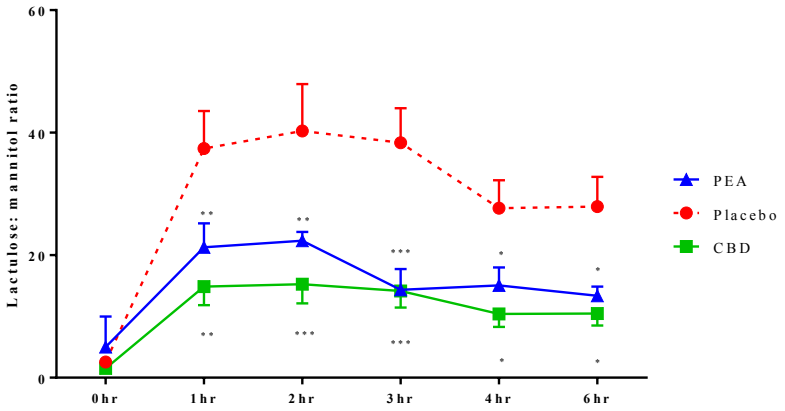

Figure 1 – The concentration ratios of urinary lactulose and mannitol over time in healthy participants treated with aspirin and either placebo, CBD or PEA, measured by LC MS. Results are expressed as mean ratios +/- SEM. Time points between groups were compared using two-way ANOVA using Dunnett's multiple comparisons test comparing to placebo at the same time point (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

CANNABIDIOL – AN INNOVATIVE STRATEGY FOR THE TREATMENT OF GRAFT VERSUS HOST DISEASE (GVHD)

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Background: GVHD is a common potentially life-threatening complication following allogeneic hematopoietic cell transplantation (alloHCT). GVHD is the leading cause of post-transplantation morbidity and non-relapse mortality. Importantly, complete response to front-line therapy with glucocorticoids is achieved in only 30-40% of patients with acute GVHD and among patients with steroid refractory severe (grade 3-4) acute GVHD, mortality rate is over 80%. The ability to treat acute GVHD is a major unmet need and currently there is no FDA-approved therapy.

We have completed three phase 2 clinical studies clearly suggesting that pure oral cannabidiol (CBD) is a safe and promising strategy for the prevention and treatment of acute GVHD. Here we present the data on a phase 2a study in 10 patients with severe acute GVHD that were refractory to high-dose steroids.

Methods: Ten patients (male=5, median age 58 (range, 31 to 70) years) with hematologic malignancies (acute leukemia, n=9, lymphoma, n=1) were enrolled. Five of these patients were diagnosed with Grade 3 acute GVHD and 5 patients with Grade 4 acute GVHD. All patients except for one were refractory to first line therapy consisting of high-dose (1-2 mg/kg/day methylprednisolone) steroids and a calcineurin inhibitor. Patients were administered oral CBD at a dose of 150 mg twice daily.

Results: CBD consumption was safe and no significant adverse effects were reported. Nine out of 10 patients responded to treatment, 7 of them achieved complete remission and 2 achieved very good partial response. Most patients showed maximal response within 14 days.

At the time of last analysis, 6 out 10 enrolled patients (60%) are still alive with a median follow up period of 13 months (range 5-30 months). Two patients died from leukemia relapse and 2 patients from GVHD-related infectious complications. No patient deaths were related to CBD treatment.

Conclusions: Considering the dismal prognosis of patients with severe (grade 3-4) refractory acute GVHD the results of this pilot Phase 2a study are encouraging, albeit with the limitation of the small number of trial participants. Further clinical research is warranted.

MECHANISMS UNDERLYING THE PRO-COGNITIVE EFFECTS OF CANNABIDIOL: INSIGHTS FROM THE RAT BRAIN AND SEX-SPECIFIC IMPLICATIONS

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Cognitive impairment is a major source of disability in neuropsychiatric disorders, including schizophrenia and autism, with greater deficits observed in male patients. An imbalance in excitatory (glutamate) and inhibitory (GABA) neurotransmission plays a role in cognitive impairment. The endocannabinoid system represents a therapeutic target for cognitive deficits, as it regulates the release of glutamate and GABA (via the CB1 receptor) to maintain optimal cognitive function. The aim of this study was to examine the effects of the phytocannabinoid, cannabidiol (CBD), on endocannabinoid, glutamatergic and GABAergic markers in the brain, using a model relevant to the aetiology of schizophrenia/autism. Maternal immune activation (MIA) was induced in pregnant Sprague-Dawley dams by intravenous administration of polyinosinic-polycytidilic (poly I:C; 4 mg/kg) acid on gestational day 15. Controls received an equivalent volume of saline. Male and female control/MIA offspring were treated with CBD (10 mg/kg, i.p.; twice daily) or vehicle from postnatal day 56 to 80. Cognitive function and social interaction was assessed, and brains were collected (n=8 per group) for post-mortem analysis.

We have previously shown that male MIA offspring exhibit working and recognition memory impairment in adulthood, while female offspring have intact working memory. CBD treatment was able to prevent cognitive deficits in adulthood. Reflecting the results of the cognitive testing, male and female MIA offspring brains differed in neurochemistry, with males displaying greater dysfunction. CBD treatment prevented CB1 receptor binding deficits in the prefrontal cortex of males, which correlated with an improvement in working memory. CBD also prevented hippocampal GAD_{67} deficits and increased parvalbumin (a subset of GABAergic neurons) expression in males. On the other hand, CBD treatment in female MIA offspring had opposing effects on the CB1 receptor, and restored deficits in glutamatergic ionotropic signalling. Taken together with our previous findings, these results suggest that CBD may improve cognition by restoring glutamatergic/GABAergic imbalances via the CB1 receptor. Male and female MIA offspring showed differential patterns of cognitive impairment, and interestingly their neurochemical response to CBD treatment differed. These results highlight the importance of considering sex differences in neuropsychiatric trajectory when testing preventative treatments that target the endocannabinoid system.

Acknowledgements: Funded by a University of Wollongong Advancement Grant to KWG and XFH (2015/SPGA-S/02).

WIDESPREAD EFFECTS OF ACUTE CBD ON THE ENDOGENOUS CANNABINOID RELATED LIPIDOME IN 8 BRAIN REGIONS OF WT AND NAPE-PLD KO MICE

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Derived from the cannabis plant, cannabidiol (CBD) lacks traditional psychoactive effects. However, CBD must impact signaling in the brain to have therapeutic benefits on neurological disorders, such as childhood epilepsy and schizophrenia. The endogenous cannabinoids (eCBs), *N*-arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG) are members of an interconnected lipidome that includes an emerging class of AEA structural analogs, the lipoamines, additional 2-acyl glycerols, free fatty acids, and prostaglandins (PGs). Previous work suggested that CBD inhibits AEA's hydrolysis via FAAH because CBD increased AEA levels. CNS lipidomics from FAAH KO mice showed that indeed AEA and other *N*-acyl ethanolamines (NAEs) increase relative to WT; however, many other lipids decreased throughout the brain in a predictable way, providing a clearer lipid fingerprint of FAAH inactivity. Recent data from our lab showed that chronic CBD increased AEA in some brain regions; however, the lipids that would have predictably decreased showed either no change or increases. This suggested that CBD may act at an alternative site to FAAH. Here, we test the hypothesis that CBD acts through NAPE-PLD to regulate AEA and related lipids in the brain. Using WT and NAPE-PLD KO mice, levels of ~80 bioactive lipids were measured in 8 brain regions 2 hours after a 3 mg/kg CBD or vehicle injection. Methanolic extracts of frozen brain areas were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS.

Acute CBD has a broad, region-dependent impact on lipid regulation. Across all 8 brain regions in WT mice, 38% of the detected lipids were significantly up or downregulated and 79% of the changes were increases. In contrast, levels of 20% of the detected lipids changed across NAPE-PLD KO brain areas and 45% of changes were increases. Concentrations of AEA and at least 1 other NAE increased in the WT hippocampus, cerebellum, thalamus, cortex, midbrain, and brainstem. The increases in AEA and other NAEs measured in the WT brain were absent in the NAPE-PLD KO mice. Further suggesting that FAAH inhibition is not driving the increases in NAEs, many of the lipoamines downregulated in all 8 brain areas of FAAH KO increased with CBD in some regions in the WT. For example, levels of the AEA-metabolite *N*-arachidonoyl glycine (NAGly) increased in the WT striatum, hippocampus, thalamus, cortex, and midbrain, and decreased in the hypothalamus only. NAGly was upregulated in the NAPE-PLD KO striatum, hippocampus cerebellum, and thalamus. In addition, the eCB 2-AG changed in just 2 of 8 regions of the WT brain, increasing in the striatum and decreasing in the hypothalamus. 2-AG levels increased in 2 brain areas of NAPE-PLD KO, the thalamus and brainstem, and decreased in the hypothalamus. Suggesting that CBD may have broad anti-inflammatory effects, PGs were downregulated across the entire brain of both WT and NAPE-PLD KO mice. Analysis of CBD levels 2 hours post-injection revealed an unequal distribution across these 8 brain areas. The highest levels were in the striatum for WT mice, but in the brainstem for NAPE-PLD KO mice. Both genotypes had the lowest CBD levels in the hypothalamus. This data illustrate that CBD alters lipid regulation in the brain and presents a novel potential mechanism by which an acute dose of CBD upregulates brain levels of NAEs – an increase in NAPE-PLD activity.

PHYSIOLOGY AND FUNCTION OF AMYGDALO-CORTICAL ENDOCANNABINOID SIGNALING

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Stress exposure is a ubiquitous risk factor for the development of psychiatric illnesses such as generalized anxiety disorder, major depression, and post-traumatic stress disorder. However, a causal link between stress-induced neuropathology and resultant pathological behavioral states remains elusive. Stress induces a wide variety of morphological and neurochemical adaptions in the prelimbic prefrontal cortex (plPFC), a key nodal structure that is implicated in the top down regulation of emotional behavior. The plPFC has strong reciprocal glutamatergic connectivity with the basolateral amygdala (BLA), and several studies have suggested that reciprocal excitatory coupling between these two regions is involved in generating cognitive, behavioral, and autonomic responses to stress. Interestingly, the anxiolytic properties of cannabinoid agonists appear to be mediated at least in part by the activation of CB1 receptors on glutamatergic axon terminals in the medial prefrontal cortex.

Using optogenetic projection targeting combined with retrograde labeling, we show that glutamatergic input to the plPFC from the BLA is highly regulated by endocannabinoid (eCB) signaling in a laminar and output specific manner. Furthermore, we show that exposure to traumatic stress impairs eCB regulation of BLA- Layer 2 plPFC reciprocal circuit. This impairment of eCB signaling can be rescued by incubation with the Monoacylglyercol Lipase (MAGL) inhibitor JZL184. We also demonstrate that selective deletion of CB1 from BLA cells that project to the plPFC, or deletion of DAGL-alpha from plPFC neurons, induces a ‘stress like’ state, characterized by heightened basal anxiety. These data demonstrate a critical role for eCB signaling in regulating a neural circuit implicated in the pathophysiology of affective disorders.

Acknowledgement: These studies were supported by NIH grant MH107435

DELTA-9-TETRAHYDROCANNABINOL AND CANNABIDIOL PRODUCE DISTINCT EFFECTS ON EMOTIONAL MEMORY FORMATION AND SALIENCE ATTRIBUTION THROUGH ACTIONS IN THE VENTRAL HIPPOCAMPUS

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Disturbances in emotional processing and salience attribution are core features of schizophrenia and other neuropsychiatric disorders. Clinical and preclinical evidence suggest that exposure to the phytocannabinoids delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) differentially impact affective memory processing and neuropsychiatric risk through actions on dopamine (DA) signaling in corticolimbic regions. We recently reported that cannabinoid type 1 receptor activation in the ventral hippocampus (VHipp) strongly modulates both fear and reward-related associative memory formation through modulation of DA activity states in the ventral tegmental area (VTA). Additionally, we demonstrated that CBD can prevent both the amplification of DA activity and psychotomimetic behaviours produced by amphetamine sensitization. Nevertheless, the precise molecular and neurophysiological mechanisms by which THC and CBD modulate affective memory remain unknown. The current study tested the hypothesis that intra-VHipp THC and CBD elicit opposing effects on emotional memory processing via distinct neuronal and molecular signaling mechanisms in the mesolimbic pathway. Male Sprague Dawley rats receiving intra-VHipp THC, CBD, or THC+CBD were assessed using assays of morphine place conditioning, social reward, and fear conditioning. Additionally, western blot assays identified candidate proteins associated with acute phytocannabinoid exposure, and in-vivo electrophysiology was used to assess neuronal activity profiles and neural oscillations (LFP) in the VTA and prelimbic medial prefrontal cortex (mPFC) simultaneously in rats under urethane anesthesia.

THC induced deficits in social memory formation but potentiated the salience of contextual cues associated with morphine reward and aversive footshocks. Western blot analysis of VHipp tissue revealed a dramatic increase in phosphorylated extracellular-signal regulated kinase (ERK) following acute THC that was inhibited by co-administered CBD. Although CBD produced no behavioural changes alone, the combination of THC+CBD remarkably enhanced social memory relative to vehicle, and disrupted THC-induced effects on reward and aversion-related memory formation. Inhibition of ERK signaling using ‘U0126’ blocked THC-induced changes in emotional memory formation, and activation of ERK signaling using ‘eicosapentaenoic acid' combined with THC+CBD was sufficient to re-induce THC-like behavioural deficits. In-vivo electrophysiology data indicated that intra-VHipp THC increased VTA DA neuronal activity and bursting rates, and reduced mPFC neuronal activity. Furthermore, disruptions in alpha and gamma LFPs across brain regions were observed following THC administration. These neurophysiological changes were prevented by combining THC with either CBD, or U0126. Collectively, our findings indicate the ERK signaling pathway as a molecular switch in the VHipp that controls phytocannabinoid-induced modulation of emotional memory processing, and suggest important implications for understanding effects of specific cannabis-derived phytochemicals in neuropsychiatric disorders.

Acknowledgements: Natural Sciences and Engineering Research Council of Canada (NSERC)

EXPLORING THE EFFECTS OF GENETIC AND EPIGENETIC VARIATION ON CANNABINOID RECEPTOR-1 GENE EXPRESSION; IMPLICATIONS FOR PERSONALISED AND STRATIFIED MEDICINE

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Personalised and stratified medicine represent key goals of modern medicine. Genetic and epigenetic variation influence disease susceptibility and is also likely to cause significant variation in drug efficacy and side effects within patient groups. The cannabinoid receptor-1 (CB_1) gene is involved in regulating appetite, addiction and pain. As the cell specific regulation of CB_1 is critical to these processes, we analysed the putative regulatory sequences that control CB_1 gene expression. Additionally, we examined the effects of genetic variation and DNA methylation on the activity of these sequences and how they may influence CB_1 expression or drug efficacy.

We show that the CB_1 gene promoter is active in cells from the hypothalamus, hippocampus and the dorsal root ganglia (DRG). We also show that epigenetic variation such as DNA methylation significantly impacts promoter activity in hippocampus and DRG. Moreover, we demonstrate that a polymorphic enhancer region (ECR1), associated with obesity and substance abuse, enhances CB_1 promoter activity in its major allele but represses promoter activity in its minor, disease-associated allele. ECR1 also affects promoter response to pharmacological stimuli. Critically, we have successfully disrupted ECR1 in mice using CRISPR/Cas9 and present evidence that CB_1 expression in regions such as the hippocampus is reduced, drug response is altered and alcohol intake is significantly reduced suggesting an important role for this highly conserved enhancer in cannabinoid driven brain homoeostasis. Taken together, these studies provide compelling evidence of a role for genetic and epigenetic variation in CB_1 gene regulation in disease susceptibility and drug response.

EFFECTS OF THE TYPE 1 CANNABINOID RECEPTOR POSITIVE ALLOSTERIC MODULATOR GAT211 ON ABSENCE SEIZURES AND THE ANXIETY-LIKE PHENOTYPE OF GENETIC ABSENCE EPILEPSY RATS FROM STRASBOURG

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Absence epilepsy is a developmental epileptic encephalopathy syndrome, characterized by short (10-20 sec) lapses in awareness (90% occurring in children < 4-14 years of age). Unfortunately, the most widely accepted anti-epileptic treatments ethosuximide and valproic acid, can produce side effects such as drowsiness, insomnia, confusion and hepatotoxicity, respectively. Additionally, these medications successfully manage absence seizures only in approximately 66% of patients. Targeting the type 1 cannabinoid receptor (CB1R) is considered as a potential therapeutic intervention for many forms of epilepsy, including absence seizures via reducing hyperexcitability of neurons presumably potentiating gamma-Aminobutyric acid (GABA)-ergic activity. Nevertheless, orthosteric agonists of CB1R produce undesirable intoxicating and desensitizing effects. Alternatively, positive allosteric modulators bind a distinct receptor site and enhance the efficacy of endogenous cannabinoid signaling and may exert its anti-seizure effect with limited side effects. Thus, the aim of this study was to explore the effects of acute treatment CB1R positive allosteric modulator GAT211 (10 mg/kg i.p.) on seizure activity and associated anxiety-like behavior in a rat model of childhood absence epilepsy - Genetic Absence Epilepsy Rats from Strasbourg (GAERS). Adult male GAERS and non-epileptic control rats (NEC) were implanted with recording electrodes in hippocampus and sensorimotor cortex to monitor seizure activity. After recovery from surgery, electroencephalography (EEG) was recorded twice for 3 h on separate days in well-habituated rats to a recording chamber. . Group of rats were treated with either vehicle or GAT211 1 h after recording was initiated.
Initial analyses revealed that GAT211 treatment decreased the total duration of seizure activity for 1 h after treatment. In addition, other groups of male and female GAERS and NEC were injected with either vehicle or GAT211 and tested on the elevated plus maze and acoustic startle for anxiety behavior. Vehicle-treated GAERS showed decreased open arm time on the elevated plus maze and increased startle (i.e. elevated anxiety) compared to NEC. Importantly, GAT211 treatment normalized both behaviours in GAERS without significant effects in NEC. These results suggest that positive allosteric modulation of CB1R may be therapeutically effective target for ameliorating absence seizures and their comorbidities such as anxiety.

Acknowledgements: This work was supported by grants from the Canadian Institutes of Health Research (CIHR; 386247 to RBL, 594325 to JGH) and the National Eye Institute at NIH (NEI; EY024717 to GAT). RBL is supported by as an endowed chair by GlaxoSmithKline.

PREFRONTOCORTICAL ENDOCANNABINOID-CB1 TRANSMISSION MEDIATES THE ANTIDEPRESSANT-LIKE BEHAVIOURAL AND NEUROPLASTIC EFFECTS OF STRESS CONTROLLABILITY

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Affective disorders such as major depression can be exacerbated by a perceived lack of control over stressors, a phenomenon that is linked to perturbed prefrontal-raphe and hippocampal neurotransmission. Conversely, cognitive-behavioural remediation ameliorates mood-related symptoms by enhancing behavioural control. We developed a rodent model of cognitive remediation based on a modified Morris water maze (with vs. without an escape platform) in mice. Animals were first exposed to at least six weeks of chronic mild stress (CMS), then were submitted to behavioural control training (BCT), where BCT+ animals were repeatedly allowed to learn to evade swim stress, while BCT- were subjected to inescapable swim stress. CMS-exposed animals exhibited depressive-like behaviours in the sucrose consumption, fruit loops and forced swim tests, as well as anxiety-like reactivity in the novelty-suppressed feeding and elevated plus maze tests. CMS also increased plasma corticosterone levels and decreased plasma levels of brain-derived neurotrophic factor (BDNF). The CMS-induced behavioural deficits were reversed by 8-10 days but not by 3 days of BCT in CMS-exposed animals (BCT+), an effect that was not observed in CMS-exposed BCT- animals. The therapeutic-like effects of BCT was nullified by administration of the cannabinoid CB1 receptor antagonist AM251 and recapitulated by the endocannabinoid enhancer URB597. We recorded long-term potentiation (LTP) in the dentate gyrus, and found that CMS disrupted LTP, and this impairment was rescued by BCT. This rescuing effect was abrogated by AM251. Electrophysiological recordings of prefrontocortical and raphe neuronal activity after CMS and BCT (BCT+ vs. BCT-), as well as assessment of CMS and BCT-induced changes in CB1 receptor density and fatty acid amide hydrolase (FAAH) expression are under way.

Acknowledgements: Funded by the Natural Sciences and Engineering Council of Canada

MATERNAL CANNABIS VAPOR EXPOSURE DOSE-DEPENDENTLY IMPAIRS BEHAVIORAL FLEXIBILITY IN ADULT OFFSPRING

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Cannabis is the most commonly used illicit substance among pregnant women, yet the effects of prenatal cannabis exposure on cognitive functioning remain largely unknown. With recreational cannabis laws now in effect in 8 states and counting, there is growing concern that maternal cannabis use during pregnancy could increase dramatically in the coming years. Thus, there is an urgent need to better understand the impact of prenatal cannabis exposure on cognitive functioning later in life.

In the current study, we investigated whether chronic exposure to vaporized cannabis during pregnancy alters cognitive flexibility in male and female offspring using an automated attentional set-shifting task. Female dams were passively exposed to vaporized cannabis extract (29.2% THC; 50 or 400mg/mL; 1 puff every 2 min for 1 hr, twice daily) or vehicle vapor throughout mating and gestation. Separate cohorts of dams were not exposed to any vapor during pregnancy. Beginning at postnatal day 55, all offspring were trained to press a lever that was paired with delivery of a cue light to receive a sugar pellet reward. On the day after learning criteria were reached, rats were tested in the set-shifting task, whereby they had to disregard the previously learned strategy in favor of an egocentric spatial strategy (i.e., ignore the cue and always press the left, or right, lever). On the final day of testing, rats were tested in a reversal-learning task that required them to press the lever opposite of the previous task. The number of trials required to meet criterion and the number errors, along with error type (perseverative, regressive, or never reinforced) and spatial reference of distractor (i.e., toward or away from cue) were tabulated and compared across groups.

Results indicate that rats prenatally exposed to cannabis vapor showed no impairment in visual cue discrimination, suggesting that they are capable of learning rule contingencies in a manner comparable to non-exposed rats. However, exposure to high (400 mg/ml) but not low (50 mg/ml) concentrations of vaporized cannabis extract resulted in significant impairment in attentional set shifting compared to no vapor control rats. Specifically, high-dose prenatal cannabis exposure led to an increased number of never-reinforced and regressive errors, which is indicative of an inability to acquire and maintain the new rule. There were no apparent deficits in reversal learning at either dose of cannabis and no significant sex differences in any endpoints of interest.

These data indicate that maternal cannabis vapor exposure dose-dependently impairs behavioral flexibility when tested in adulthood. Inability to acquire and maintain a new strategy mirrors the effects of inactivation of the nucleus accumbens core in similar tasks (Floresco et al., 2006). Accordingly, ongoing research is exploring differences in ventral striatal function and prenatal cannabis-induced alterations in dopamine- and endocannabinoid-related gene expression.

Acknowledgements: Funded by Washington State University's Alcohol and Drug Abuse Research Program (Dedicated Marijuana Account)

FETAL HEPATIC AND PLACENTAL CANNABINOID RECEPTOR 1 ($CB1_a$ and $CB1_b$) TRANSCRIPT VARIANTS IN MATERNAL UNDERNUTRITION

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Introduction: The endogenous cannabinoid system (ECS) plays an essential role in metabolism. Transcript variant of cannabinoid receptor 1 - $CB1_b$ – a strong inhibitor of adenylate cyclase- is the main receptor isoform in adult liver and fetal pancreas. Fetal growth and metabolism depends on maternal nutrition and fetal hepatic responses to maternal undernutrition (MNR) are well documented. However, no data are available regarding $CB1_b$'s role in fetal hepatic and placental responses to MNR. *The aim of this study* was to determine fetal gender-specific nutritional regulation of $CB1_a$ and $CB1_b$ transcript variants in liver and placenta in baboon (*Papio* spp.) MNR near term [165 dGA (days gestation)]**.**

Methods: Fetal liver and placental samples of a control group [CTR; male liver (ML) n=13; female liver (FL) n=13; male placenta (MP) n=9; female placenta (FP) n=9], in which pregnant mothers were fed *ad libitum* and an MNR group [ML n=9; FL n=9; MP n=8, FP n=6)], in which mothers received 70% of global feed eaten by CTR were studied. Quantitative RT-PCR analyses were carried out with isoform specific primers. Mann-Whitney U statistical test was performed.

Results: There were no significant differences between CTR and MNR groups ($p>0.05$). Expression of $CB1_a$ and $CB1_b$ was increased in CTR male fetal liver compared to CTR female fetal liver ($CB1_a$, $p=0.006$; $CB1_b$, $p=0.02$), this difference was absent in the MNR group. (Fig.1) Placental CB1R transcript variants were expressed but no significant differences were found. (Fig.2).

Conclusions: Nutritional regulation of $CB1_a$ and $CB1_b$ transcript variants in fetal liver is gender-specific. Fetal expression of CB1b isoform in hepatocytes might explain differences development of insulin resistance between male and female fetuses.

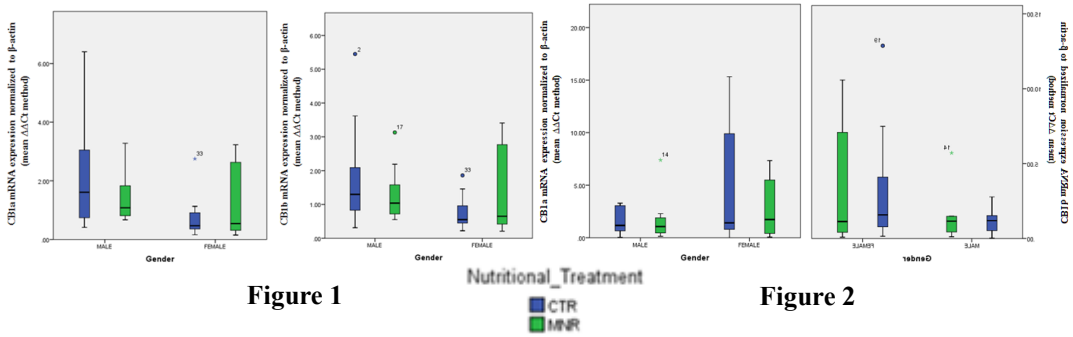

Figure 1 **Figure 2**

Figure 1. Fetal liver near term [165 dGA (days of gestation)]. CTR group [blue boxes; male (M) n=13; female (F) n=13] pregnant mothers fed *ad libitum*. MNR group [green boxes; M, n=9; F, n=9] mothers fed 70% of the global feed eaten by CTR during pregnancy. There were no significant differences between treatments ($p>0.05$). Expression of $CB1_a$ and $CB1_b$ was significantly increased in fetal liver male compared to female (Mann-Whitney U, $CB1_a$ $p=0.006$; $CB1_b$ $p=0.023$).

Figure 2. Placenta tissue at 165 dGA (days of gestation). CTR group [blue boxes; male (M) n=9; female (F) n=9] pregnant mothers fed *ad libitum*. MNR group [green boxes; M, n=8; F, n=6] mothers fed 70% of the global feed eaten by CTR during pregnancy. There were no significant differences between treatments in the expression of $CB1_a$ and $CB1_b$ transcript variants ($p>0.05$).

GESTATIONAL CANNABINOID EXPOSURE INFLUENCES EXTRACELLULAR KYNURENIC ACID AND GLUTAMATE LEVELS IN THE MEDIAL PREFRONTAL CORTEX OF ADOLESCENT OFFSPRING

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Cannabis is the illicit drug most commonly abused by pregnant women. The main psychoactive component of marijuana, delta^9–tetrahydrocannabinol (Δ^9-THC), can reach the fetus through the placenta and the blood-brain barrier. Several longitudinal studies of children and adolescents prenatally exposed to marijuana reported a significant impairment of higher cognitive functions, as well as a link to psychiatric disorders. Preclinical studies indicate that prenatal exposure to cannabinoids induces cognitive deficits in rat offspring. Moreover, these impairments are associated with alterations of aminoacidergic neurotransmission in the hippocampus and the prefrontal cortex (PFC). In particular, some of the deleterious effects on cognitive functions resemble those observed in adult rats, which had been prenatally exposed to the tryptophan metabolite (L-) kynurenine, the direct bioprecursor of the neuroactive compound kynurenic acid (KYNA). We therefore investigated whether alterations in KYNA levels in the rat brain might play a role in the short- and long-term consequences of prenatal cannabinoid exposure. Pregnant Wistar rats were treated daily with Δ^9-THC [5 mg/kg or vehicle (sesame oil) by oral gavage] from gestational day (GD) 5 through GD 20. Three vehicle-treated and five Δ^9-THC-treated dams were euthanized at GD 20, and the levels of kynurenine and KYNA were determined in maternal and fetal plasma and brain. The remaining dams (n=4 per group) gave birth, and one adolescent [postnatal day (PD) 35–45] male rat per litter was used to determine the extracellular levels of KYNA and glutamate by *in vivo* microdialysis in the medial PFC (mPFC).

No changes were found in kynurenine and KYNA levels in the fetal and maternal plasma or in the fetal and maternal brain. However, extracellular basal KYNA levels in the mPFC were significantly higher in prenatally Δ^9-THC-exposed adolescent rats ($p<0.01$) compared to the vehicle group. In addition, following gestational Δ^9-THC treatment, adolescent rats had significantly lower extracellular glutamate levels than the vehicle group ($p<0.05$). The present data demonstrate that prenatal cannabinoid exposure leads to long-term alterations of KYNA and glutamate levels in the mPFC in adolescence. As an increase in KYNA levels has been associated with cognitive dysfunction and psychiatric disorders, the possibility that this mechanism could underlie the detrimental effects of prenatal marijuana exposure is hypothesized.

Acknowledgements: This work was supported by USPHS grant, P50 MH103222; FAR2017 (LF).

ENDOCANNABINOID SYSTEM-RELATED GENETIC MODIFIERS IN A MOUSE MODEL OF DRAVET SYNDROME

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Recent evidence suggests that cannabinoids are effective in the treatment of Dravet syndrome (DS), a severe form of childhood epilepsy that is resistant to conventional antiepileptic drugs. DS patients experience multiple seizure types, cognitive and psychomotor delays and an increased risk of sudden death. 70-80% of DS patients carry mutations in *SCN1A* and heterozygous deletion of *Scn1a* (*Scn1a*$^{+/-}$) in mice engenders the hallmark features of DS, including spontaneous seizures, thermally-induced seizures and premature death. Interestingly, there exists a strain-dependent difference in phenotype severity with *Scn1a*$^{+/-}$ mice, where on a 129 strain the mice have a mild phenotype, while mice on a 129xC57BL/6J (F1) strain exhibit a severe epileptic phenotype. The strain-dependent difference suggests that there may be genetic modifiers driving the severity of the epileptic phenotype. *Gabra2* has already been established as a genetic modifier. The F1 strain displays reduced expression of *Gabra2* and treatment with clobazam, an agonist of the alpha2 subunit of the GABAA receptor, protects against hyperthermia-induced seizures. In this study we aimed to identify additional genetic modifiers of the *Scn1a*$^{+/-}$ mouse model in genes related to the endocannabinoid system and assess the functional implications of the identified genes. Whole-transcriptome RNAseq analysis of hippocampal tissue identified changes in the expression of cannabinoid-related genes; *Cnr1*, *Fabp5*, *Htr1a* and *Gpr55*. Within the F1 strain, which exhibits the more severe phenotype, expression of *Htr1a* and *Cnr1* were decreased while *Fabp5* and *Gpr55* expression were significantly increased compared to the 129 strain. Hippocampal endocannabinoid levels and lysophosphatidylinositol (LPI), the endogenous ligand of GPR55, were measured. Significant differences in 2-AG and LPI levels were observed between background strains, which could be contributing to the neuronal hyperexcitability and subsequent seizure susceptibility seen in the F1 strain. The involvement of GPR55 in the pathophysiology of DS is further supported by a recent study that demonstrated the anticonvulsant action of CBD was mediated through GPR55 antagonism. We have identified potential genetic modifiers of *Scn1a* and are assessing functional implications within the *Scn1a*$^{+/-}$ mouse model to determine their potential as novel drug targets in the treatment of DS.

ANTICONVULSANT EFFICACY OF PHYTOCANNABINOIDS IN A MOUSE MODEL OF DRAVET SYNDROME

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Dravet syndrome is a catastrophic epileptic encephalopathy that typically begins during the first year of life with febrile seizures and subsequently develops into other seizure types. Children with Dravet syndrome respond poorly to currently available antiepileptic drugs and have an increased risk of sudden unexpected death in epilepsy. This has led many families in Australia to resort to using illegal cannabis extracts, as there have been numerous reports of cannabis reducing seizures in Dravet syndrome. Cannabis, however, is a complex mixture containing hundreds of cannabinoids so the active constituent(s) need to be elucidated. Recently, a placebo-controlled trial of cannabidiol (CBD), a major component of cannabis, showed a reduction in seizures for childhood epilepsy. Here we used the *Scn1a*$^{+/-}$ mouse model to assess the efficacy of cannabinoids for the treatment of Dravet syndrome. The *Scn1a*$^{+/-}$ mouse model mimics the hallmark features of Dravet syndrome, including spontaneous seizures, thermally-induced seizures and premature death; thereby, providing an ideal platform to screen novel cannabinoids. We evaluated the anticonvulsant potential of ten cannabinoids (CBC, CBD, CBDA, CBDV, CBDVA, CBG, CBN, THC, THCA and THCV) on hyperthermia-induced seizures in *Scn1a*$^{+/-}$ mice. As expected, CBD treatment significantly increased the temperature threshold for thermally-induced seizures. CBDV and THC treatments also increased the temperature threshold but with much greater potency than CBD. Additionally, we found that the anticonvulsant potential of THC was potentiated by sub-threshold CBD. Surprisingly, THCV treatment appeared to have proconvulsant effects, exacerbating hyperthermia-induced seizures in *Scn1a*$^{+/-}$ mice. The *Scn1a*$^{+/-}$ mouse model has been used to identify cannabinoids other than CBD with anticonvulsant properties that could provide promising therapeutic leads in the treatment of Dravet syndrome.

SYSTEMS PHARMACOLOGY OF AN ENDOCANNABINOID SYSTEM MODULATOR IN ZEBRAFISH LARVAE

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The zebrafish is increasingly being used as an alternative pre-clinical *in vivo* model in drug research. Although widely used in drug toxicology and efficacy studies, the pharmacological aspects of this model still need to be established. Recently we quantified the pharmacokinetics of paracetamol in the zebrafish larvae. Here, we used endocannabinoid system modulator PF-04457845, a clinically proven inhibitor for FAAH, as a model drug compound to study the dynamic effect of the drug on 5-day-old zebrafish larvae using a system pharmacology approach. Activity based protein profiling (ABPP) was performed to investigate the serine hydrolase interaction profile of PF-04457845 and lipidomics to evaluate the drug effect on zebrafish lipid network.

PF-04457845 treatment showed a concentration-dependent increase of endogenous anandamide (AEA) levels. Through MS-based chemoproteomics, 55 serine hydrolases were identified in vehicle treated zebrafish larvae. We observed that *in vivo* exposure to PF-04457845 completely inhibited FAAH2 activity without cross-reacting with the other detected serine hydrolases. Using LC-MS based lipidomics, 235 lipids from 15 different classes were identified in vehicle treated zebrafish. Upon PF-04457845 treatment, N-acylethanolamine levels were increased, proving drug engagement with FAAH. The levels of other lipid species were not altered, demonstrating the absence of off-target effects with regards to lipid metabolism. Furthermore, internal exposure of PF-04457845 was assessed by taking a blood samples of the zebrafish larvae. Overall these results indicate that zebrafish is an attractive pre-clinical vertebrate model to study the system pharmacology of the drugs. We could prove target engagement of the drug, on-target effects and the absence of off-target effects with regards to a part of the protein landscape and lipid metabolism in zebrafish larvae.

QUALITATIVE AND QUANTITATIVE PROPERTIES OF TONIC CANNABINOID SIGNALING AT HIPPOCAMPAL GABAERGIC SYNAPSES

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CB_1 cannabinoid receptors have essential functions in the fine-tuning of neurotransmitter release probability. STochastic Optical Reconstruction Microscopy (STORM) super-resolution imaging and paired whole-cell patch-clamp recordings were used to delineate the molecular principles governing a tonic form of cannabinoid signaling at hippocampal GABAergic synapses. Correlating physiological, anatomical and molecular information obtained from single synapses between perisomatic interneurons and CA1 pyramidal cells, the receptor/effector ratio, a molecular parameter reflecting of how many CB_1 receptors regulate the voltage-gated calcium channels as effectors showed a strong inverse correlation with release probability. Notably, only the intrasynaptic/perisynaptic receptor/effector ratio, but not the extrasynaptic receptor/effector ratio predicted successful synaptic events. Administration of the CB_1 antagonist AM251 disrupted inverse correlation demonstrating that CB_1 signaling tonically controls GABA release. Surprisingly, in the absence of diacylglycerol lipase-α (DGLα), the enzyme synthesizing the endocannabinoid 2-arachidonoyl-glycerol, AM251 treatment could still increase GABAergic synaptic transmission. Conversely, depolarization-induced suppression of inhibition (DSI), a widespread form of phasic endocannabinoid signaling was not observed in DGLα knockout mice and DSI did not correlate with the intra/perisynaptic receptor/effector ratio. Taken together, these data demonstrate that the canonical synaptic endocannabinoid-synthesizing enzyme DGLα is not required tonic cannabinoid signaling, and imply that constitutive receptor signaling keeps a tight control over GABA release.

Acknowledgements: Funded by the Momentum Program (LP2013-54/2013) of the Hungarian Academy of Sciences.

PRADER-WILLI SYNDROME-INDUCED OSTEOPOROSIS BY *MAGEL2* LOSS IS REVERSED BY A NOVEL DERIVATIVE OF OLEOYL SERINE

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Prader-Willi Syndrome (PWS) is the most common known genetic cause of childhood-onset morbid obesity. Among a multitude of complications, such as hypotonia, cognitive impairment endocrine dysfunction, and altered endocannabinoid 'tone', subjects with PWS demonstrate marked bone abnormalities including decreased bone mineral density, osteoporosis, and subsequent increased fracture risk. While a number of causative loci within the imprinted Prader-Willi region have been implicated in the development of the disorder, the identity of the exact contributor to the development of PWS-induced bone disorders remains unknown.

Here we show, that loss of *Magel2*, a maternally imprinted gene in the PWS critical region, in mice resulted in a low bone mass phenotype, corresponding to that observed in humans with PWS as well as in individuals suffering from Schaaf-Yang syndrome (SYS). Reduced bone mass in *Magel2*$^{-/-}$ mice was associated with a reduced bone formation rate, increased osteoclastogenesis, and trans-differentiation of osteoblasts-to-adipocytes. Intriguingly, reduced skeletal and circulating levels of the endogenous regulator of bone homeostasis, *N*-Oleoyl serine (OS), was found in *Magel2*$^{-/-}$ mice as well as in individuals with PWS and SYS. Moreover, levels of OS were positively correlated with the osteoporotic phenotype of these individuals. A full reversal of PWS-induced skeletal abnormalities was achieved by treating *Magel2*$^{-/-}$ mice with oleoyl α-methyl serine (HU-671) a novel synthetic derivative of OS.

Taken together, *Magel2* plays a key role in modulating bone remodeling and mass in PWS by affecting OS levels and activity. Furthermore, these findings provide the rationale for further clinical testing of potent synthetic analogs of OS as bone therapeutics for treating osteoporosis.

Acknowledgments: This work was supported by the Foundation for Prader Willi Research (FPWR), the Israel Science Foundation (ISF) (grant #617/14), the NIH grant U54HD08302 (IDDRC, Clinical Translational Core), and the Tzvi Yanai grant provided by the Israeli Ministry of Science and Technology.

IDENTIFICATION OF P62 AS A NEW PROTEIN INTERACTION PARTNER OF THE CANNABINOID RECEPTOR 2

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The cannabinoid system displays important functions in the regulation of skeletal remodeling and bone mass. To better understand the molecular mechanisms in bone physiology, we aimed to identify protein interaction partners of CB2 receptors to shed new light on CB2 receptor function in bone modulation.

For the identification of putative interaction partners of CB2 receptors we used a method including systematic affinity purification and mass spectrometric analysis, which is specifically applicable for membrane receptors. Among the proteins with the highest probability of interaction we identified the scaffold and adapter protein p62 as a potential functional interaction partner of CB2. Mutations of the p62 gene in humans cause a disease called Paget Disease that is characterized by bone deformation caused by giant osteoclasts. In addition, mice deficient for p62 develop mature-onset obesity, impaired glucose tolerance, as well as insulin and leptin resistance with age.

Based on the common functions of the cannabinoid system and p62 in bone, we further investigated this interaction and we verified it by co-immunoprecipitation. Next, we identified a binding motif in p62 for the interaction with CB2 receptor. Furthermore, we investigated if the interaction of CB2 and p62 could affect bone homeostasis. Therefore we treated wild type (WT) and p62 knockout mice (KO) with CB2 agonist (JWH133), inverse agonist (AM630) and vehicle (DMSO) for 5 consecutive days. Subsequently, bones were analyzed by μCT-studies and histomorphometry. The cellular histomorphometry indicate that CB2 receptor activation and inhibition had a significant effect on bone cells in the homeostatic state of bone maintenance in p62 KO but not in WT mice. The treatment led to a significant increase in number and surface of osteoblasts and an increased number of osteoclasts. Additionally, a mild effect on mineral apposition rate was present in p62 KO mice only.

In summary, we present the identification of p62 as a novel CB2 receptor interacting protein and demonstrate that this interaction could help to balance bone homeostasis.

Acknowledgements: Funded by the DFG to MK (KA 2306/3-1).

DISCOVERY AND CHARACTERISATION OF AN *IN VIVO* ACTIVE NAPE-PLD INHIBITOR

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N-acylethanolamines (NAEs) are a family of signaling lipids biosynthesized by the enzyme *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD). A lack of potent and *in vivo* active inhibitors for this enzyme has hampered the study of these signaling molecules. Here, we report EM243 as the first brain-penetrant, *in vivo* active inhibitor for NAPE-PLD. Compound EM243 was identified through high-throughput screening and an extensive hit optimization campaign. EM243 showed nanomolar potency (K_i = 27 nM) for NAPE-PLD in a biochemical substrate assay and was selective over the other proteins of the endocannabinoid system. EM243 lowered a broad range of NAEs in a mouse Neuro2A neuroblastoma cell line, but not in NAPE-PLD$^{-/-}$ cells. Mice exhibited reduced NAE levels, including anandamide, in the brain after two hours of *i.p.* administration of EM243. Furthermore, the compound induced locomotor depression, hypothermia and elevated hot plate latencies in the mouse tetrad assay, but no signs of catalepsy or anti-nociception in the tail flick assay. These effects were also observed in cannabinoid CB_1-receptor knock-out mice. In a mouse model for inflammatory pain, EM243 was able to fully reverse lipopolysaccharide-induced allodynia. These findings suggest that lowering basal NAE levels has profound neurophysiological effects and induces an analgesic response via a non-CB_1-receptor mediated pathway.

Acknowledgements: Leiden University, Faculty of Science "Profiling Programme: Endocannabinoids"; to E.D.M., V.K., T.H., M.v.d.S. The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.

PRECLINICAL AND EARLY CLINICAL CHARACTERIZATION OF THE MONOACYGLYCEROL LIPASE INHIBITOR ABX-1431 FOR THE TREATMENT OF NEUROLOGICAL DISORDERS

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ABX-1431 is a first-in-class, small molecule inhibitor of monoacylglycerol lipase (MGLL), a serine hydrolase enzyme that regulates metabolic flux of the endocannabinoid 2-arachidonoylglycerol (2-AG). Inhibition of MGLL enhances 2-AG concentrations in the central nervous system (CNS) and potentiates 2-AG signaling through the cannabinoid receptors, while also reducing brain concentrations of arachidonic acid and downstream pro-inflammatory prostanoids, resulting in therapeutic effects in preclinical models of pain, anxiety, epilepsy and neurodegeneration.

ABX-1431 is a potent inhibitor of MGLL across species that inhibits 2-AG hydrolysis in human brain and peripheral blood mononuclear cell (PBMC) preparations. ABX-1431 was extensively profiled by gel- and mass spectrometry-based activity-based protein profiling (ABPP) in human tissue and cells and found to be highly selective. Oral administration of ABX-1431 to mice and rats resulted in potent and sustained inhibition of MGLL and accumulation of 2-AG in the brain and periphery. A pharmacokinetic/pharmacodynamic (PK/PD) relationship was defined in the rat formalin model of pain and used to predict the clinical dose.

ABX-1431 was well-tolerated following single- and multiple-ascending doses to healthy human volunteers with no serious adverse events. Dose- and time-dependent target engagement of MGLL was confirmed in PBMC using a novel substrate biomarker assay. CNS manifestations were observed at higher doses of ABX-1431, consistent with activation of the endocannabinoid system. Additional clinical assessments of mood, suicidality, cutaneous nociception and cognition revealed no significant abnormalities. In a placebo-controlled exploratory Phase 1b study in Tourette Syndrome (TS), a single dose of ABX-1431 showed benefit on TS symptoms, supporting MGLL inhibition as a novel CNS mechanism for the treatment of TS and other movement and neuropsychiatric disorders.

PALMITOYLETHANOLAMIDE PROMOTES A PRO-RESOLVING MACROPHAGE PHENOTYPE AND ATTENUATES ATHEROSCLEROTIC PLAQUE FORMATION IN MICE

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Palmitoylethanolamide (PEA) is an endogenous fatty acid mediator that is synthetized from membrane phospholipids by N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD). Its biological actions are primarily mediated by type-α peroxisome proliferator-activated receptors (PPARα) and the orphan receptor GPR55. PEA exerts potent anti-inflammatory actions and its metabolism is disturbed under inflammatory conditions. However, the role of PEA and its promise as a therapeutic agent in atherosclerosis remain unexplored.

We first studied the expression of NAPE-PLD in human atherosclerosis samples. NAPE-PLD mRNA levels were downregulated in unstable advanced plaques and they positively associated with smooth muscle cell (SMC) markers and negatively with macrophage markers. Secondly, apolipoprotein E deficient ($ApoE^{-/-}$) mice were fed a high-fat diet for 4 or 16 weeks and treated with either vehicle or PEA (3 mg/kg/day, 4 weeks) to study the effects of PEA on early and pre-established atherosclerosis. Without affecting body weight or plasma cholesterol level, PEA treatment reduced plaque size in both early and advanced atherosclerosis (by 34% and 32%, respectively). PEA also promoted signs of plaque stability as evidenced by reduced necrotic core size, increased collagen deposition and downregulation of M1-type macrophage markers. Mechanistically, we found that PEA increases the expression of the phagocytosis receptor MerTK and enhances macrophage efferocytosis, indicative of pro-resolving properties. These effects were dependent on GPR55 activation.

The present study demonstrates that PEA protects against atherosclerosis by promoting an anti-inflammatory and pro-resolving phenotype of lesional macrophages, representing a new therapeutic approach to resolve arterial inflammation.

FUNCTION OF THE BIOACTIVE LIPID PALMITOYLETHANOLAMIDE (PEA) AND ITS HYDROLYZING ENZYME N-ACYLETHANOLAMINE ACID AMIDASE IN TNBC PROGRESSION, INFLAMMATION AND METASTASIS

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Triple negative breast cancer (TNBC) has a high propensity to form brain metastases. When this disease reaches the brain, it is incurable and highly fatal. Tumors in the brain pose a complex problem as they are often surgically inaccessible and most of the therapeutic drugs cannot penetrate the blood-brain barrier (BBB). Therefore, there is an urgent need to selectively target TNBCs.

Recent studies showed that lipids play important roles in metastasis and cancer progression. Although fatty acid synthesis (FAS) overexpression confers a survival advantage to cancer cells, yet new compounds that target de novo FAS, are not yet in the clinic. Palmitoylethanolamide (PEA) is an endogenous bioactive lipid synthesized "on demand" by most mammalian cells, and is involved in the regulation of inflammation and pain processes. N-acylethanolamine acid amidase (NAAA) is a lysosomal enzyme which plays a central role in the deactivation of PEA. PEA reduces peripheral inflammation and exerts neuroprotective and antinociceptive effects and may putatively act as a modulator of inflammation and lipid deregulation in cancer. Thus, enhancement of endogenous PEA levels, by inhibition of NAAA enzyme, using specific NAAA inhibitors, represents a novel approach to treat tumor inflammation, tumor growth and metastasis using the body's own defense mechanisms. To this end, our Center of Drug Discovery at NEU has generated a new specific NAAA inhibitor named AM11095.

We hypothesize that the bioactive lipid PEA plays important roles in breast cancer metastasis via modulation of lipid signaling and inflammation. Thus, inhibition of PEA's catalytic enzyme NAAA via AM11095 should lead to changes of lipid signaling important in tumor-promoting signals in the tumor niches and inhibition of inflammation, angiogenesis and metastasis. We observed: 1) Significant increase in the expression of full length and splice variants of NAAA in human breast cancer cells, as compared to normal breast epithelial cells; 2) Exposure of breast cancer cells to PEA or AM11095 inhibitor resulted in significant decrease of secretion of inflammatory cytokines/chemokines from tumor cells as well as inhibited secretion of VEGF, endothelin-1, and endoglin, all angiogenic important factors in breaching the BBB integrity and facilitating tumor migration and tumor growth; 3) NF-kB signaling Multiplex pathway (6-plex-panel) was significantly inhibited in breast cancer cells treated with either PEA or AM11095 as compared to untreated cells; and 4) Both PEA and AM11095 inhibited tumor cell invasion, proliferation and migration. Using in vitro cell systems and in vivo mouse models for TNBC, we aim to further investigate the functional aspects of PEA/NAAA pathway using pharmacological and genetic approaches in TNBC tumor growth and metastasis via fatty acid networks in vivo. Regulation of PEA levels by blocking its hydrolysis with NAAA inhibitor AM11095, constitutes a potentially novel approach in inhibiting TNBC tumor growth, inflammation and metastasis.

EFFECT OF ORAL VCE-004.8, A CANNABIDIOL QUINOL DERIVATIVE, ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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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 the inflammatory stage and novel remyelination therapies are being pursued to restore lost function. While angiogenesis and blood-brain barrier (BBB) disruption are potentially negative in inflammatory phases, angiogenesis mediated by the stabilization of HIF-1α could be beneficial for neuroprotection and axonal regeneration in remitting phases and progressive forms of the disease. Therefore, the development of complementary treatments aimed at axonal repair and remyelination and/or slowing the progression of the disease through neuroprotection and axonal regeneration remain the most important objective in the clinical management of chronic progressive MS.

VCE-004.8 is a CBD derivative acting as a PPARγ/CB_2 dual agonist that ameliorates symptomatology in Experimental Autoimmune Encephalomyelitis (EAE) and Theiler's Murine Encephalitis Virus (TEMV) models of MS (Navarrete et al., 2018. J Neuroinflammation, in press). VCE-004.8 enhances $Arg1^+$ expression in macrophages and microglia cells trough PPARγ and CB_2 independent pathways, and shows hypoximimetic activity by stabilizing HIF-1α and HIF-2α in different cell types including human microvascular endothelial cells. In addition, VCE-004.8 induces angiogenesis and upregulates the expression of erythropoietin (EPO) and vascular endothelial growth factor (VEGFA). EHP-101, an oral, lipid-formulation of VCE-004.8, showed an excellent dose-dependent efficacy profile in (EAE). In the spinal cord, a transcriptomic analysis using RNA-Seq demonstrated that EHP-101 downregulated the expression of several genes, including chemokines, cytokines and adhesion molecules, which are closely associated with MS pathophysiology. Histopathological analysis revealed that EHP-101 treatment prevents microgliosis, demyelination and axonal damage. In conclusion, we provide evidence that VCE-004.8 is a promising small molecule to modulate relevant MS targets, being endowed with PPARγ and CB_2-mediated anti-inflammatory activity and may enhance remyelination through the induction of HIF-dependent neuroprotective factors such as VEGF and EPO.

Acknowledgements: Partially funded by MINECO grants SAF2014-53763-P and SAF2017-87701-R to EM.

ENDOCANNABINOIDS AS CHEMOTAXIC REGULATORS OF EARLY STAGE CORNEAL WOUND HEALING

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The cornea is an unusual and complex tissue that serves several vital roles. More than just a window into the world, the cornea is a highly innervated barrier that must rapidly heal in response to injury without many of the usual tools available to skin. Failure can result in infection and scarring or even blindness. Corneal healing is therefore highly choreographed, with an initial wound site cleanup – the lag phase – followed by migration toward the wound to cover it over. After this the corneal epithelial cells proliferate to replenish their accustomed numbers. It is likely that these epithelial cells respond to directional cues to guide their migration and a likely source of such a cue is chemical, i.e. chemotaxis.

The cannabinoid signaling system consists of G protein-coupled receptors, lipid messengers and the enzymes to produce and metabolize these messengers. Cannabinoid signaling is implicated in corneal wound healing since cannabinoid CB1 receptor knockout mice are slower to heal after corneal injury (Yang et al., 2013). We have tested *in vivo* wound healing in knockout mice for various components of the cannabinoid signaling system. In addition we have used *in vitro* migration assays as well as immunohistochemical and lipid profiling analyses to dissect the cannabinoid contribution to corneal wound healing. We have reported that corneal epithelial cells use cannabinoids and CB1 receptors as a chemoattractive guidance cue for migration (Murataeva et al., 2015). Extending this work, we confirm the slowed corneal healing in CB1 knockouts as reported by Yang et al., but find that at least one other cannabinoid receptor affects healing and chemotaxis, but appears to do so through chemo*repulsion*. We propose a complex chemotaxic regulation of corneal wound healing involving at least two, and possibly more, cannabinoid receptors to regulate the migration of epithelial cells during early stage corneal healing. In addition, we observe a role for immune cells positive for Lymphocyte antigen 6 complex locus G6D (ly6G) marker. Loss of CB2 receptor halts post-injury leukocyte migration towards wound site, altering wound healing.

OVERACTIVITY OF CB_1 RECEPTORS (CB_1R) ON MYELOID CELLS PROPAGATE FIBROGENIC MICROENVIRONMENT IN LUNG FIBROSIS

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Overactivity of CB_1R contributes to the pathophysiology of fibrosis in different organs, including the lung (*Cinar et al., JCI Insight 2017 2(8):92281*). Idiopathic pulmonary fibrosis (PF) is a progressive disease with high mortality, characterized by scarring of lung tissue. PF pathophysiology involves complex, multicellular interactions, and the contribution of distinct cell populations to disease progression is not well understood. Resident pulmonary macrophages have been found to express CB_1R, the specific contribution of which to fibrosis progression remains unexplored. We have examined the potential pathogenic role of CB_1R on alveolar macrophages (AM) and myeloid cells in a rodent model of bleomycin-induced PF and AM injury, using wild-type (WT), *Cnr1*$^{-/-}$, and myeloid cell-specific *Cnr1*$^{-/-}$ (*myCnr1*$^{-/-}$) mice. Fibrosis development was assessed by gene expression profiling of fibrogenic markers, hydroxyproline content, and histological staining of lung tissue collected 14 days after oropharyngeal administration of bleomycin (1U/kg). Bronchoalveolar lavage fluid (BALF) was collected to investigate the effects of CB_1R gene deletion on the status of infiltrating immune cells and AM phenotype by flow cytometry.

In vitro exposure of isolated AM to bleomycin resulted in increased CB_1R expression and increased production and secretion of anandamide (AEA). Bleomycin or ACEA treatment increased the expression of inflammatory regulators such as IL1β and interferon regulatory factor-5 (IRF5). The development of PF and the associated weight loss induced in WT mice by in vivo bleomycin treatment was fully prevented in *Cnr1*$^{-/-}$ mice and partially attenuated in *myCnr1*$^{-/-}$ mice. Bleomycin induced similar increases in total BAL cell number in the three strains, but infiltration of $GR1^+$ neutrophils and $CD19^+$, $CD8a^+$ and $CD4^+$ lymphocytes was almost completely absent in both *Cnr1*$^{-/-}$ and *myCnr*$^{-/-}$ mice. In addition, genetic deletion of *Cnr1* globally or in myeloid cells only similarly blunted the bleomycin-induced activation of AM, as quantified by CD11b and CD206 cell surface expression intensity. Furthermore, bleomycin-induced expressions of IRF5 and chemokine (C-X-C motif) ligand 13 (CXCL13) in WT mice were similarly attenuated in *Cnr1*$^{-/-}$ and *myCnr1*$^{-/-}$ mice. AM are sensitive to activate endocannabinoid/CB_1R system upon injury. Activation of myeloid CB_1Rs exacerbated fibrosis progression in bleomycin-induced PF in mice, and deletion of *Cnr1* in myeloid cells prevented fibrosis progression. Notably, myeloid CB_1Rs regulate AM phenotype and functionality by inducing a pro-inflammatory state after lung injury through increased expression of IRF5. Additionally, myeloid CB_1Rs control the infiltration of lymphocytes and neutrophils by regulating their chemoattractant production. These findings establish myeloid CB_1R as a therapeutic target in lung fibrosis, which could be engaged by peripherally restricted CB_1R antagonists for therapeutic benefit.

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

ACETAMINOPHEN ANTAGONIZES CB1 CANNABINOID SIGNALING AND EXERTS DUAL OPPOSING YET CB1-DEPENDENT EFFECTS ON MURINE INTRAOCULAR PRESSURE

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Acetaminophen is a widely used medicine for the treatment of pain and inflammation, in use for more than 140 years. Oral and injected acetaminophen have been reported to lower intraocular pressure (IOP), raising the possibility acetaminophen as an anti-glaucoma therapy. The hypothesized mechanism of action is indirect action on cannabinoid CB1 receptors: Acetaminophen is metabolized to AM404, a blocker of endocannabinoid uptake and/or metabolism, raising levels of anandamide, which then activate CB1 to lower IOP. The preferred treatment method for IOP-lowering drugs is via application of topical eye drops and acetaminophen has been shown to cross the cornea.

To explore the mechanism of action of acetaminophen we measured intraocular pressure after application of acetaminophen and its metabolite AM404 in mouse models, including knockouts for the cannabinoid CB1 receptor. We also measured the cannabinoid-related lipidomic profile of the mouse eye after acetaminophen treatment. Lastly we tested acetaminophen in autaptic hippocampal neurons to determine whether acetaminophen interacts with CB1-mediated cannabinoid signaling.

We find that direct topical application of acetaminophen *raises* IOP in a CB1-dependent manner. This rise in IOP is absent in CB1 knockout mice. However, when injected acetaminophen *lowers* IOP, also in a CB1-dependent manner. Furthermore, an acetaminophen secondary metabolite AM404 also lowers IOP in a CB1-dependent manner, though AM404 appears to also act at a second target, raising IOP in CB1 knockout mice. The lipidomic profile is only modestly altered by topical treatment with acetaminophen. Surprisingly, direct application of acetaminophen inhibits endogenous cannabinoid signaling in a concentration-dependent manner.

We propose that unmetabolized acetaminophen antagonizes CB1 cannabinoid signaling at concentrations routinely seen in human patients, a surprising finding. For this reason acetaminophen applied topically to the eye *raises* IOP by antagonizing endogenous CB1 tone. However, when acetaminophen is injected or ingested, it is converted via hepatic metabolism to the secondary metabolite AM404 that ultimately enhances CB1 signaling. Unfortunately, AM404 also raises ocular pressure via a second non-CB1 mechanism, though in our model the pressure-lowering effect is dominant. Our results show that even after 140 years there are still new things to be learned about one of the world's most widely used pain medications.

REGULATION OF NOCICEPTIVE BEHAVIOUR AND FEAR-CONDITIONED ANALGESIA BY 2-AG IN THE RAT MEDIAL PREFRONTAL CORTEX

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Fear-conditioned analgesia (FCA) is the robust pain suppression that occurs upon re-exposure to a context previously paired with an aversive stimulus. The endocannabinoid system has been shown to play a key role in mediating FCA (Finn et al., Eur J Neurosci. (2004) 848-52; Butler et al., Pain (2008) 491-500). The prefrontal cortex (PFC) plays a role in the expression of FCA in rats (Butler et al., Physiol. Behav. (2011) 1075-1081). The medial PFC (mPFC) consists of three subregions, the infralimbic (IL), prelimbic (PrL) and anterior cingulate (ACC) cortices which have been shown to differentially regulate the expression of contextually-induced fear and nociceptive behaviour (Almada et al., Neuroscience (2015) 988-97; Vidal-Gonzalez et al., Learn Mem (2006) 728-33). The aim of the present study was to investigate the role of 2-AG in the IL, PrL and ACC on conditioned fear, formalin-evoked nociceptive behaviour and FCA.

Male Lister-Hooded rats (n=7-11 per group) received footshock (10x1s, 0.4mA) or no footshock (controls) in a conditioning arena. 23.5 hours later, rats received intraplantar injection of formalin (2.5%, 50µl) into the right hind paw. 15 minutes later, rats received a bilateral microinjection (0.3µl) of either vehicle (VEH; 100% DMSO), the monoacylglycerol lipase inhibitor MJN110 (2µg/µl) or the CB_1 receptor antagonist AM251+MJN110 (AM251: 2mM, MJN110: 2µg/µl) into the IL, PrL or ACC and in a follow up study either VEH (100% DMSO), MJN110 (2µg/µl), the CB_2 receptor antagonist AM630 (5mM) or MJN110+AM630 into the ACC. 30 minutes post-formalin, rats were re-exposed to the conditioning arena for 30 minutes, and nociceptive and fear-related behaviour were assessed. Animals were euthanised post behavioural testing, the brain removed, and liquid chromatography-tandem mass spectrometry used to determine endocannabinoid and N-acylethanolamine levels in the three regions of the mPFC. Data were analysed by two-way ANOVA (with or without repeated measures) followed by Tukey's post-hoc tests.

Re-exposure to the context previously paired with an aversive stimulus resulted in robust expression of FCA. Administration of MJN110 into the PrL and ACC, but not the IL, attenuated FCA over the full 30 min trial, effects unaltered by co-administration of AM251.Co-administration of MJN110 and AM251 into the IL attenuated FCA. MJN110 had no effect on formalin-evoked nociceptive behaviour in non-fear-conditioned rats or on freezing behaviour in fear-conditioned rats. In our follow-up study, intra-ACC administration of MJN110 again attenuated FCA, an effect blocked by AM630. Intra-ACC AM630 reduced nociceptive behaviour in non-fear-conditioned rats, an effect attenuated by MJN110. Intra-ACC MJN110 increased the levels of 2-AG in the ACC of FC rats. Intra-IL and intra-PrL administration of MJN110 had no effect on 2-AG levels in the subregions of the mPFC.

These findings suggest an important role for MAGL in the PrL and ACC in the modulation of FCA. The data also suggest that FCA is attenuated by 2-AG-CB_2 receptor signalling in the ACC, and that CB_2 receptors in the ACC may facilitate formalin-evoked nociceptive behaviour.

Acknowledgements: Science Foundation Ireland (10/IN.1/B2976) and a PhD fellowship from Irish Research Council.

PALMITOYLETHANOLAMIDE, VIA PPAR-ALPHA RECEPTOR, RESTORES THE ALTERED PLASTICITY AND AMELIORATES THE COMPROMISED PAIN-RELATED BEHAVIORS IN THE HIPPOCAMPUS OF NEUROPATHIC MICE

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The hippocampus is an integral part of the Papez circuit involved in learning, memory, emotion, and motivation. Patients with chronic pain exhibit increased anxiety, depression, and deficits in learning and memory. Long-term potentiation (LTP) in the hippocampus has received attention as the biological substrate at the base of learning and memory. The activation of cannabinoid receptors, either directly by natural or synthetic agonists, or indirectly by selective inhibitors of the inactivation of endogenous cannabinoid receptor ligands (endocannabinoids), is widely supported by recent studies on neuropathic pain management (Goya et al.,2003; Cravatt et al., 2004; Maione et al., 2006). There is evidence that palmitoylethanolamide (PEA) is able to reduce pain-related behaviors and to restore glutamatergic synapses homeostasis in the medial prefrontal cortex of neuropathic mice (Guida et al., 2015). In this study, to investigate the impact of chronic pain condition on the hippocampal synaptic plasticity and on the related behavioral responses, electrophysiological, behavioural and biochemical analysis were performed, in a murine model of spared nerve injury (SNI), 30 days post-surgery (Decostered and Woolf, 2000). Moreover, the possible neuroprotective effect of chronic treatment with PEA, was evaluated, in both wild-type and Ppar-α -/- SNI mice. Our results showed, in 30 days SNI mice, a reduction of alternation in the Y-maze task, of recognition index in the Novel Object Recognition (NOR) test and of open-arm choice in the elevated plus-maze test, whereas neuropathy induced an increase of the time of immobility in the tail suspension test, as compared to the control group (Sham mice). Moreover, both neuropathic wild-type and PPARα null mice showed either an altered spatial memory retention and an impairment of LTP in the granule cells of dentate gyrus induced by theta-burst stimulations (TBS) of the perforant path (PP) in the entorhinal cortex (Jedlicka et l., 2009). In fact when the entorhinal cortex was electrically stimulated, a great potentiation of the EPSP (LTP), was observed in the ipsilateral hippocampus, in sham mice. PEA chronic treatment (14 days) increased the alternation in the Y-maze task, the recognition index in the NOR test and decreased the immobility time in the tail suspension test, suggesting that PEA was able to improve memory deficits and the depressive-like behavior but not the anxiety-like behavior associated to neuropathic pain. Finally, PEA partially restored the LTP in the dentate gyrus and ameliorated the altered spatial memory in wild-type SNI mice but not in PPARα/SNI null mice. These results suggest that neuropathic pain negatively affect the limbic and cognitive functions, which may underlie the deficiency of LTP and memory. Moreover, it opens new perspectives for the possible use of natural compounds such as PEA for the treatment of neuropathic pain and its central behavioural sequel

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AN EXPLORATORY ANALYSIS OF CIRCULATING ENDOCANNABINOID-RELATED LIPIDOME ASSOCIATED WITH THE TRANSITION FROM ACUTE TO CHRONIC LOW BACK PAIN

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Alterations within the endocannabinoid system have direct mechanistic effects on inflammation as well as peripheral and central sensitization which are processes involved in the transition from acute to chronic pain. As a possible molecular mechanism underlying pain chronicity we sought to examine the levels of endocannabinoid lipid-based molecules in a group of participants with acute low back pain (LBP). The endogenous cannabinoids *N*-arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol are members of an interconnected family of lipids that includes AEA's lipoamine structural analogs, additional 2-acyl glycerols, free fatty acids, and prostaglandins. Lipids in this lipidome share many biosynthetic and metabolic pathways, and play an important role in inflammatory and pain signaling. The aim of this study was to identify plasma endocannabinoid-related lipids that discriminate patients with acute low back pain that resolves within the first six-weeks since onset (acute resolvers) versus patients that develop chronic low back pain versus no-pain healthy controls. We conducted a pilot analysis among plasma samples from 16 acute resolvers, 16 patients who developed chronic low back pain (at baseline and 6 months following low back onset, yielding 32 samples) and 16 age- and gender-matched no-pain healthy controls. Methanolic extracts from human plasma samples were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole mass spectrometer. We observed significant elevation of circulating arachidonic acid in the plasma of those developing chronic LBP. We also observed elevated levels of other free fatty acids, 2-acyl glycerols and *N*-acyl glycines, including the AEA metabolite *N*-arachidonoyl glycine, as well as lowered AEA levels among the chronic group. This pilot analysis suggests that there are distinct alterations in the arachidonic acid cascade between acute resolvers, chronic low back pain, and no-pain healthy controls. Thus, lipidomic analysis may be able to discriminate at the onset of low back pain in patients who transition to chronic low back pain. Further research in this area is needed in order to fully understand the impact of the endocannabinoid-related lipidome in the process of pain sensitization and vulnerability to chronic pain.

Funding sources: This research was funded by the National Institute of Nursing Research (Starkweather, PI; R01 NR013932) and the UCONN Office of Research Scholarship Facilitation Funds (Starkweather, Ramesh, PI)

HORMONALLY DRIVEN SEX-DIFFERENCES AND SPINAL CHANGES IN ENDOCANNABINOID LEVELS IN HIV- 1 GP120-INDUCED NEUROPATHIC PAIN

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Pain is part of the clinical picture associated with Human Immunodeficiency Virus-1 (HIV-1) and Acquired Immune Deficiency Syndrome (AIDS), affecting 55-67% (Parker et al., 2014) of the 36.7 million infected individuals (http://www.who.int/hiv/en/). Unfortunately, many conventional agents utilized as pharmacologic therapies for neuropathic pain do not provide satisfactory analgesia in HIV-1 related neuropathic pain. The growing therapeutic use (self-medication) of cannabinoids by HIV-1 infected people, and the recent interest in the possible medicinal use of cannabinoids, particularly pain management, create an urgent need to identify potential interactions with HIV-1. HIV- viral protein glycoprotein 120 (HIV-1gp120) has been shown to induce neuropathic pain in rodent (Herzberg and Sagen 2001).

The goal of this study is to determine whether and how gp120-induced neuropathic pain impairs endocannabinoid (EC) system in male and female mice. Mice were treated with HIV-1 gp120 (50-200 ng) or control (inactive HIV-1 gp120). HIV-1 gp120 was given intrathecally on day 1, 3 and 7. Mechanical and cold allodynia were evaluated daily using (digital von Frey and acetone) for 4 weeks. We also analyzed the estrous cycle by assessing cytology of vaginal smear on a daily basis throughout the experiments, and used liquid chromatography-tandem mass spectrum (LC-MS) to determine the spinal level of 2-arachidonyl glycerol (2-AG). Our results show for first time that females are more sensitive to gp120-induced neuropathic pain-like behaviors, and this viral protein disturbs estrous-cycle, but this disturbance does not correlate with pain severity. Also, we found an impairment of endogenous level of 2-AG in spinal cord in female mice. Our results suggest that HIV-1 gp120 increase pain sensitivity in female mice (compared to male) independently of estrous-cycle, and impairs 2-AG levels. A better understanding of the hormonal and endocannabinoid levels changes occurring in HIV-induced pain is needed to improve treatment and find novel therapies.

Acknowledgements: Funded by The CH Foundation (Grant 241865 to JG), NIDA (Grant R01-361014 to KB) and Texas Tech University Health Sciences Center School of Medicine (Grant 121035 to JG).

PERIPHERAL CANNABINOID CB_1 RECEPTOR BLOCKADE REDUCES ETHANOL DRINKING IN MICE VIA THE GUT-BRAIN AXIS, BY LIMITING GHRELIN ACYLATION IN THE STOMACH

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There is a dynamic interplay between energy homeostasis and motivational behavior as exemplified by common mediators involved in the regulation of both, such as leptin, endocannabinoids, orexin and ghrelin. Endocannabinoids acting via CB_1 receptors (CB_1R) promote food intake and support addictive behavior, including alcohol seeking. Similarly, the stomach-derived hormone ghrelin stimulates energy intake and promotes alcohol drinking and craving. Although neural networks controlling consummatory and reward-seeking behaviors are located in the CNS, here we report that the peripheral CB_1R antagonist JD5037 reduces voluntary ethanol drinking in mice via an interaction with the stomach-derived hormone ghrelin. Daily oral doses of JD5037 reduced voluntary ethanol intake in wild-type mice, but not in mice deficient in CB_1R, ghrelin, or ghrelin receptor, all of which displayed lower baseline ethanol preference and intake than their respective wild-type littermates. Ethanol-drinking mice had significantly higher plasma ghrelin levels than control mice, and JD5037 treatment normalized the levels of the biologically active octanoyl-ghrelin without affecting its precursor desacyl-ghrelin. Ghrelin-producing cells of mouse gastric oxyntic mucosa and MGN3-1 mouse ghrelinoma cells can generate octanoic acid from long-chain fatty-acid precursors and also express components of the endocannabinoid system including CB_1R. Exposure of MGN3-1 cells to JD5037 suppressed octanoyl-ghrelin but not desacyl-ghrelin production by reducing the amount of octanoyl-carnitine generated from palmitic acid due to an increase in mitochondrial fatty acid oxidation, as reflected by increased activities of carnitine-palmitoyl-transferase and acyl-CoA dehydrogenase. We conclude that blockade of CB_1R in ghrelin-producing cells inhibits ghrelin acylation by decreasing the availability of octanoic acid as substrate, which limits the formation of biologically active acyl-ghrelin and its signaling to facilitate voluntary ethanol intake. Thus, peripheral CB_1R blockade affecting the gut-brain axis may have therapeutic potential in alcoholism.

Δ^9-TETRAHYDROCANNABINOL (THC) PRODUCES BI-PHASIC REWARDING AND AVERSIVE EFFECTS IN THE ANTERIOR VS. POSTERIOR NUCLEUS ACCUMBENS SHELL THROUGH DISSOCIABLE MU VS. KAPPA OPIATE RECEPTOR MECHANISMS AND DIFFERENTIAL MODULATION OF MEDIUM SPINY NEURON ACTIVITY STATES

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The phytochemical in cannabis primarily responsible for its psychoactive and motivational properties, Δ^9-tetrahydrocannabinol (THC), produces seemingly contradictory effects on reward and aversion at different doses. In addition, previous studies demonstrate that cannabinoid receptor transmission can strongly modulate opiate-related motivational processing. Emerging evidence suggests that there are anatomical and functional differences between the poles of the shell region of the nucleus accumbens (NASh). Stimulation of a so-called "hedonic hotspot" on the anterior pole produces reward, while stimulation of the rest of the structure either has no effect or produces aversion. Using a combination of conditioned place preference, social interaction, and *in vivo* electrophysiology we sought to elucidate how the unique properties of the NASh could be responsible for the biphasic effects of THC. Targeted microinfusions of THC into the anterior shell (+2.5mm from bregma) produces reward but microinfusions into the posterior shell (+1.5mm from bregma) produces aversion. Both these effects were challenged by either κ-opioid or μ-opioid receptor blockade. Reward in the anterior NASh was selectively blocked by co-administration of a μ-opioid receptor antagonist whereas aversion in the posterior NASh was selectively blocked by a κ-opioid receptor antagonist. Next, we wanted to examine if the effects of THC in the NASh generalized beyond producing reward and aversion. Using a social interaction test we demonstrated that infusions into the posterior (but not anterior) NASh induces deficits in natural sociability and social recognition memory. Conversely, THC infused into the anterior (but not posterior) NASh potentiates the reward salience of a normally subthreshold dose of morphine. Neither infusions into the anterior or posterior NASh have any effect on sucrose consumption, indicating these effects are specific to drug-related reward processing. We have previously demonstrated that cannabinoid modulation of medium spiny neuron activity states in the NASh potently control rewarding or aversive motivational states. Thus, using *in vivo* neuronal recording in rats, we demonstrate that intra-cranial ventricular (ICV) infusions of THC produce a predominate decrease in medium spiny neuron (MSN) activity in the anterior NASh (consistent with an accumbal neuronal reward signal) and an increase in the power of high-frequency gamma oscillations. Recordings in the posterior NASh following ICV THC infusions, however, show a predominate increase in MSN activity and a decrease in the power of high-frequency gamma oscillations (consistent with an accumbal aversion signal). Collectively, these data further categorize the functional differences present in the NASh and suggest that interactions with distinct opioid receptor substrates in the NASh are important for the effects of THC on affective processing.

CANNABINOID MODULATION OF THE ANALGESIC EFFECTS OF OPIOIDS IN HUMANS

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There is a rich literature of preclinical studies demonstrating cannabinoid agonist-enhancement of the analgesic effects of μ-opioid agonists. The aim of this study was to examine the analgesic effects of dronabinol alone and in combination with oxycodone in humans, using an array of laboratory pain models predictive of the clinical pain response.

Healthy participants (*n*=10) without current drug use or pain conditions completed this within-subject, double blind, placebo-controlled, randomized outpatient study. Nine 8-hr sessions were completed during which oral dronabinol (0, 2.5, 5 mg) was administered 1hr prior to oral oxycodone (0, 5, 10 mg) for a total of 9 test conditions. Sensory threshold and tolerance outcomes from a battery of experimental pain measures (cold pressor, pressure algometer, menthol-induced cold hyperalgesia, heat testing) were collected. Participant-rated, performance and physiological outcomes were also assessed.

Oxycodone (5, 10 mg) produced miosis and analgesic responses. Dronabinol alone did not produce consistent analgesic or pupillary effects. Depending on the dose combination, dronabinol attenuated or did not alter oxycodone analgesia. For example, dronabinol blocked the analgesic effects of 10 mg of oxycodone on heat threshold, pressure tolerance, and cold pressor tolerance. Oxycodone-induced miosis and nausea/vomiting (n=4) were not altered by dronabinol.

In contrast to previous animal research, this human study demonstrates that dronabinol attenuated the analgesic effects of oxycodone at select dose combinations. These data suggest that dronabinol may not be an effective opioid adjuvant and could potentially even increase opioid dose requirements necessary for acute pain relief. Future studies should examine chronic pain models and cannabinoid modulation of opioid analgesic tolerance.

Acknowledgements: Research funded by NIH (NCATS KL2TR000116) and the UK CTSA (UL1RR033173).

RATS LACKING FATTY ACID AMIDE HYDROLASE (FAAH) ACTIVITY SHOW REDUCED INTAKE AND MOTIVATION IN MODELS OF OPIOID ADDICTION

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The opioid epidemic has grown considerably in just the past 5 years, with overdose fatalities being estimated at about 60,000-70,000 for this year in the United States alone, representing a tripling of all drug overdose deaths in the span of a decade. At previous ICRS meetings, we have presented data demonstrating that FAAH inhibition may be a promising therapeutic target for excessive opiate use, and its ability to confer stress resilience may impede the transition from recreational use to compulsive dependency. In an effort to produce converging lines of evidence and expand our studies, we presented on the generation of a Wistar rat with a targeted deletion within exon 1 of the FAAH gene. Initial behavioral screening suggesting some similar anxiolytic effects under high-stress conditions, like that seen in the FAAH knockout mouse. However, there was also some concern over an apparent duplication of the FAAH gene specific to the laboratory rat genome. We have since confirmed that the duplicate gene, even if downstream of the original FAAH gene, does not produce measurable mRNA or functional enzyme activity.

We have also performed initial studies, in both males and females, looking for differences in opioid intake under extended-access self-administration conditions. Over a course of several weeks, rats will tend to escalate their intake, doubling or tripling their use, while also showing increased motivation for drug infusions. FAAH knockout rats demonstrate similar acquisition of opioid self-administration to drugs such as heroin and oxycodone, but show decreased intake of opioids following weeks of extensive self-administration, in agreement with data from daily inhibition using PF-3845. FAAH knockout rats show no altered lever pressing behavior when examining saccharin/sucrose preference or quinine sensitivity, suggesting no non-specific deficits in reward or aversion. We are currently working on examining motivation for drug seeking using progressive ratio performance, where rats must work harder for each progressive infusion, as well as traditional reinstatement (drug, cue, and stress) models of drug relapse to opiates.

Acknowledgements: Funding provided by VCU School of Medicine Startup, along with NIH grant R00DA037344.

MODULATION OF MORPHINE ANTINOCICEPTIVE EFFECTS AND MU OPIOID RECEPTOR BINDING BY A SELECTIVE CB2 RECEPTOR AGONIST IN MICE

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We and others have previously demonstrated that CB1 receptor activation interacts with morphine antinociception. It is unclear however whether modulation of the CB2 receptor affects morphine antinociception or the development of morphine antinociceptive tolerance. This is important to determine as CB2 agonists have also shown promise preclinically as neuroprotective agents and could potentially be given alongside opioid treatment following CNS injury.

In the first set of experiments, increasing doses of morphine and a selective CB2 agonist O-1966 were administered alone and in combination and their ability to alter thermal sensitivity on the hot plate was compared. Latency to attend to the hind paw on the hotplate was then assessed 30 min post last injection. Next, the effect of chronic morphine and O-1966 administration alone and in combination on the development of morphine antinociceptive tolerance was assessed. During the morphine tolerance dosing regimen, mice were treated with a twice daily dosing regimen for 5 days of vehicle, morphine, O-1966, or their combination. Based on these findings, we then assessed the effects of O-1966 on [H^3] DAMGO binding and on morphine-stimulated GTPγS binding. We also applied computer modeling and FRET analysis to determine whether O-1966 may be interacting with the mu-opioid receptor to affect mu-opioid receptor homodimerization.

Morphine (0.3 – 32 mg/kg IP) produced dose dependent antinociception, while O-1966 was without effect. Simultaneously injected morphine + O-1966 produced a 4-fold rightward shift in the morphine dose response curve (a decrease in morphine's antinociceptive effect) and was blocked by co-administration of the selective CB2 antagonist SR144528. Chronic administration of morphine produced antinociceptive tolerance, and co-administration of O-1966 during the morphine tolerance dosing regimen could potentiate the development of tolerance. O-1966 also attenuated [H^3] DAMGO binding to the mu opioid receptor and inhibits morphine's response in the GTPγS binding assay. Forced-Biased Metropolis Monte Carlo simulated annealing showed that O-1966 may bind to an exterior site in the TMH3-4-5 region of the mu opioid receptor, which has been previously shown to be necessary for mu opioid receptor homodimerization. Lastly, this finding was supported by FRET analysis results which showed that the presence of O-1966 decreased fluorescence intensity and inhibited FRET by approximately 25%.

These data suggest that the CB2 receptor agonist O-1966 and structurally similar compounds have the potential to modulate morphine signaling at the mu-opioid receptor, leading to a decrease in morphine's antinociceptive effects and an enhancement of morphine tolerance. These results are important giving the importance in mitigating morphine tolerance while improving efficacy, coupled with growing interest in determining the safety and efficacy of opioid/cannabinoid combinations.

Acknowledgements: Funded by NIDAT32DA007237, DA013429

BRAIN KINETICS OF NEUROTRANSMISSION DURING THC INTOXICATION

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Cannabis is the most commonly used illicit drug in the world. The acute effects of THC, the main psychoactive component of cannabis, on subjective and behavioral state are well known. However, the mechanisms underlying these acute effects have yet to be fully elucidated. Rodent studies assessing the acute actions of THC suggest that it selectively activates dopaminergic neurons in the ventral tegmental area, dose-dependently increasing dopamine neuron firing rates. This activation subsequently results in an increase of dopamine in the limbic system, which is implicated in the euphoric and rewarding effects of cannabis. However, there is less direct evidence from human studies regarding the effects of THC on the dopamine system. Instead, recent evidence suggests that THC may not act directly on dopamine firing, but acts indirectly by inhibiting glutamate release. The present study was therefore designed to assess the acute influence of two doses of THC on brain kinetics of glutamate, GABA, and dopamine, in relation to subjective experience, and cognitive performance. Twenty occasional cannabis users participated in a double-blind, placebo-controlled, crossover design. Participants received acute doses of cannabis (300 μg/kg THC) and placebo during two separate testing days. Magnetic resonance spectroscopy was used to assess glutamate and GABA levels in key areas of the limbic system, including the anterior cingulate cortex (ACC) and striatum. Dopamine was assessed indirectly by measuring functional connectivity between the nucleus accumbens and (sub)cortical areas during resting-state fMRI. The effects of THC on subjective high and sustained attention were also measured. In addition, THC kinetics in blood was assessed. Spectroscopy data shows that THC significantly increased glutamate levels in the striatum. Functional connectivity analysis showed that THC decreased connectivity between the nucleus accumbens and broad areas of the brain, indicating an increase of dopamine. Alterations in functional connectivity were correlated with changes in metabolite and behavioral measures. Preliminary results suggest that dopamine and glutamate may play an important role in the effects of THC on behavior.

DEVELOPMENT OF A CORE ASSESSMENT BATTERY FOR OBSERVATIONAL CANNABIS RESEARCH STUDIES

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Background: Cannabis use has been legalized for medicinal and non-medicinal purposes widely in recent years. This has occurred predominantly through legislative mechanisms rather than through traditional drug development mechanisms. As a result, there is an urgent need for data to capture the impact of cannabis legalization at both the public health and individual user levels. In response, many researchers have launched observational research studies to capture longitudinal data from individuals engaged in cannabis use, ranging from brief to very intensive data collection methods. A major limitation is that researchers often use a variety of assessments to measure the same target endpoint. A volunteer workgroup of cannabinoid scientists was convened to develop a core assessment battery recommended for participant self-reports in longitudinal observational research studies.

Methods: The research committee convened on bi-weekly conference calls throughout the fall of 2017 to discuss key assessment domains that are critical for collection in observational studies of individual level cannabis use outcomes. For each assessment domain, the committee reviewed known measures and discussed the merits of each. Preference was given to measures that 1) were validated and brief, 2) did not require purchase of a license to use, and 3) had good potential for international/cross-cultural use (e.g. available in multiple languages, not specific to culture of a single country). The experience of the participating scientists with each assessment also weighed in on decision making. Discrepancies were resolved through consensus discussion.

Results: The research group identified 9 assessment domains as critical for a core battery across studies for which validated assessments were available. These domains, with recommended assessment tool presented in parentheses, are as follows: 1) quality of life (World Health Organization Quality Of Life, short form; WHOQOL-BREF); 2) general health and well-being (36-item Short Form Survey; SF-36); 3) daily functioning (Brief Inventory of Psychosocial Functioning; B-IPF); 4) pain (Brief Pain Inventory; BPI); 5) mood (Inventory of Depression and Anxiety Symptoms, IDAS); 6) sleep (Insomnia Severity Index, ISI); 7) motives for cannabis use (Comprehensive Marijuana Motives Questionnaire, CMMQ); 8) cannabis use type and frequency (Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory, DFAQ-CU); and 9) effects of cannabis use (Marijuana Effect Expectancies Questionnaire, MEEQ-short).

In addition to the core battery, the committee recognized the importance of consistency in collecting demographic information. Demographics questions should include the following: Age, sex & gender, race/ethnicity, education, total annual household income, employment status, and military status. For any survey conducted evaluating medicinal cannabis use, it is also recommended that each participant report which health condition(s) they have for which the use of cannabis is intended to help and also that they provide a list of prescription and OTC medications (to include name of medication, dose, and frequency of use) they also use for the same health conditions(s) for which they are using cannabis.

Conclusions: There is an urgent need for "big" data sets about the impact of legalized cannabis at the level of the individual user, whether for medicinal or non-medicinal purposes. The best way of achieving this is to have researchers adopt a common set of instruments for measuring key outcome domains that will enable data sharing. This assessment battery is not intended to cover everything, but rather represents a minimum data set for evaluating health-related outcomes of cannabinoid product use that is agnostic with respect to any particular health condition. Each measure is validated. Additional measures that are specific to individual health conditions should be added for studies that are targeted for specific indications. Our hope is that these can be widely adopted for future or even ongoing research studies as a method of enhancing comparison of data across studies and provide an opportunity to combine data for more nuanced analyses of outcomes. Widespread use of a common battery will accelerate our understanding of the impacts of cannabis on health and hopefully will steer policy accordingly.

Acknowledgements: There was no financial support dedicated for this initiative.

DOES CANNABIS USE FACILITATE EXERCISE BEHAVIOR AMONG OLDER ADULTS? RESULTS FROM A SUPERVISED EXERCISE INTERVENTION

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As of this year, 63% and 21% of the U.S. population will live in a state with legal access to medical and recreational cannabis, respectively. Given studies that suggest that cannabis users experience increased caloric intake during acute intoxication, there are particular concerns that higher rates of cannabis use could exacerbate rates of obesity. Paradoxically, however, cross sectional data demonstrate associations between chronic cannabis use and lower body mass index, prevalence of obesity, insulin resistance, waist circumference, and rates of type 2 diabetes, despite data supporting higher caloric intake acutely. One route by which cannabis use may influence these metabolic outcomes is physical activity. There is some evidence that cannabis use may be associated with more frequent participation in physical activity (Vidot et al., 2017), either via psychological influences on motivation or on decreased perceptions of pain during or after exercise (Gillman, Hutchison, & Bryan, 2015). However, the literature is limited by the use of retrospective survey methodology.

We are currently conducting a randomized controlled trial examining the extent to which increasing physical activity in adults age 60 and over has positive impacts on cognitive function and downstream effects on social and emotional functioning. Inactive but otherwise healthy older adults are randomized to low intensity continuous exercise or moderate intensity continuous exercise+interval training and participated in a 16 week long supervised exercise intervention. Our location in Colorado, where cannabis has been legal since 2014, means that a proportion of older adults in our study are current cannabis users, giving us the opportunity to examine exercise adoption in older adults users versus non-users. A sample of non-users (n=15) were matched by age, gender, race, and experimental condition to a sample of current cannabis users (n=15). Half-way through the supervised exercise intervention, participants completed the 7-day Physical Activity Recall interview which queried their total exercise participation (supervised visits+any exercise done outside of the study) over the previous seven days. Regression analyses controlling for baseline physical activity and intervention condition showed that cannabis users were engaging in more total physical activity than non-users (p = .076). Though the data are preliminary, and the mechanisms for the effect as yet unknown, results will be discussed in terms of the potential for cannabis to facilitate physical activity in older adults.

Acknowledgements: Funded by NIH/NIA 1R01AG043452 (PI: A. Bryan)

FIELD SOBRIETY TEST FOR CANNABIS

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Cannabis and its active ingredient delta-9-tetrahydrocannabinol (THC) have been shown to impair memory, reaction time and attention. However, it is difficult to assess these impairments in the nonlaboratory setting. We have developed a prototype for a phone application called *Am I Stoned?*. As a first step, we tested the app in a within-subjects double-blind placebo-controlled study with THC (0, 7.5, 15 mg). Participants completed both iPhone-based and standard computer tests of cognitive speed, reaction time, fine motor ability, and memory. As a secondary aim we also assessed participants' ability to estimate their performance impairment. Twenty-four healthy experienced non-daily cannabis users completed a laboratory study, which included three 4-hour experimental sessions in which participants consumed a capsule containing THC (7.5, 15 mg) or placebo. They completed all tasks at both two and three hours after taking the capsule. Performance was impaired by THC in three of the four computer tasks, but only one of the iPhone tasks. It is likely that the computer tasks were more sensitive to THC impairment than the app task because the computer tasks were longer (15-20 min compared to 5-7 min), providing more opportunity to detect a drug effect. With regard to self-assessments, subjects were in general accurate in their awareness of impairments on the tasks. In a follow-up study we will improve the tasks used in the phone application to increase their sensitivity to THC impairment. This research is likely to lead to sensitive field tests that will allow users to objectively evaluate their ability to perform psychomotor or cognitive tasks. The research will also identify conditions under which individuals are or are not aware of their impaired state.

Acknowledgments: Funded by the National Institute for Drug Abuse (Grant. No. DA02812)

THE TRUTH IS IN TITRATION: IMPROVING SAFETY OUTCOMES OF THE WORLD'S FIRST INHALED CANNABIS PRESCRIPTION DRUG

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Canada benefits from a world-leading federally-regulated medical cannabis program that creates opportunities for cannabis research and the development of cannabis-based drugs. Hundreds of thousands of medical cannabis patients are currently authorized in Canada; however, cannabis products are not yet recognized as medical treatments and no universal cost coverage is available. The development of herbal cannabis products as prescription drugs presents challenges and requires collaboration between experts in drug development, cannabinoid medicine and clinicians. Such a partnership was made between the authors in 2017 to deploy the world's first Phase 1 study to investigate an inhaled herbal cannabis product, PPP001, a standardized pellet of dried cannabis containing 25 mg tetrahydrocannabinol (THC) and 5.5 mg cannabidiol (CBD) per dose. The researchers selected this formulation of THC-CBD based on published data (Andreae MH et al., J Pain. 16 (2015) 1221–32) and clinical experience at Santé Cannabis, a leading medical cannabis clinic located in Montreal, Canada. Inhaled cannabis was identified as the best approach to treat breakthrough pain and the ideal complement to long-acting medicine including oral cannabinoids for managing continuous symptoms in advanced cancer patients (Maida V et al., Curr Oncol. 23 (2016) 398–406).

The phase 1 study was designed as a double-blind, placebo-controlled trial of single and multiple daily ascending doses of inhaled PPP001 in 48 healthy male and female volunteers. Initially, a single dose of PPP001 or placebo-to-match pellet (0% THC/ 0.8%(2.2mg) CBD) was administered after randomization once (cohort A1), twice (cohort A2) and three times (cohort A3) on day 1, in a fasted state; 6 subjects per cohort received active drug, 2 received placebo; safety data was analysed prior to initiating the subsequent cohort. Secondly, doses of PPP001 or placebo were administered every day during a period of 7 days, at varying frequencies, once (cohort B1), twice (cohort B2) and three times (cohort B3) per day. THC and CBD were detectable in plasma in measurable concentrations 1 minute after initiation of dosing and remained detectable up to 3 hours post-administration. As expected, the peak plasma concentration (Tmax) was achieved within 5m post-administration and peak plasma concentrations (Cmax) were highly variable from 30 to 180 ng/ml (Figure 1). However, the rapid absorption of THC and CBD resulted in moderate to severe adverse events. Thus, a titration strategy based on experience at Santé Cannabis was implemented prior to proceeding to the 7-day administration to facilitate tolerance (Table 1). The titration approach led to a significant improvement in the adverse event profile of subjects in cohort B, who developed only mild or no side effects (Figure 2). Importantly, there was no accumulation of THC, CBD or the metabolite (11-OH-THC) and steady state was not achieved after multiple doses. Safety outcomes supported the design of the first Phase III trial of inhaled PPP001 for improving quality of life in advanced cancer patients (ClinicalTrials.gov NCT03339622). This trial was recently approved by Health Canada and is in the recruitment phase.

Acknowledgments: Study sponsored by Tetra Bio-Pharma Inc. and performed in collaboration with Algorithme Pharma.

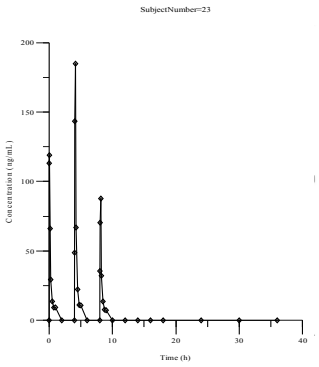

Figure 1. Pharmacokinetics of inhaled PPP001 in healthy volunteers

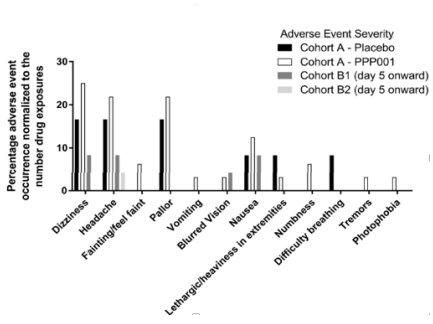

Figure 2. Adverse events after the administration of PPP001

Days	Number of inhalations	Time allowed to consume pellet
1	2	15 min
2	3	15 min
3	4	15 min
4	5	15 min
5-7	Until pellet was completed	15 min

Table 1. Titration regimen of inhaled PPP001

A GENETIC VARIANT OF FATTY ACID AMIDE HYDROLASE (FAAH) EXACERBATES GLUCOCORTICOID-MEDIATED METABOLIC OUTCOMES

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Glucocorticoid (GC) excess, either from endogenous overproduction or from exogenous medical therapy, is recognized to cause adverse metabolic outcomes. We have previously demonstrated that an endocannabinoid-mediated mechanism is responsible for GC-induced metabolic changes. Specifically, GCs significantly increase levels of the endocannabinoid anandamide, decrease the expression of the fatty acid amide hydrolase (FAAH, the primary catabolic enzyme responsible for inactivating anandamide signaling), and increase weight gain and adiposity. Interestingly, a common polymorphism (C385A) in the human FAAH gene is also associated with adverse metabolic outcomes. Subjects expressing the FAAH 385 A/A genotype present decreased FAAH expression, increased anandamide levels, and an increased risk of obesity. The aim of this study was to determine the effect of the FAAH C385A polymorphism on GC-mediated metabolic outcomes using a knock-in mouse model that recapitulates the human FAAH C835A mutation. Homozygous FAAH A/A mice were more susceptible to GC-induced hyperphagia and weight gain within the first week of GC exposure. Homozygous A/A mice were likewise more vulnerable to GC-induced changes in glucose tolerance. By contrast, there was no effect of the FAAH C385A polymorphism on either substrate utilization or energy expenditure despite a strong GC treatment effect. These findings agree with the increased body mass index and risk of obesity associated with the human FAAH A/A genotype. Furthermore, these findings extend the previous literature by demonstrating that the FAAH C385A polymorphism modifies GC-induced weight gain through its effect on feeding rather than energy expenditure.

Acknowledgements: Funded by the Killam Trusts, CIHR

ALTERED GUT MICROBIOTA AND ENDOCANNABINOID SYSTEM TONE IN VITAMIN D DEFICIENCY-MEDIATED CHRONIC PAIN

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Low-serum vitamin D (VitD) levels have been linked to chronic illness associated with weakened immune system, inflammation and pain states, particularly in intestinal mucosa. Here changes in VitD signaling induce modifications in composition and functions of the intestinal bacterial community, by affecting the healthy microbe–host interactions. We have previously demonstrated that the intestinal endocannabinoid system and related bioactive lipids strongly contribute to the development of some behavioral dysfunctions induced by antibiotic-induced microbiota perturbation. In this study we used a free VitD dietary intake to induce hypovitaminosis in mice and hypothesized that VitD deficiency may contribute to pain development through the modulation of endocannabinoid system. The free VitD diet induced gut microbiota unbalance characterized by the decrease of short chain fatty acids (SCFA)-producers, as compared with mice fed with a normal diet. VitD deficient mice developed tactile allodynia sustained by an enhanced neuronal activity at spinal cord level. Interestingly, pain behaviour was accompanied by reduced spinal Cannabinoid type 1 receptor (CB1R) expression levels and the administration of synthetic CB1R agonist ACEA failed to ameliorate pain symptoms. By contrast, the injection of the fatty acid amide Palmitoylethanolamide (PEA) significantly reverted tactile allodynia and reduced the neuronal excitability mediated by low VitD condition. Interestingly, the employment of spared nerve injury (SNI) model in VitD deficient animals caused a further reduction of serum VitD levels and, at the same time, a significant increase of gut Ruminococcus OTUs levels, classified in Lachnospiraceae family and Akkermansia. In these animals, PEA reverted allodynia in a similar fashion to control neuropathic animals, whereas ACEA was not effective. Collectively, these data indicate that VitD deficiency can lead to selective alterations in endocannabinoid signaling associated with pain development, where PEA treatment seems to ameliorate the related chronic pain symptoms and electrophysiological changes. Moreover, we prove that altered VitD status is responsible for deep changes in microbiota composition. The interaction between microbiota and endocannabinoid system in normal health and VitD deficiency condition remained to be elucidated.

POSTPRANDIAL EFFECTS ON ENDOCANNABINOID PLASMA PROFILES AND FAT TISSUE CB1 EXPRESSION WITH A HIGH CALORIE MIXED-MEAL TEST IN OBESE HUMANS UPON A 12 WEEKS RANDOMISED CONTROLLED TRIAL WITH 2 DIFFERENT ENERGY-RESTRICTED DIETS

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The endocannabinoid system (ECS) is dysregulated during obesity and its associated metabolic complications. Interestingly, alternatively to a potential pharmaceutical approach, nutritional intervention has recently emerged as a possible way to modulate the ECS and return to homeostatic conditions. An important aspect of metabolic health is the individual's metabolic flexibility or resilience to respond to a meal. Here, we assessed how plasma EC response curves and fat tissue gene expression is influenced by different diet interventions both fasted and in response to a meal. Hundred participants (aged 40-70 yrs; BMI: 31.3 ± 3.5 kg m^{-2}; non-diabetic) with abdominal obesity completed a 12 weeks parallel-designed, randomized intervention trial. Men and women were allocated to either a standard, Western-style, 25% energy restricted (ER) diet (n=39), a Targeted 25% ER (n=34) diet or were assigned to a control group (n=27). Before and after the 12 weeks intervention, participants were subjected to a high calorie mixed meal test (MMT). Postprandial plasma dynamics were determined for anandamide (AEA), 2-arachidonoylglycerol (2-AG), palmitoylethanolamide (PEA), oleoylethanolamide (OEA), stearoylethanolamide (SEA), *N*-arachidonylglycine (NAGly), docosahexaenoylglycine (DHAGly) and docosahexaenoylethanolamide (DHEA) at 6 time points; Fat biopsies taken at t=0 and t=4 hr were used for microarray analysis. For statistics linear mixed models was used.

Both the Western-style ER diet and Targeted ER diet resulted in significant weight loss (mean ± SD: -6.3 ± 3.9 and -8.4 ± 3.2 kg, respectively) and improved metabolic parameters upon 12 weeks intervention. Furthermore: 1) All measured ECs and related compounds, except 2-AG, showed a similar characteristic time curve in response to the MMT. Interestingly, at 5 min after food intake (t=10 min.), EC levels displayed a sharp rise, gradually declined till T=120min and successive raised till T=360min. CB1 expression in subcutaneous fat biopsies decreased 4 hr after MMT in all groups ($p<0.001$). 2) Within the Targeted ER diet group which was enriched in monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), complex carbohydrates and vegetable-derived proteins, AEA ($P<0.001$) and 2-AG ($P=0.047$) response curves were significantly decreased upon intervention while curves for DHEA ($P=0.009$) and DHAGly ($P=0.003$) had increased. Upon intervention, CB1 expression was significantly increased at baseline ($P=0.008$) and decreased in response to MMT ($p=0.048$) within the Targeted ER group only. Concluding, within a Targeted ER diet designed to improve metabolic health postprandial AEA and 2-AG profiles were significantly reduced and DHEA and DHAGly curves increased and CB1 expression sensitised. This well-controlled trial provides evidence that postprandial EC responses and CB1 expression alter by dietary intervention during energy restriction (ER), supporting a role for n-3 PUFA enriched diets to modulate endocannabinoid signalling during obesity.

MUSCLE CANNABINOID 1 RECEPTOR GOVERNS PHYSICAL PERFORMANCE AND WHOLE-BODY METABOLISM

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Westernized diets and a sedentary lifestyle result in obesity and associated metabolic disorders such as insulin resistance in muscle and other peripheral tissues, and lead to accumulation of fat in liver, muscle and adipose tissue. The cannabinoid 1 receptor (CB1R) plays a critical role in the regulation of whole-body energy metabolism because of its involvement in controlling food intake thorough its role in the central nervous system, and controlling distribution and utilization of fuel in the periphery, particularly in fat and liver. Additionally, inhibition of CB1R improves insulin secretion from pancreatic beta cells and enhances insulin sensitivity in both pancreatic beta cells and hepatocytes. We now describe the development of a skeletal muscle-specific CB1R-knockout (Skm-CB1R$^{-/-}$) mouse to study the specific role of CB1R in muscle.

We examined the phenotype of the Skm-CB1R$^{-/-}$ mouse in models of acute insulin resistance, diet-induced obesity and in aging. We found that ablation of CB1R in muscle prevents insulin resistance. Importantly, ablation of CB1R in muscle led to a significant increase in lean body mass and delayed loss of muscle mass with age. Skm-CB1R$^{-/-}$ mice also displayed increased physical endurance and energy expenditure compared to control mice. In fact, Skm-CB1R$^{-/-}$ mice showed increased lean/fat mass ratio when fed a high fat/high sugar diet and were able to run for 175% longer time on a treadmill than control mice. We further determined the specific mechanisms by which muscle-CB1R regulates mouse physiology. Our main findings are that ablation of CB1R in muscle enhances AKT signaling, which leads to reduced myostatin (MSTN) expression and increased IL-6. These changes in the myokines MSTN and IL-6 lead to increased myogenesis through their actions on MAPKs-mediated myogenic genes expression, favoring type 1 slow-twitch (oxidative) fibers, and improved mitochondrial performance in muscle by enhancing the oxidative phosphorylation coupled to ATP production. Therefore, chronic activation of CB1R in muscle, as occurs in obesity, would negatively impact body composition, favor fat formation and reduce whole-body insulin sensitivity and physical endurance.

Acknowledgments: This work was supported by NIH/NIA Intramural Research Program. S961 was donated by Novo Nordisk.

XIE2-64, A NOVEL CB2 RECEPTOR INVERSE AGONIST, INHIBITS COCAINE SELF-ADMINISTRATION AND OPTOGENETIC BRAIN-STIMULATION REWARD IN RATS AND MICE

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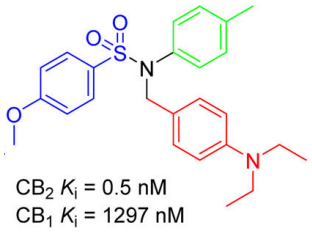
Cocaine addiction continues to be a serious public health problem, and so far, no effective pharmacotherapies are available for its treatment. We have recently reported that cannabinoid 2 receptors (CB2R) are expressed in midbrain dopamine (DA) neurons and functionally inhibit DA neuron activity and cocaine self-administration, suggesting that brain CB2Rs may be a new target in medication development for the treatment of cocaine addiction. JWH133 is a commonly used CB2R agonist in experimental animals. However, JWH133 displays species differences in its pharmacological action (more effective in mice than in rats), and therefore may have less translational potential for use in humans.

Xie2-64 is a novel CB2R inverse agonist reported recently (*Yang et al., J Med Chem, 2013, 56: 2045-2058, compound #57*). As shown in the attached figure, Xie2-64 is a highly potent and highly selective CB2R inverse agonist compared to JWH133 (Ki = 3.4 nM, CB1/CB2 = 200). Xie2-64 also displays favorable PK profiles (~10% oral bioavailability, $T_{1/2}$ ~10 hours after oral administration and ~ 17 hours after i.v. injections, unpublished data). Therefore, in the current study, we explored the potential utility of Xie2-64 in the treatment of cocaine abuse and addiction using multiple experimental animal models.



CB_2 K_i = 0.5 nM
CB_1 K_i = 1297 nM
Selectivity CB_1/CB_2 = 2594

Systemic administration of Xie2-64 (10, 20 mg/kg, i.p.) dose-dependently inhibited cocaine self-administration and cocaine intake under both fixed-ratio 2 and progressive-ratio schedules of reinforcement in rats. At 10 mg/kg, Xie2-64 significantly inhibited cocaine self-administration in wildtype mice, but not in CB2-knockout mice. However, at 20 mg/kg, Xie2-64 produced a significant reduction in cocaine self-administration in both genotypes of mice, suggesting that non-specific CB2R mechanisms are recruited at higher doses. To determine whether a DA-dependent mechanism underlies Xie2-64's action, we used optogenetic and transgenic approaches to express light-sensitive channelrhodopsin (ChR2) in DA neurons in the ventral tegmental area (VTA) in DAT-cre mice. We found that optical stimulation of VTA DA neurons induced robust intracranial self-stimulation (ICSS) behavior in a stimulation frequency-dependent manner, which was significantly suppressed by systemic administration of Xie2-64 in a dose-dependent manner. Optical stimulation of VTA DA neurons also produced strong place preferences for a stimulation-paired side of a two chamber-apparatus. Xie2-64 did not alter place preferences for DA stimulation, suggesting this drug does not block DA reward when response requirements for reward are minimal. Taken together, these findings suggest that the CB2R inverse agonist Xie2-64 may have efficacy as a new pharmacotherapy for psychostimulant abuse and addiction.

Acknowledgements: Funded by the NIDA Intramural Research Program (Z1A DA000389).

CANNABINOIDS AS METABOLIC REPROGRAMING AGENTS IN PROSTATE CANCER CELLS

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[2]University of Brescia, Department of Molecular and Translational Medicine, Italy

Cancer is characterized by uncontrolled proliferation of cells, invasion of neighboring tissues and metastasis. As an additional hallmark, all types of cancers are linked to impaired mitochondrial function and dysregulated energy metabolism generally supporting a view of cancer as a mitochondrial metabolic disease (Seyfried, Front Cell Dev Biol. 3 (2015)). Cannabinoids affect mitochondrial functions (Fisar et al., Toxicol Lett. 231 (2014) 62-71; Singh et al., J Mol Neurosci. 56 (2015) 926-931; Harkany et al. Front Neurosci. 11 (2017)).

We previously demonstrated that CBD (alone and in combination with CBG) significantly reduced ($p<0.05$ and $p<0.001$, respectively) tumor progression in TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice, which uniformly and spontaneously develop multistage autochthonous (orthotopic) prostate tumors following the onset of puberty. We also demonstrated that CBD:CBG (1:1) significantly reduced ($p<0.001$) the percentage of pathological adenomers and tumor progression from prostatic intraepithelial neoplasia (PIN) towards well-differentiated cancer. Finally, we set up an *in vivo* model of hormone refractory prostate with TRAMP mice and showed that the CBD:CBG mixture (1:1) significantly reduced tumor relapse ($p=0.0052$) in animals under hormone refractory status as compared to a standard chemo used for metastatic castration-resistant prostate cancer, Enzalutamide (MDV3100) ($p=0.02$).

Here, we investigate whether purified plant cannabinoids (CBD and CBG) affect respiration rates and mitochondrial function in an *in vitro* model (TRAMP-C2 cells) derived from TRAMP tumors as well as in a MDV3100-resistant phenotype of TRAMP-C2 cells. Preliminary results indicate that CBD modulates mitochondria membrane potential in TRAMP-C2 cells using fluorescence imaging. The effect in MDV3100-resistant cells is currently under investigation. Data on the effect of both phytocannabinoids (CBG and CBG) on different mitochondrial protein complex functions and membrane parameters will be presented.

Acknowledgments: The authors thank GW Pharmaceuticals for financial support.

HIGH POTENCY CANNABIS PRODUCTS: PUBLIC HEALTH THREAT OR RED HERRING?

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As of 2018, Canada has legalized access to cannabis at the national level while 63% and 21% of the U.S. population lives in a state with legal access to medical and recreational cannabis, respectively. Over the last decade the THC potency of cannabis flower has increased substantially with recent strains reaching THC potency in the 20-25% range. In addition, sales of concentrates with THC potencies up to 90% have increased dramatically. Recent increases in potency have sparked concerns about the impact of potency on public health outcomes. Because of the legal status of cannabis at the federal level in the U.S. and because potency of cannabis flower available for research at the federal level has lagged beyond potency of products in state-regulated markets (see Vergara et al., 2016), there have been no published studies on the impact of high potency products on health or behavioral outcomes. To address this gap in the literature, we have conducted a study on the acute effects of moderate (16% THC) and high potency (24%) flower as well as medium (70%) and high potency concentrates (90% THC) using a novel methodological approach that allows for the research to be consistent with federal laws in the U.S. To that end, regular users (n=90) were assigned to use cannabis flower or concentrates for 5 days. On the fifth day, participants were assessed in a mobile pharmacology lab immediately prior and after self-administering either flower or concentrate in order to assess the acute effects of the product on blood levels of THC as well as cognition, subjective drug effects, and mood. The blood data indicated significant differences in exposure to THC across the conditions in the expected direction ($p < .05$). The concentrate users demonstrated approximately twice the blood levels of THC as compared to the flower users. Consistent with our previous research (Bidwell et al., in press), the only cognitive measure that demonstrated a significant change before and after cannabis use was delayed verbal recall errors ($p < .05$). Likewise, subjective ratings of "High" showed significant increases after use. Surprisingly, there was no differences between the high and low potency conditions in terms of cognition or subjective effects of the cannabis, despite the fact that blood levels were twice as high in the concentrate group. The data suggest that product potency and blood levels are not associated with acute objective or subjective indicators of intoxication. History of use and tolerance are likely to emerge as important moderators of the acute, deleterious effects of THC. Further, the higher THC blood levels in concentrate users raise questions about longer-term effects of these high potencies products. Additional outcomes and analyses will also be discussed.

Acknowledgements: Funded by NIDA (Hutchison) and the state of Colorado (Bidwell)

THE THERAPEUTIC POTENTIAL OF CANNABIDIOL (CBD) FOR ALZHEIMER'S DISEASE – A PRECLINICAL PERSPECTIVE

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Background: In Alzheimer's disease (AD) pathological brain changes include the accumulation of amyloid-β (Aβ) and tau hyperphosphorylation causing neurodegeneration, neuroinflammation and oxidative stress. Current AD treatments do not stop or reverse the disease progression, highlighting the need for more effective therapeutic alternatives. Cannabidiol (CBD) has demonstrated anti-oxidant, anti-inflammatory and neuroprotective properties. Furthermore, we previously reported that chronic CBD treatment (20 mg/kg) reverses social recognition memory deficits in an AD transgenic mouse model (i.e. APPxPS1 mice). The current project compares the therapeutic-like effects of 50 mg/kg CBD in APPxPS1 mice and a mouse model for Tau pathology (i.e. Tau58/2 mice).

Methods: Male APPxPS1 and Tau58/2 mice as well as control littermates were treated with vehicle or CBD (50 mg/kg CBD, daily i.p. injections) starting 3 weeks prior to behavioural testing ($n >= 8$). A variety of cognitive domains including object and social recognition memory, spatial memory, and fear-associated memory were evaluated. Tau58/2 mice were also tested for anxiety and motor functions. Brain tissue of APPxPS1 mice was analysed for AD-relevant brain pathology including Aβ40 and Aβ42 levels.

Results: Vehicle treated male APPxPS1 mice demonstrated impaired social recognition memory and impaired reversal learning in the cheeseboard task. These deficits were absent in CBD-treated APPxPS1 mice. CBD also trended to reduce insoluble Aβ40 levels in the hippocampus of these mice. Early analysis suggests moderate effects of CBD on the behavioural impairments characteristic for Tau58/2 transgenic mice.

Conclusions: This study investigated the therapeutic-like effects of 50 mg/kg CBD on cognition and brain pathology of AD transgenic males. Chronic CBD treatment reversed cognitive deficits in APPxPS1 mice and had subtle effects on Aβ brain pathology. The therapeutic properties of CBD for a preclinical model of Tau pathology appear less significant.

Acknowledgement: CBD was provided free-of-charge by GW Pharmaceuticals (UK).

CANNABIS SIGNIFICANTLY REDUCES THE USE OF OPIOIDS AND IMPROVES QUALITY OF LIFE IN PATIENTS; PRELIMINARY RESULTS OF A LARGE PROSPECTIVE STUDY

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Background

This presentation will examine the preliminary findings from a large multi-site national prospective study of Canadian medical cannabis patients, with a focus on the impacts of cannabis use on prescription opioids and quality of life over a 6 month period.

Methods

The Tilray Observational Patient Study (TOPS) is underway at 18 different medical clinics in 4 provinces throughout Canada, enrolling 1118 patients as of February 14, 2018. Detailed baseline characteristics are gathered in person on REDCap via iPad during an initial patient clinic visit, with follow up at 1, 3, 6 and 12 months. A comprehensive cannabis use inventory and the World Health Organization Quality of Life Bref (WHOQOL-BREF) is self-administered by patients, and a detailed prescription drug use questionnaire is completed by the physicians at each visit. Preliminary analysis involved patients enrolled by Sept. 30, 2017 (N=789) of whom 540 had at least 1 follow-up visit, and 490 used medical cannabis post baseline.

Findings

The sample is 48% male (n=237) with a mean age of 47.9 (SD=14.2), and the top 3 symptoms reported by participants were chronic pain (77.1%; 378), insomnia (41.2%; n=202), and anxiety (35.3%; n=173). Mean cannabis use per week was 8gms per week at 1 month, and the most common method of use was *oral ingestion* (32.4%; n=159), followed by *joints* (30.8%; n=151) and *vaporization* (22.2%; n=109). Baseline opioid use was reported by 26.7% of patients, dropping to 13.1% of total study participants at 6 months. Average mgs. per day of use among the 39 patients using opioids at baseline that completed a 6 month follow-up dropped from an average of 104.3mgs (SD= 260.4) to 66.4mgs (SD=169.2) per day, a 36.5% decline, with 51.3% ceasing opioid use altogether. All 4 domains of the WHOQOL-Bref – physical health, psychological health, social relationships, environment – improved in the total population, with the most significant changes seen in physical health (11.3point/28.7% increase between baseline and month 6; [95% CI = 8.7-13.8]) and psychological health (9.3 point/17.3% increase [95% CI 6.8-11.7]).

Discussion

While cannabis substitution effect for prescription drugs has been identified and assessed via cross-sectional and population level research, this study provides a granular individual-level perspective of cannabis substitution for opioids and other prescription drugs and associated improvement in QOL over time. The high rate of cannabis use for the treatment of chronic pain and subsequent substitution for opioids suggests that cannabis may play a harm reduction role in the ongoing opioid dependence and overdose crisis, and improve the quality of life of patients.

CANNABIDIOL AND Δ^9-TETRAHYDROCANNABINOL TREATMENT OF HINDPAW PAIN AND INFLAMMATION IN MALE VS FEMALE RATS

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Using tests of acute pain in healthy rats, Δ^9-tetrahydrocannabinol (THC) is a more potent antinociceptive agent in females compared to males. However, females develop greater tolerance than males to THC's antinociceptive effect, suggesting that the effects of chronic THC may be similar between the sexes. THC also produces anti-inflammatory effects that are greater in males than females, suggesting that THC may be more immunosuppressive in males. A few studies, in males only, have shown antinociceptive effects of cannabidiol (CBD) against inflammatory pain. The purpose of this study was to compare the effects of acute vs. chronic THC or CBD exposure on inflammatory pain in male and female rats. In Experiment 1 (behavior), on day 1, baseline measurements were taken and inflammation was induced by intraplantar injection of complete Freund's adjuvant (CFA). THC (0.0, 1.0, 2.0 or 4.0 mg/kg, i.p.) or CBD (0.0, 1.25, 2.5, or 5.0 mg/kg, i.p.) was administered twice daily on days 1-3. On day 4, rats that had previously received THC or CBD were injected with the same dose of drug, while rats that had previously received vehicle were injected with vehicle or an acute dose of drug. Allodynia, hyperalgesia, biased weight-bearing, locomotor activity, and edema were assessed 30-240 min post-injection. In Experiment 2 (immune activity), rats received an intraplantar injection of CFA or mineral oil (no-pain control). THC (0.0 or 2.0 mg/kg, i.p.) was administered in the same manner as Experiment 1. Two h post-injection on day 4, serum samples were collected and analyzed via ELISA for interleukin (IL)-6 and -1β.

CFA induced hindpaw inflammation resulting in allodynia, hyperalgesia, biased weight-bearing, and decreased locomotor activity. Acute and chronic THC caused dose-dependent anti-allodynia in both sexes; tolerance did not develop to THC's anti-allodynic effect in either sex. Acute THC also caused dose-dependent anti-hyperalgesia and antinociception (effects >100% baseline), and antinociception was greater in females than males. Both males and females developed tolerance to THC's antinociceptive effect, but not to its anti-hyperalgesic effect. Neither acute nor chronic THC reduced biased weight-bearing in either sex. Acute THC dose-dependently reduced locomotor activity in both sexes, whereas rats treated with THC chronically did not show locomotor suppression. Chronic, but not acute, THC reduced paw edema in both sexes. In contrast to THC, neither acute nor chronic CBD altered allodynia, hyperalgesia, biased weight-bearing, locomotor activity, or edema. In Experiment 2, females had higher serum IL-6 levels than males, and CFA increased IL-6 and -1β levels in both sexes. THC did not alter IL-6 levels in either sex, but increased IL-1β levels in males. In contrast, THC decreased IL-1β in females with inflammation, but had no effect in females that received mineral oil. These results suggest that in both sexes, THC can reduce inflammation and pain, and that tolerance develops to the sedative but not therapeutic effects of THC. THC's pain-relieving effects may be due in part to its attenuation of inflammatory cytokines in females but not males.

Acknowledgements: Funded by the National Institute on Drug Abuse (DA016644).

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

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The CB1 N-terminal membrane proximal region has been revealed to have effects on the orthosteric ligand binding site if deleted or mutated.(Fay & Farrens, Biochemistry, 2013; Iyer et al., Mol Pharmacol, 2015; Murphy & Kendall, Biochem Pharmacol, 2003) The N-terminus has also been proposed as a extracellular cover or lid over the orthosteric pocket.(Fay & Farrens, Biochemistry, 2013) A recently reported x-ray structure of Taranabant bound to the inactive state of CB1 showed deep insertion of the N-terminus into the orthosteric pocket with residues F102 and M103 in direct contact with the co-crystallized ligand.(Shao et al., Nature, 2016) The deep penetration of the N-terminus, particularly F102 and M103, also forced K3.28, the residue shown to be a primary interaction site for most cannabinoid ligand classes (both antagonists and agonists) out of the ligand binding pocket.

It is not uncommon for receptor regions in GPCR x-ray crystal structures, particularly N-termini, to have unexpected contacts with the ligand binding pocket caused by crystal packing effects that are not physiologically relevant. A case in point is His54 in the N-terminus of the mu opioid receptor with agonist, BU72.(Huang et al., Nature, 2015) To test if the positions of F102 and M103 were physiologically relevant, we undertook a combined mutation and modeling study to test the importance of F102, and M103 to the binding of Taranabant in the CB1 inactive state. Binding studies used [^{3}H]-CP55,940 as the radioligand because CP55940 is unaffected by any of the mutations. The M103A and M103G mutations had no effect on Taranabant binding and an F102A mutation had a small (5-fold) effect. These results suggest that F102 and M103 do not have direct contact with Taranabant. However, in an F102A/M103A double mutant, Taranabant had a 40 fold drop in binding affinity. Modelling studies of the CB1 WT N-terminus in the inactive state suggest that F102 lies above and over the EC2 loop and M103 has favorable Van der Waal's contacts with this loop. These stabilize the EC2 loop F268 interaction with Taranabant. While loss of F102 or M103 via single point mutation does not change the EC2 loop conformation, the F102A/M103A double mutation results in an N-terminus that no longer interacts with the EC-2 loop, and allows the EC2 loop to move away from Taranabant and destabilize the ligand interaction with F268. Taken together, these results suggest that F102 and M103 are not in contact with Taranabant in the CB1 inactive state.

Support provided by NIDA DA003934 and School of Medical Sciences, University of Auckland.

CHARACTERIZATION AND MODULATION OF HUMAN CAV3.2 CHANNELS BY SYNTHETIC CANNABINOIDS *IN VITRO*

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$Ca_v3.2$ channels are members of the Low-voltage-activated calcium channel (T-type I_{Ca}) family. They play a critical role in several physiological processes including regulating rhythmicity in the heart and brain. Altered T-type activity has been implicated in several disease states including; cardiac arrhythmia, epilepsy and pain. Interestingly, some synthetic cannabinoids have been associated with severe adverse reactions that closely resemble the disease states associated with disruption of T-type I_{Ca} function. Several laboratories, including our own, have recently reported that some cannabinoid-based compounds can potently block and modulate T-type activity. The aim of this study is to determine whether synthetic cannabinoids modulate T-type I_{Ca} and how these drugs interact with human $Ca_v3.2$ in vitro.

Whole-cell voltage clamp recordings were made from HEK293 cells expressing $hCa_v3.2$. A screen of 14 synthetic cannabinoids at 10 μM revealed 2 compounds that potently blocked $hCa_v3.2$. One of these compounds, Methyl-2-[[1-(cyclohexylmethyl)indole-3-carbonyl]amino]-3,3-dimethylbutanoate (MDMB-CHMICA) has been associated with severe toxic side-effects including death. The other compound, Methyl 2-((1-(cyclohexylmethyl)-1H-indazole-3-carbonyl)amino)-3-methylbutanoate (AMB CHMINACA), is a less commonly reported drug. We further explored the block of Cav3.2 by these drugs and compared them with the well characterized effects of THC and CBD.

When cells were held at –100 mV and stepped to -30 mV, MDMB-CHMICA and AMB CHMINACA rapidly blocked $hCa_v3.2$ with $IC_{50's}$ of 1.5 and 0.74 μM respectively, compared with EC_{50}'s of approximately 10 μM for THC and CBD. We reported previously and confirmed here that both THC and CBD significantly shift the half activation and steady state inactivation kinetics of $hCa_v3.2$ to more negative potentials but in contrast, MDMB-CHMICA and AMB CHMINACA did not affect these channel properties. Frequency dependent block of $Ca_V3.2$ by THC but not CBD was also consistent with previous findings. When currents were evoked at 0.2 and 0.5 Hz neither of the 2 synthetic compounds showed any significant change in the rate of block vs control. However, at 1 Hz, AMB CHMINACA showed a significant increase in channel block. Finally, slow inactivation block of $hCa_v3.2$ was assessed in presence and absence of drug using a protocol where a test pulse (P2) follows a 10-s conditioning pre-pulse of -80mV to elicit a peak current amplitude that is approximately 40 to 50% of the initial test pulse (P1). Under these conditions, all 4 drugs showed significant increase in channel block. This suggests that both the synthetic cannabinoids and THC and CBD preferentially bind to $hCa_v3.2$ while in this conformation. These results are the first to systematically test the effects of synthetic cannabinoids on human $Ca_v3.2$. *in vitro* and although it is likely the hallucinogenic and psychotomimetic effects of these drugs are due to their increased potency on CB1 receptors, other severe side effects, including arrhythmia and seizures are yet to be established. These data suggest that one possible cause may be the high affinity and interaction of these drugs with Cav3.2 and potentially other ion channels of similar structure.

THE SEIZURE-INDUCING ACTIVITY OF VARIOUS CLASSES OF CANNABINOIDS

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Synthetic cannabinoid agonists like JWH-073 and AM2201 are commonly found in preparations currently on the market as recreational drugs (i.e. Spice or K2). Several studies document serious cardiac and central nervous system toxicities including myocardial infarctions and seizures.

In our laboratory we happened to notice that some mice being treated with the high-potency synthetic cannabinoid agonist AM2201 appears to have convulsions. Subsequent experiments were conducted to determine ability of natural and synthetic cannabinoids to cause seizures using mice as a model. Since the use of *Cannabis sativa* has not been associated with seizures in humans, it was hypothesized that the primary active constituent, Δ^9-tetrahydrocannabinol (THC) would cause fewer, if any, seizures than synthetic cannabinoids. Three different chemical classes were investigated. The classes included plant-derived cannabinoids and analogs, two indole synthetic cannabinoids and an analog of the endocannabinoid anandamide. Specific drugs in the first group were THC, cannabidiol, HU-210, CP55940; the indole agonists were JWH-073 and AM2201; and the analog of the endocannabinoid was methanandamide.

Groups of male C57BL/6J mice were given intraperitoneal (IP) injections with various doses of each compound, their change in body temperature was measured, and their activity was recorded for 3 hours. An observer of the video recordings noted the time and relative severity of each observable seizure (convulsion). Preliminary dose-response experiments determined the doses needed to produce the maximum number of convulsions for each drug, and confirmed the relative efficacy of each drug by change in body temperature 45 and 360 min after injection of each drug.

Preliminary results indicate that THC and HU-210 produce far fewer observable seizures than the synthetic agonists CP55940, JWH-073 or AM2201, which all produced similar number of seizures at the maximum effective doses. Methanandamide did not produce any seizures at a dose that produced a decrease in body temperature as great as that caused by any other agonist at any dose.

Future experiments will determine whether cannabidiol can induce seizures in mice and whether cannabinoid agonist-induced seizures are attenuated by CB_1 or CB_2-selective antagonists or cannabidiol.

GENETIC VARIATION IN ENDOCANNABINOID SIGNALING AND BRAIN AND BEHAVIORAL MECHANISMS UNDERLYING FEAR EXTINCTION IN CHILDREN AND ADOLESCENTS

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The endocannabinoid system modulates emotion-related behavior and is implicated in fear-based disorders (e.g., anxiety, posttraumatic stress disorder). Neuroimaging studies in adults have linked genetic variation in endocannabinoid signaling with variation in fear-related neural circuitry. For example, individuals carrying the A allele of the fatty acid amide hydrolase (*FAAH*) gene (rs324420) – associated with lower enzymatic degradation and higher brain endocannabinoid levels – demonstrate increased resting-state functional connectivity between the amygdala and the ventromedial prefrontal cortex (vmPFC). Previous studies link higher amygdala-vmPFC connectivity with lower anxiety and better extinction recall ability, suggesting that the endocannabinoid system is involved in fear-extinction learning. However, despite evidence that these disorders frequently begin in childhood and adolescence, no studies to date have examined the impact of the endocannabinoid system on fear-extinction neural circuitry in youth. This study reports on 48 children and adolescents (ages 6-17 years) who completed fear-extinction learning using a novel virtual reality paradigm. Twenty-four hours later, functional magnetic resonance imaging (fMRI) and skin conductance response (SCR) data were collected while completing a test of extinction recall, and during a standard 10-min resting-state paradigm. Genetic data were collected from saliva for the *FAAH* rs324420 variant by Taqman Genotyping.

During extinction recall, *FAAH* A-alleles demonstrated lower response in the dorsomedial prefrontal cortex, a region involved in conditioned fear responding, relative to youth with the CC genotype. A-alleles also demonstrated lower SCRs during recall, but this effect did not reach significance (p = 0.062). Consistent with better recall of extinction learning, *FAAH* A-alleles also demonstrated increased amygdala-vmPFC resting-state functional connectivity. Our data suggest that genetic variation in endocannabinoid signaling alters fear-extinction related neural circuitry in children and adolescents, and may therefore play a role in susceptibility to fear-based disorders.

Acknowledgements: The Wayne State University Department of Pharmacy Practice and American Cancer Society and Karmanos Cancer Institute Institutional Research Grant 14-238-04-IRG (CAR). Dr. Marusak is supported by American Cancer Society award 129368-PF-16-057-01-PCSM. Dr. Rabinak is supported by NIH National Institute of Mental Health grant K01MH101123 and R61MH111935.

A CHRONIC LOW DOSE OF Δ^9-THC RESTORES FAILING CANNABINOID SIGNALLING AND THUS BRAIN AGEING IN OLD MICE

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Brain aging is accompanied by a number of cellular and molecular changes that may ultimately lead to cognitive deficits. One of these changes is a decline in the activity of the endocannabinoid system characterised by diminished 2-AG levels and reduced coupling between CB1 receptors and Gi-protein. Reduced CB1 receptor signalling in $CB1^{-/-}$ or $GABA/CB1^{-/-}$ mice leads to accelerated brain ageing, therefore we asked whether activation of CB1 receptors could alleviate symptoms of brain ageing. We showed that the normal age-related decline of cognitive functions can be counteracted with a chronic low dose of Δ9-tetrahydrocannabinol (THC). Thus, twelve month-old mice receiving THC showed improved spatial learning and memory accompanied by an enhanced expression of synaptic marker proteins and an increased spine density in the hippocampus. Most strikingly, THC treatment facilitated a rebalanced hippocampal gene transcription in old mice so that their expression profiles closely resembled that of young THC-free animals. The transcriptional effects of THC were critically dependent on histone acetylation, because its pharmacological inhibition completely blocked all beneficial effects of THC. Thus, restoration of CB1 signalling in old individuals could be an effective strategy to treat or prevent age-related cognitive impairments.

EVIDENCE FOR AGONIST-SPECIFIC MECHANISMS OF CANNABINOID TOLERANCE IN PATHOLOGICAL PAIN

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Although cannabinoids such as delta-9-tetrahydrocannabinol (Δ^9-THC) exhibit clinical efficacy in pain, tolerance to the antinociceptive and analgesic effects develops with repeated treatment. The focus of our work is to investigate the mechanisms responsible for tolerance to different cannabinoid agonists. We previously found that tolerance to Δ^9-THC (30 mg/kg) is delayed in S426A/S430A mutant mice expressing a desensitization-resistant form of cannabinoid receptor 1 (CB_1) that disrupts the classic mechanism of G protein-coupled receptor kinase (GRK)/βarrestin2-mediated CB_1 desensitization. We have also found that tolerance to Δ^9-THC is partially mediated by c-Jun N-terminal kinase (JNK) signaling mechanisms that coordinate with GRK/βarrestin2 to mediate tolerance for Δ^9-THC. The objective of our current work is to assess the contribution of these different GRK/βarrestin2 and JNK-mediated mechanisms of tolerance for WIN55,212-2 and CP55940, two synthetic, high potency cannabinoid agonists. Tolerance to the antinociceptive effects of CP55,940 (0.3 mg/kg) is modestly disrupted in S426A/S430A mutant mice using the formalin and tail-flick tests. For the formalin test, intraplantar formalin injection produces a biphasic nociceptive response consisting of an acute and inflammatory pain phase. In contrast to CP55,940, tolerance to the antinociceptive effects of WIN55,212-2 (3 or 10 mg/kg) are strongly disrupted, but not completely abolished, in S426A/S430A mutant mice using these tests. The S426A/S430A mutation disrupts tolerance for the antiallodynic effects of WIN 55,212-2 (3 mg/kg) but not CP55,940 in mice with cisplatin-evoked neuropathic pain. The effect of the JNK inhibitor, SP600125, on cannabinoid tolerance was also examined. Pre-treatment with SP600125 (3 mg/kg) attenuates tolerance for the antinociceptive effects of Δ^9-THC in the tail-flick test and both the acute and inflammatory pain phases of the formalin test. Interestingly, inhibition of JNK signaling is sufficient to strongly disrupt tolerance to Δ^9-THC (6 mg/kg) in wild-type mice with cisplatin-evoked neuropathic pain. In contrast to Δ^9-THC, disruption of JNK signaling has no effect on tolerance for WIN55,212-2 but accelerates tolerance for CP55,940, specifically in S426A/S430A mutants but not in wild-type mice. These results demonstrate complex and partially agonist-specific roles for these two mechanisms of cannabinoid tolerance.

Acknowledgements: Funded by NIH grants DA036385 (DM), DA037355 (DM), The CH Foundation (JG) and Texas Tech University Health Sciences Center School of Medicine 121035 (JG).

CHARACTERIZATION OF CYTOSOLIC PHOSPHOLIPASE A_2Ε: PHOSPHATIDYLSERINE-STIMULATED PRODUCTION OF *N*-ACYL-PHOSPHATIDYLETHANOLAMINE

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Background: Bioactive fatty acyl ethanolamides, including the endocannabinoid anandamide, are mostly derived from *N*-acyl-phosphatidylethanolamine (NAPE). In mammals, NAPE production proceeds through the transfer of an acyl chain from certain glycerophospholipids to the amino group of phosphatidylethanolamine (PE) by Ca^{2+}-dependent or -independent *N*-acyltransferases. Recently, Ogura *et al*. identified the Ε isoform of mouse cytosolic phospholipase A_2 ($cPLA_2$Ε) as a Ca^{2+}-dependent *N*-acyltransferase (Ca-NAT). However, its human orthologue has not been characterized as Ca-NAT. Moreover, $cPLA_2$Ε has not been studied with its purified preparation.

Methods and Results: In this study, we first purified two isoforms of recombinant human $cPLA_2$Ε and detected Ca-NAT activity with both of them. Next, we examined catalytic properties of purified recombinant $cPLA_2$Εs from mouse and human. All $cPLA_2$Εs showed the highest *N*-acyltransferase activity at pH around 8.0. The Michaelis constants for dipalmitoyl-phosphatidylcholine (acyl donor) were found to be more than 400 μM and 660 μM for the mouse and human enzymes, respectively. $cPLA_2$Ε contained a C2 domain, which was first recognized in protein kinase C as a conserved domain to direct the host protein to membrane in the presence of Ca^{2+}. The activity of $cPLA_2$Ε exclusively required Ca^{2+}, and similar to many other C2 domain-containing proteins, this Ca^{2+}-dependent activity was dose-dependently enhanced by phosphatidylserine (PS). In fact, 200 μM PS increased the activity 25-fold in the presence of 1 mM $CaCl_2$ with a decrease in the EC_{50} value of Ca^{2+} more than eightfold. As observed by confocal laser microscopy using a PS-specific marker, $cPLA_2$Ε was largely co-localized with PS in the organelles involved in the endo/lysosomal pathway, suggesting that PS at least partly regulates the intracellular localization of $cPLA_2$Ε. Finally, we showed that [^{14}C]ethanolamine-labeled $cPLA_2$Ε-expressing cells produced [^{14}C]NAPE more than tenfold in response to the Ca^{2+}-ionophore ionomycin which, on the other hand, did not increase the NAPE level in the cells expressing the Ca^{2+}-independent *N*-acyltransferase PLAAT-1.

Conclusion: Two human isoforms of $cPLA_2$Ε functioned as Ca-NAT. PS strongly stimulated the Ca^{2+}-dependent *N*-acyltransferase activity of purified mouse and human $cPLA_2$Εs.

Acknowledgements: Grants-in-Aid for Scientific Research (C) (T.U., grant number 15K08278; K.T., 17K01852; N.U., 16K08589) from the Japan Society for the Promotion of Science, Young Scientists Fund for 2017 of Kagawa University Research Promotion Program (KURPP) (to T.U.) and the Japan Foundation for Applied Enzymology (to T.U.).

DIACYLGLYCEROL LIPASE-β KNOCKOUT MICE DISPLAY A SURVIVAL PROTECTIVE PHENOTYPE FOLLOWING TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI)-induced increases of endogenous cannabinoid (eCB) brain levels suggest that the eCB system plays a role in compensatory repair mechanisms. The eCB 2-arachidonyl glycerol (2-AG) serves as a rate-limiting precursor for arachidonic acid (AA), which in turn is a precursor for the production of pro-inflammatory eicosanoids in brain. As neuroinflammation impacts TBI-induced cognitive impairments, injury-induced increases in AA and its metabolites may contribute to secondary mechanisms of neurodengeneration. Studies showing that genetic deletion or pharmacological inhibition of the 2-AG biosynthetic enzyme diacylglyercol lipase-β (DAGL-β) reduces *in vitro* inflammatory responses, prompted us to examine whether DAGL-$\beta^{-/-}$ mice display a protective phenotype from functional deficits of experimental TBI. Male DAGL-$\beta^{-/-}$and $^{+/+}$ mice were subjected to a left lateral moderate Fluid Percussion injury (FPI) (1.94±0.1 atm), and assessed for spatial memory performance in a Morris water maze (MWM) Fixed Platform task, as well as motivational and sensory-motor performance in a Cued task. In addition, we assessed neurological motor impairments using the Neurological Severity Score (NSS) and the Rotarod assay, as well as physiological measures of body weight and body temperature.

DAGL-$\beta^{-/-}$ mice displayed similar magnitudes of FPI-induced cognitive impairments in the MWM Fixed Platform task, and neurological motor deficits in the NSS and Rotarod assays, as DAGL-$\beta^{+/+}$ mice. While injured mice had reduced body weights for the duration of experimental testing, no changes were seen in baseline body temperature, post-MWM swim body temperatures, or Cued task performance in DAGL-$\beta^{-/-}$ compared to DAGL-$\beta^{+/+}$ mice. Unexpectedly, DAGL-$\beta^{-/-}$ mice demonstrated a significant survival protective phenotype (100% survival) in response to brain injury compared to DAGL-$\beta^{+/+}$ mice (77% survival), despite equal injury severities between groups. We therefore tested whether this DAGL-$\beta^{-/-}$ survival phenotype would persist after increasing the magnitude of lateral FPI to 2.0±0.1 atm and 2.17±0.1 atm. Male DAGL-$\beta^{-/-}$ mice continued to demonstrate a significant survival protective phenotype at both 2.0 atm (100% survival) and 2.17 atm (90% survival) compared to male DAGL-$\beta^{+/+}$ mice (75% survival at 2.0 atm, and 60% survival at 2.17 atm). In contrast, female DAGL-β mice generally survived both injury magnitudes regardless of genotype (DAGL-$\beta^{+/+}$; 93% survival at 2.0 atm and 86% survival at 2.17 atm, DAGL-$\beta^{-/-}$; 100% survival at both injury levels), demonstrating sex differences in survival from FPI, and suggesting their survival phenotype is likely occurring through alternative mechanisms in addition to DAGL-β gene deletion. In sum, these findings suggest the provocative possibility that DAGL-β activity contributes to TBI-induced mortality, but not to the evolution of TBI cognitive or motor impairments in male mice. Accordingly, DAGL-β inhibition represents a potential strategy to ameliorate post-traumatic fatality.

Acknowledgements: Ruth L. Kirschstein National Research Service Award, 1F31NS095628-01A1, and grants NS-056247, NS-057758, DA032933.

ELUCIDATION OF THE ROLE OF CANNABINOID CB2 RECEPTORS IN MODULATING NEUROPATHIC PAIN USING A CB2 REPORTER MOUSE

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Cannabinoid CB2 receptors are a promising therapeutic target because their activation suppresses pathological pain without unwanted psychotropic effects in the central nervous system (CNS). However, the cell types that contain CB2 receptors and mediate these anti-allodynic effects remain poorly understood, at least in part, due to questionable specificity of existing CB2 antibodies. In this study, we used transgenic mice with a CB2-promoter driven expression of green fluorescent protein (GFP) to study the cell types that contain CB2 receptors and mediate their anti-allodynic effects. GFP labeled CB2 f/f mice developed and maintained neuropathic pain that was induced by treatment with the chemotherapeutic agent paclitaxel. In CB2 GFP reporter mice, multiple CB2 agonists (i.e. AM1710, LY2828360) suppressed paclitaxel-induced mechanical and cold allodynia without producing tolerance. These results are consistent with previous observations by our group using wildtype mice (Deng et al. (2015) Biological Psychiatry 77: 475-487; Lin et al. (208) Molecular Pharmacology 93: 49-62). CB2f/f mice showed native CB2 expression, marked by GFP positive cells, in dorsal root ganglion cells of heterogeneous cell sizes under conditions in which CB2 receptors were not detected in neurons of the central nervous system. CB2 protein was also observed in keratinocytes in the epidermis; GFP was differentially expressed on epidermal keratinocytes in stratified patterns that shifted and increased in intensity, especially after repeated paclitaxel injections. Moreover, these studies also revealed novel expression of CB2 on dendritic and Langerhans cells that invaded the epidermis. Anti-allodynic effects of the CB2 agonist AM1710, were also blocked by SR144528, a CB2 antagonist that exhibits limited CNS penetration. Our studies suggest potential targets for cannabinoid CB2 agonists in suppressing chemotherapy-induced neuropathic pain.

Acknowledgements: Supported by DA041229 (AGH, KM), CA200417 (AGH) and DA009158 (AM, KM, AGH). LMC is supported by T32DA024628-01A1 and the Harlan Research Scholars Program.

FATTY ACID BINDING PROTEINS REGULATE CANNABINOID METABOLISM

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The lipophilic nature of cannabinoids necessitates protein-mediated cytoplasmic transport to their intracellular catabolic enzymes. Fatty acid-binding proteins (FABPs) mediate endocannabinoid degradation by acting as an aqueous shuttle, bringing anandamide to the endoplasmic reticulum (ER)-bound enzyme fatty acid amide hydrolase (FAAH). Phytocannabinoids, including Δ^9-tetrahydrocannabinol (THC), are metabolized and inactivated principally by the cytochrome P450 (CYP450) enzymes of the liver. Like FAAH, the CYP450s are localized to the ER and therefore some aqueous transport mechanism is presumably required. Currently, no hepatic proteins that facilitate intracellular shuttling of exogenous cannabinoids have been described, highlighting a major gap in our knowledge of phytocannabinoid inactivation. The high expression level of liver-type fatty acid binding protein (FABP1, L-FABP) in the liver coupled to its promiscuous binding to structurally diverse lipophilic xenobiotics make this protein an ideal candidate to mediate hepatic phytocannabinoid transport.

The work presented here tests the central hypothesis that FABP1 is an important regulator of phytocannabinoids transport and subsequent inactivation by the CYP450s. We show that recombinant human FABP1 binds both THC and CBD with low micromolar affinities *in vitro*. The *in vivo* contributions of FABP1 to phytocannabinoid activity was assessed by comparing relative cannabimimetic effects by tetrad tests (e.g. hypothermia and hypomotility) in FABP1-knockout (FABP1-KO) and wild type (WT) mice following treatment with THC (10 mg/kg, i.p.). Compared to their WT counterparts FABP1-KO mice exhibit significantly potentiated cannabimimetic responses following THC administration. The stark pharmacodynamic differences we observed prompted a comparative pharmacokinetic investigation. LC-MS/MS analysis of brain and blood plasma from THC-treated mice indicate that FABP1-KO animals exhibit reduced rates of THC metabolite formation and bioelimination. Importantly, crude microsomes isolated from WT and FABP1-KO livers showed no obvious differences in *in vitro* THC biotransformation, indicating that these effects are likely not resulting from altered CYP450 expression levels between the two genotypes. Collectively, these results suggest that FABP1 is a hepatic THC transport protein and further positions the FABP family as a critical mediators of cannabinoid inactivation.

Acknowledgements: This work was funded by NIDA grants DA035923 and F31-DA042545.

TARGETING THE MU OPIOID AND CANNABINOID RECEPTORS WITH HETEROBIFUNCTIONAL LIGANDS

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In order to obtain novel pharmacological tools and to investigate a multi-targeting analgesic strategy, the CB_1 and CB_2 cannabinoid receptor agonist JWH-018 was conjugated with the opiate analgesic oxycodone or with an enkephalin related tetrapeptide. The opioid and cannabinoid pharmacophores were coupled via spacers of different length and chemical structure. The resulting heterobifunctional ligands were characterized in *in vitro* radioligand binding experiments. In order to obtain adequate cannabinoid receptor binding data the radioactive labeling and characterization of JWH-018 as a radioligand were also performed. The functionality and ability of the bivalent ligands to activate the CB_1/CB_2 and/or μ opioid receptors were tested in [^{35}S]-GTPγS binding assays.

In *in vitro* radioligand binding experiments revealed that 11- and 19 compound definitely bound to both MOR and CB receptors. Furthermore, in [^{35}S]-GTPγS binding assays 11 preferred MOR and CB_2-, while 19 preferred MOR and CB_1-mediated signaling. Finally, for parent ligands and 11, 19 compounds *in vivo* experiments were performed in order to evaluate their effects in chronic inflammatory model of pain. The parent ligands exhibited strong antinociceptive effects applied supraspinally, while synergism was observed in the case of 11 and 19 after intrathecal administration. These results support the development of lead compounds targeting multiple G-protein coupled receptors.

Acknowledgements: This work was supported by the ÚNKP-16-3 New National Excellence Program of the Ministry of Human Capacities of Hungary.

INHIBITION OF FAAH PROTECTS AGAINST MICROGLIAL ACTIVATION AND NEUROTOXICITY INDUCED BY HIV-1 TAT PROTEIN *IN VITRO*

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In the era of combined antiretroviral therapy (cART), human immunodeficiency virus type 1 (HIV-1) is considered a chronic disease that specifically targets the brain and causes a high prevalence of mild forms of neurocognitive impairments, also referred to as HIV-associated neurocognitive disorders (HAND). The HIV-1 transactivator of transcription (Tat) protein is detectable in the brains of HIV-1 patients on cART, and is known to produce neurotoxic effects directly through neuronal pathways and indirectly through microglial proinflammatory actions. Interventions with neuroprotective or anti-inflammatory activity are in high demand to stop these chronic neurodegenerative processes. Compounds targeting the catabolic enzymes responsible for endocannabinoid degradation show promise in reducing pain and inflammation with minimal side effects in rodents. We previously demonstrated in a murine prefrontal cortex (PFC) neuron culture model that inhibiting a major enzyme responsible for the degradation of anandamide, Fatty Acid Amide Hydrolase (FAAH), with use of PF3845, blunts the direct Tat neurotoxic effects. In the present study we assessed the effects of PF3845 on Tat-induced proinflammatory responses in microglial cells. Cultured murine microglia were incubated with Tat and/or PF3845. After 24 hours, microglial conditioned media was collected and applied to PFC neuron cultures at different dilutions and neurotoxicity was assessed using live cell Ca^{2+} imaging. Fura-2AM was used to visualize dynamic changes in neuronal $[Ca^{2+}]_i$ during exposure to medium derived from Tat-treated microglia. Medium from microglia exposed to Tat significantly increased neuronal $[Ca^{2+}]_i$ levels compared to control medium. Importantly, medium derived from microglial cultures pretreated with PF3845 and Tat increased $[Ca^{2+}]_i$ less in PFC neurons than medium derived from Tat treatment alone, indicating that FAAH inhibition attenuates microglial response to Tat. These data suggest that targeting FAAH expressed in microglia may be useful in treating neuroinflammation in HAND and other neurocognitive diseases.

Acknowledgements: Funded by NIDA R21 DA041903, R00 DA033878, T32 DA007244, UNC CFAR P30 AI50410, R01 DA032933, and K05 DA021696. B. Ignatowska-Jankowska is supported by the Fellowship from the Japan Society for Promotion of Science (JSPS)

THE 2-AG METABOLITE PGD_2-G DECREASES INFLAMMATORY PAIN IN MICE

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2-Arachidonoylglycerol (2-AG) is known to exert anti-inflammatory effects in vivo. We previously showed that the anti-inflammatory effects of increasing 2-AG levels were in part cannabinoid receptors dependent, and in part due to the oxidative metabolism of 2-AG by cyclooxygenase-2 (COX-2) to give the anti-inflammatory mediator prostaglandin D_2 glycerol ester (PGD_2-G).[1] Inflammation is characterized by the release of multiple mediators, some of which can act on nerve endings, thus triggering inflammatory pain. As PGD_2-G exerts anti-inflammatory effects in vitro and in vivo, we decided to investigate its effect in inflammatory pain. To this end, we used the carrageenan-induced model of inflammatory pain to assess the effect of PGD_2-G. This model is well-described and broadly used to test new anti-inflammatory/analgesic drugs.[2] PGD_2-G (20 µg) was injected in the mouse paw 30 minutes before carrageenan injection (0.1 mg in saline). In our studies, we tested in parallel the effects of PGD_2 (20µg), a possible metabolite of PGD_2-G, in order to evaluate its involvement in the effects of PGD_2-G.

We monitored over time the effect of PGD_2-G and PGD_2 on carrageenan-induced edema and hyperalgesia, using von Frey filaments. The expression of pro-inflammatory markers was assessed in the paw tissue and the sciatic nerve at different time points using RT-qPCR. Histological studies and myeloperoxidase activity (a marker for neutrophils infiltration) were used to monitor immune cell infiltration.

Mice receiving PGD_2-G recovered faster from carrageenan-induced hyperalgesia while PGD_2 delayed the recovery time. We also confirmed the anti-inflammatory effect of PGD_2-G with a decrease of edema formation, an effect not reproduced by PGD_2. Moreover PGD_2-G, but not PGD_2, decreased the carrageenan-induced expression of inflammatory markers (TNFα, IL-6 and COX-2) in the mouse paws at 24h following carrageenan injection. Concerning immune cell infiltration, PGD_2-G decreased the histological score compared to the carrageenan group and the same effect was observed in mice receiving PGD_2. However, there was no difference in MPO activity between the treated and untreated carrageenan groups at 24h. Additional studies are underway to further investigate the mechanism by which PGD_2-G affects edema formation and hyperalgesia recovery. In conclusion, the anti-inflammatory and anti-hyperalgesia effects of PGD_2-G support the interest to investigate its pathophysiological role.

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Acknowledgements: MA is a post-doctoral researcher of the FRS-FNRS (Fonds de la Recherche Scientifique).

GUIDELINES FOR PUBLIC HEALTH AND SAFETY METRICS TO EVALUATE THE POTENTIAL HARMS AND BENEFITS OF CANNABIS REGULATION IN CANADA

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Canada is preparing to become the first major industrialized country to implement a regulatory framework for non-medical cannabis. This unprecedented national policy shift provides an opportunity to re-imagine the system of metrics necessary to assess the effectiveness of drug policy, including a prioritization of public health and safety over traditional indicators of drug supply and demand. In this paper, we recommend a comprehensive set of indicators to guide the evaluation of cannabis regulation in Canada, and discuss the ways in which these indicators may shift to demonstrate positive or negative public health and safety impacts in the era of non-medical cannabis regulation.

Five scientific databases were searched to compile a list of cannabis-related issues of interest to public health and safety. A set of indicators was developed based on topics and themes that emerged. Evidence was summarized on the short-term impact of regulation on each indicator from other North American jurisdictions that have legalized medical and/or non-medical cannabis (e.g., Colorado, Washington).

In total, 30 indicators under five broad themes were identified: public safety; cannabis use trends; other substance use trends; cardiovascular and respiratory health; and mental health and cognition. Assessment of preliminary trends from other jurisdictions revealed little consensus regarding the effect of cannabis legalization on public health and safety harms, and an emerging body of evidence to support potential benefits (e.g., reductions in opioid use and overdose). Many proposed indicators, including those linked to longer-term outcomes, have not yet been assessed in other jurisdictions.

This is the first comprehensive review to establish guidelines for evaluating the public health and safety impacts of wide-scale legalization and regulation of non-medical cannabis. In addition to indicators that correspond with the more commonly discussed possible public health and safety challenges (e.g., cannabis-related hospitalizations, cannabis-impaired driving), this review led to the recommendation of several indicators to monitor for possible public health and safety improvements, supported by preliminary evidence from other North American jurisdictions that have passed cannabis legislation. Canada's monitoring and evaluation system should incorporate the proposed guidelines to promote a balanced public health and safety assessment that serves to minimize the harms and maximize the benefits of cannabis regulation.

Acknowledgments: Stephanie Lake is supported by doctoral awards from the Canadian Institutes of Health Research (CIHR) and the Pierre Elliott Trudeau Foundation. Thomas Kerr is supported by a Foundation grant from CIHR (FDN-148476). Daniel Werb is supported by the National Institute on Drug Abuse (NIDA; DP2-DA040256-01), a New Investigator Award from CIHR, and an Early Researcher Award from the Ontario Ministry of Research, Innovation and Science. Rebecca Haines-Saah is supported by a Young Innovator in Public Health Award from Petro Canada. Benedikt Fischer receives research support from the Chair in Addiction, Department of Psychiatry, University of Toronto, and from the CIHR grant #SMN-139150 (CRISM Ontario Node Team). Zach Walsh is the PI of an ongoing clinical trial of cannabis for PTSD sponsored by Tilray, a licensed producer of medical cannabis in Canada. Mark A. Ware is supported by the Fonds de Recherche du Québec-Santé (FRQS). He is a consultant to CHI, CannaRoyalty and Zynerba, and has received a grant to his institution for clinical research from CanniMed. Evan Wood receives support through a Tier 1 Canada Research Chair in Inner City Medicine. M-J Milloy is supported by the US National Institutes of Health (NIH; U01-DA021525), a New Investigator Award from CIHR, and a Scholar Award from the Michael Smith Foundation for Health Research (MSFHR). His institution has received unstructured funding from NG Biomed Ltd., a private firm seeking a license to produce medical cannabis from the Canadian government, to support his research.

ESTIMATING PLASMA PHARMACOKINETICS OF THC, THC-OH, TCH-COOH AND THC-COOH-GLUCURONIDE

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Pharmacokinetics of THC, but not including those of the major metabolites, have been performed in the setting of a clinical research setting[1]. Plasma concentrations of the THC-COOH and the THC-COO-glucuronide metabolites often persist above the limits of quantification of drug toxicology assays, while those of THC and THC-OH do not. Therefore, in order to interpret isolated plasma concentrations of THC and its metabolites, a comprehensive pharmacokinetic model that includes both THC and its metabolites would have utility. We extracted mean plasma THC and metabolite concentration data, using WebPlotDigitizer (https://apps.automeris.io/wpd/) from 3 publications from the Huestis group[2-4]. They enrolled 10-15 subjects for each clinical trial, obtained baseline and then frequent blood samples for up to 75 hours following smoking a single marijuana cigarette containing 54 mg of THC over 10 minutes. The blood samples were assayed for concentrations of THC and the 3 metabolites referenced above using an LC-MS/MS analysis. To enable us to reasonably assume steady-state prior to THC administration, we only included studies in which the subjects were daily users. Mean daily cannabis consumption was 4.5, 4.9 and 8 cigarettes/day and the mean estimated times since last use (before the baseline sample) were 4.1, 8.4 and 48 hours in the 3 studies, respectively. 193 THC or metabolite concentrations were obtained.

Phoenix 8.0 (Certara, Princeton, NJ) was used to conduct pharmacokinetic analyses with the naïve-pooled data specification. The resulting pharmacokinetic estimates are based on the following assumptions: 1) subjects were at steady-state, 2) 54 mg THC cigarettes smoked at regular intervals calculated as 24 hours divided by the mean daily number of cigarettes in each study group, 3) last THC consumption was the mean time since last use as reported in each study, 4) the fraction absorbed from each smoking event was 25% of 54 mg (i.e., 12.65 mg), and 5) all THC-COOH was metabolized to the glucuronide. The THC venous plasma concentration at check-in (approximately 20 hours before the THC administration), immediately before THC administration and then during smoking and for up to 75 hours after smoking were included in the model. The 3-compartment THC model parameter estimates were estimated first and fixed and the metabolite data were then fitted with the total elimination clearance for THC split between metabolism to THC-OH, THC-COOH and other. Diagnostic plots indicated the model characterized the data well. Among the uses for the full PK model would be to estimate a daily consumption of THC from a single plasma THC-COOH-glucuronide concentration by using the clearance estimates of THC (91.4 L/hr), THC-COOH production (14.6 L/hr), THC-COOH elimination (3.2 L/hr) and THC-COOH-glucuronide elimination (1.62 L/hr) and steady-state assumptions. A full population pharmacokinetic analysis of individual data with fewer mean-based assumptions would greatly refine these estimates and allow for elucidation of covariates that might better predict items of interest such as drug exposure and time since last use and other routes and forms of administration.

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FATTY ACID BINDING PROTEIN 5 INHIBITION SUPPRESSES MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 INDUCTION AND PROSTAGLANDIN E2 BIOSYNTHESIS DURING INFLAMMATION

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Fatty acid binding proteins (FABPs) are a family of intracellular proteins that regulate lipid signaling, including that of the endocannabinoid anandamide. Pharmacological or genetic inhibition of FABP5 produces antinociceptive effects in mice. Prostaglandin E_2 (PGE_2) is an eicosanoid produced at the site of inflammation that promotes inflammatory edema and hyperalgesia (i.e., heightened sensitivity to noxious stimuli). Previous work from our group has shown that FABP5 inhibition produces anti-inflammatory effects. Here, we tested the hypothesis that the anti-inflammatory effects observed after FABP5 inhibition may involve the modulation of inflammatory PGE_2 production and uncovered a novel mechanism underlying the modulation of PGE_2 biosynthesis by FABP5.

Intraplantar administration of carrageenan in wild-type mice induced edema, hyperalgesia, and elevated tissue PGE_2 levels, which were attenuated in FABP5 KO mice. Cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) are enzymes upregulated at the site of inflammation and mediate PGE_2 biosynthesis. FABP5 inhibition highly suppressed the upregulation of mPGES-1 in carrageenan-injected paws while the upregulation of COX-2 was unaffected. mPGES-1 was predominantly upregulated in macrophages, which also express FABP5. Macrophages also express the related protein FABP4 but its inhibition did not affect carrageenan-induced paw edema, hyperalgesia, mPGES-1 upregulation, or PGE_2 levels. Surprisingly, the suppression of mPGES-1 upregulation by FABP5 was not mediated by cannabinoid receptors or peroxisome proliferator-activated receptors alpha and gamma. Interleukin 1β (IL-1β) is a pro-inflammatory cytokine released at the site of inflammation and stimulates mPGES-1 expression. Upregulation of mPGES-1 by IL-1β involves activation of nuclear factor-kB (NF-kB), which binds to and activates the mPGES-1 promoter. We employed A549 cells, which express FABP5 but not express other FABP subtypes, to assess whether FABP5 inhibition modulates mPGES-1 expression through a pathway involving NF-kB. Knockdown of FABP5 suppressed IL-1β-stimulated mPGES-1 upregulation, the activation and nuclear translocation of NF-kB, as well as the activity of the mPGES-1 promoter. The activity of a mutant mPGES-1 promoter that lacks the NF-kB binding sites was no longer responsive to FABP5 inhibition, confirming the involvement of NF-kB. Collectively, our results establish FABP5 as a novel modulator of mPGES-1 induction and PGE_2 biosynthesis during inflammation.

Acknowledgements: This work was funded by NIDA grant DA035949 and DA035923.

FINDINGS FROM A RANDOMISED PLACEBO CONTROLLED TRIAL OF NABIXIMOLS IN THE TREATMENT OF CANNABIS DEPENDENCE

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Cannabis dependence is amongst the most common forms of substance use disorders internationally. Conventional treatment approaches involving psychosocial interventions (e.g. CBD) have limited effectiveness, and there are no demonstrated effective medications. Whilst cannabinoid agonist treatment has been previously shown to be effective in treating cannabis withdrawal syndrome, withdrawal treatment has limited impact on longer term substance use, with high rates of relapse.

To this end, this RCT examined the role of longer term cannabinoid agonist treatment with nabiximols, an oromucosal spray of THC-CBD. 137 treatment seeking cannabis dependent participants were randomised to 12 weeks of either nabiximols (mean daily dose 50mg THC/50mg CBD), or placebo, in combination with best practice counselling (CBT) and weekly clinical review with medical or nursing staff. Preliminary findings indicate no significant differences in treatment retention at 12 weeks. The nabiximols group reported significantly fewer days of cannabis use compared to the placebo group. The medication was well tolerated with few adverse events reported, and no medication related serious adverse events. Full analyses will be presented.

CANNABINOID-1 RECEPTOR REGULATES MITOCHONDRIAL DYNAMICS AND FUNCTION IN RENAL PROXIMAL TUBULAR CELLS

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Mitochondrial morphology is highly dynamic, changing rapidly in response to altered metabolic demand or cellular stress. The balance between ongoing fusion and fission events, shaping mitochondrial structure, is detrimental for maintaining mitochondrial homeostasis. Breach in this balance has been implicated with the progression of different metabolic diseases, including obesity and its associated complications. Endocannabinoids (eCBs) and their corresponding cannabinoid-1 receptor (CB_1R) play an important role in energy homeostasis, and are well known to modulate different aspects of mitochondrial physiology.

The eCB/CB_1R system is highly up-regulated during obesity in which renal injury, dysfunction, inflammation, and fibrosis may occur by compromising the function of the renal proximal tubular cells (RPTCs). These metabolically active cells reabsorb up to 80% of water and solute in the nephron, by using specialized ATP-dependent membrane transporters. To meet this high energy demand, RPTCs rely on mitochondrial oxidative phosphorylation to produce ATP. Thus, impairment in mitochondrial bioenergetics may result in renal dysfunction. Indeed, our recent findings (Udi et al., *JASN* 2017) demonstrate that RPTC dysfunction is accompanied by decreased utilization of fatty acids and impaired ATP production by mitochondria.

Here, we evaluated the role of the eCB/CB_1R system in modulating mitochondrial dynamics in RPTCs. We utilized mitochondrialy-targeted GFP to visualize mitochondrial morphology in live cells (wild-type and null for the CB_1R), and electron microscopy to assess mitochondrial architecture in kidney section of RPTC-$CB_1R^{-/-}$ mice and their littermate controls. Our findings show that either direct activation of CB_1R or high-fat diet-induced upregulation in eCB 'tone' *in-vivo* and *in-vitro* resulted in excessive mitochondrial fragmentation in wild-type RPTCs. This effect was mediated, at least in part, by modulating the phosphorylation levels of the fission protein, dynamin-related protein 1 (DRP1), on both S637 and S616 residues. Mitochondrial fission was associated with mitochondrial dysfunction, as documented by increased reactive oxygen species and cellular lactate content, reduced oxygen consumption and ATP levels as well as a decline in mitochondrial biogenesis. Genetic deletion of CB_1R protected RPTCs from mitochondrial fragmentation and their associated malfunction.

Taken together, these findings suggest that mitochondrial architecture is regulated by the eCB/CB_1R system, and therefore, its activation may contribute to the organelle's dysfunction during renal tubular injury associated with fatty acid flux.

Acknowledgments: This work was supported by an ERC-2015-StG grant (#676841) to J.T.

TETRAHYDROCANNABINOLIC ACID TARGETS PPARγ AND PREVENTS DIET-INDUCED OBESITY

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We have previously reported that Δ^9-tetrahydrocannabinol acid (Δ^9-THCA) is a potent PPARγ agonist exerting neuroprotective activity (Nadal et al., Br J Pharmacol. 2017. 174(23): 4263-4276). PPARγ is a nuclear receptor able to regulate lipid turnover and metabolism. Herein, we have investigated the role of Δ^9-THCA on PPARγ modulation and its efficacy in a murine model of metabolic syndrome.

We found that Δ^9-THCA mediated ubiquitination and degradation of PPARγ, and induced the expression of PPARγ-dependent genes. Using human bone marrow mesenchymal stem cells (MSCs), we found that Δ^9-THCA induced both osteoblastogenesis and adipogenesis, measured by Alizarin and Oil-Red staining respectively, and by the determination by qPCR of markers specific for osteoblasts (RUNX, SP7, IBSP, ALP), and adipocytes (PPARγ2, LPL, FABP4, CEBPA and ADIPOQ). Using docking analysis, we detected that Δ^9-THCA binds both the canonical (Ser289) and the alternative binding sites (L340 and Ser342) in the PPARγ ligand-binding pocket (LBP), and Δ^9-THC only binds to the alternative site (Ser342). Functional assays further showed that the selective PPARγ inhibitor, T0070907, almost abolished PPARγ transcriptional activity induced by rosiglitazone (RGZ), but did not affect the activity of Δ^9-THCA in a Gal4-Luc system. However, T0070907 inhibited the effects of RGZ and Δ^9-THCA on the expression of PPARγ-dependent genes upregulated in MSCs.

In addition, we have investigated the effect of Δ^9-THCA in a murine model of metabolic syndrome induced by high fat diet (HFD). Adult male mice, fed for >8-wks with either HFD or the corresponding low fat, control diet (CD), were used in this study. Daily administration of Δ^9-THCA (20 mg/kg, i.p.) for 3-wks induced a significant reduction in fat mass and body weight gain in HFD mice. Δ^9-THCA significantly ameliorated also glucose intolerance and insulin resistance in HFD animals; an effect that was even detected in CD mice. Moreover, Δ^9-THCA largely prevented liver steatosis, adipogenesis, and macrophage infiltration in fat tissues (Crown-like-structures) in HFD mice. Identification of other metabolic and inflammatory biomarkers by transcriptomic analyses in fat tissues, and by multiplex analysis in plasma will be also discussed.

In conclusion, our studies document the potent biological activity of Δ^9-THCA as a non-adipogenic PPARγ agonist highlighting its potential to ameliorate metabolic syndrome and inflammation associated to obesity.

CANNABIDIOL REDUCES INTRAVENTRICULAR HAEMORRHAGE EXTENSION AND SECONDARY BRAIN DAMAGE IN NEWBORN RATS

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Background: Intraventricular Hemorrhage (IVH) is a frequent condition in extremely low birth weight preterm newborns (ELBW) representing a major risk factor for Cerebral Palsy (CP) due to extended inflammation and oxidative-stress-related brain damage. There is no treatment reducing neither the extent of IVH once occurred nor the development of secondary CP. Cannabidiol (CBD) has shown long-lasting neuroprotective effects in newborn animals in a manner related to inflammation and oxidative-stress modulation.

AIM: to study the neuroprotective effect of CBD in a modelof IVH-induced brain damage carried out in newborn rats with a brain developmental stage similar to ELBW.

Methods: unilateral IVH brain damage was induced in newborn Wistar rats (1 days-old: P1) by injecting 0.2 U of Chlostridial collagenase VII-S into the left Germinal Matrix using a stereotaxic device and a Hamilton syringe. Twelve hours later, pups were randomly assigned to receive i.p. vehicle (VEH, n=20)or CBD 5 mg/kg single dose (CBD, n=20) (both supplied by GW Research Ltd, Cambridge, UK). 48 h post-insult brain damage was assessed by magnetic resonance image (MRI), determining blood and edema volume. Then,a set of Neurobehavioral tests were performed at P14 (negative geotaxis [motor coordination] and grip test [strength]) and P37 (Cylinder Rear Test [CRT, hemiparesis] and Novel Object Recognition [NOR, memory]). Similarly managed non-GMH rats served as controls (SHM, n=15).

Results: results from MRI and neurobehavioral studies are shown in the following table.

		GROUP		
		SHAM	**IVH+VEH**	**IVH+CBD**
P14	**GEOTAXIS[1]**	4.0(3.4,4.1)	4.5(3.5,5.5)	3.0(2.5,3.5)§
	GRIP TEST[2]	1 (1,1.5)	0 (0,1)*	1(0.5, 2)§
P37	**CRT[3]**	-0.06(-0.26,0)	0.11 (0,0.38)*	-0.18 (-0.31, -0.06)§
	NOR[4]	0.54 (0.43,0.56)	0.23(0.12,0.32)*	0.38 (0.19,0.47)
MRI	**DAMAGE[5]**	N/A	3.1(2.1,6.1)	1.7(1.1,2.4)§

Results expressed as median (IQR). (1): sec. (2): points. (3): ipsilateral paw preference ratio. (4): novel object preference ratio. (5) volume (% total brain volume). Kruskall-Wallis: (*) $p<0.05$ vs SHM; (§) $p<0.05$ vs GMH+VEH.

CBD, administered 12 h after IVH, significantly reduced the volume of brain damage. IVH led to short- and long-term motor and cognitive deficits. CBD prevented IVH-induced motor impairment. CBD exerted some beneficial effect on IVH-induced cognitive impairment but without statistical significance.

Conclusion: CBD administration to newborn rats reduced the extension of IVH and prevented IVH-induced secondary motor impairment, which makes CBD an interesting candidate to prevent IVH-induced CP.

Supported by grants from FIS PI16/0689 and GW Research Ltd. Acknowledgments to SEIC for the travel grant. .

ROLE OF CB2 RECEPTOR IN THE NEUROPROTECTIVE EFFECT OF CBD IN A NEONATAL RAT MODEL OF HYPOXIA ISCHEMIA

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Background: CB2 agonists are neuroprotective in adult animal models of brain ischemia. Neuroprotective effects of cannabidiol (CBD) administered after a hypoxic-ischemic (HI) insult are reversed by CB2 antagonists in newborn mice in vitro and newborn pigs in vivo, in the latter likely by acting on CB2-5HT1A heteromers. However, the participation of CB2 on CBD neuroprotection in immature brain has not been specifically studied.

Aim: to study the involvement of CB2 receptors in CBD neuroprotective effects in an in vivo rat model of HI.

Methods: P7-10 HI Wistar rats (left carotid artery electrocoagulation plus 112 minutes of hypoxia (10% O_2) received s.c. vehicle (VEH, n=20), or the CB2 agonist HU-308 1mg/kg (kindly provided by Prof. Mechoulam) (HU, n=13) and/or CBD 1 mg/kg (GW Research Ltd, Cambridge, UK) (CBD, n=17), alone or 10 min after the CB2 antagonist SR144528 3mg/kg (SR2, n=12). At P14, motor coordination (negative geotaxis) and paw strength (grip and grasp test) were assessed by neurobehavioural tests (NB). Then, the volume of brain loss and injury (hyperintense area) (Magnetic Resonance Imaging [MRI]) and TNFα and protein oxidation levels (Western blot) were measured in brain. Similarly managed non-HI pups served as controls (SHM, n=18).

Results: Are summarized in Table 1.

	SHM	HI+VEH	HI+CBD	HI+HU	HI+HU+CBD	HI+SR2+CBD
Vol. Lesion (%)	N/A	18.2(14,19.6)	12.0(8.8,14.1)$^{\#}$	14.1(1.5,17.1)$^{\#}$	14.1(13.1,16.5)$^{\#}$	9.8(9.5,14.7)$^{\#\,\S}$
HIA. (%)	N/A	13.0(10.6,23.6)	8.7(3.6,11.1)$^{\#}$	15.8(5.9,25.7)	9.1(7.6,10.2)$^{\#}$	11.3(8.9,13)
Geotaxis (sec)	5.7(5.5,6.8)	9.5(7,12)*	7.5(6.2,9)$^{\#}$	7.2(5.8,9.5)$^{\#}$	6.5(6,10) $^{\#}$	6.5(6.1,7.6)$^{\#\,\S}$
Grip (pts)	3(2.2,4)	1.25(1,3)*	2(1.8,3.2)$^{\#}$	2.5(2,3.2)$^{\#}$	3.5(3,4)$^{\#\,\S}$	1(1,2.6)$^{\#}$
Grasp (pts)	2(2,2)	1(0.25,1)*	1.75(1,2)$^{\#}$	1(0.8,2)*§	2(1,2)$^{\#}$	1(1,1.3)
TNFα (Int.)	0.7(0.6,1.1)	0.98(0.8,1.5)*	0.56(0.5,0.6)$^{\#}$	0.94(0.9,1.5)§	1.5(1.4,1.6)§†	1.51(1.3,1.6) §
Oxyblot (Int.)	1.7(1.1,3.1)	2.0(1.3,2.2)	2.0(1.7,2.6)	1.9(1.5,2.1)	1.4(1.2,2.0)	1.4(1.3,2.0)

Median (Interqueartilic range). Vol: volume. HIA: hyperintense area. Int.: intensity. (*)p<0.05 vs. SHM, (#) p<0.05 vs HI+VEH, (§) p<0.05 vs HI+CBD, (†) p<0.05 vs HI+HU all by Kruskal-Wallis test.

CBD reduced HI-induced brain damage as assessed by NB and MRI in a manner associated to inflammation modulation. HU led to partial neuroprotective effects improving some results of NB and MRI but with no effects on inflammation. There was no additive effect after combining CBD and HU excepting for grip. SR did not reverse the effects of CBD on NB (excepting for grasp) or MRI but either SR or HU reversed CBD effects on inflammation. There were no differences among groups regarding oxidative stress.

Conclusions: CBD was neuroprotective after HI in newborn rats sowing a pattern different from that of a CB2 agonist. Modulation of CB2 activity did not reverse CBD neuroprotection but did reverse CBD anti-inflammatory effects. These data do not support a direct action of CBD on CB2 receptors but ae consistent with the possible involvement of heteromers.

Supported by grants FPU 13/0549 and GW Research Ltd.

CANNABIDIOL REVERSES ATTENTIONAL BIAS TO CIGARETTE CUES IN A HUMAN EXPERIMENTAL MODEL OF TOBACCO WITHDRAWAL

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Cannabidiol (CBD), the non-intoxicating cannabinoid found in cannabis, may be a novel drug for smoking cessation due to its anxiolytic and potentially anti-addictive properties. It has previously been shown to reduce the salience of cues, both preclinically and clinically. Attentional bias, the ability for a drug to capture one's attention, is heightened during abstinence and can lead to relapse. The present study utilizes an experimental medicine approach with the hypothesis that CBD would reduce attentional bias to cigarette cues.

A double-blind placebo-controlled crossover design was implemented to investigate the effects of CBD, in comparison to placebo (PBO) and satiation (SAT). N=30 (Age: 20.07 ±8.66, 14F) dependent cigarette smokers (FTND: 5.56 ± 1.13) took part in two drug sessions after overnight abstinence and one fixed satiated session (SAT). On each session, participants were administered a dot probe task to assess attentional bias (AB), withdrawal (MPSS) and craving (QSU-B) questionnaires.

For the dot probe, there was greater AB after PBO compared to SAT (MDiff= -45.15 ± 10.48 ms, p=0.001, Cohens d: 0.789) and CBD (MDiff= -36.47±10.90 ms, p=0.007, Cohens d: 0.704) but not between SAT and CBD (MDiff= -8.68 ± 7.77, p=0.82) suggesting CBD normalises AB. Craving and withdrawal were greater in abstinence sessions but unaffected by CBD.

Conclusions: In the first study to assess CBD for nicotine withdrawal, we found that CBD reduced attentional bias which may be the mechanism by which CBD exerts its anti-addictive effects in humans. This effect occurred in the absence of changes in withdrawal and craving suggesting it exerts an early and direct effect.

Acknowledgements: This research was funded by a Medical Research Council (MRC) Studentship to the lead author and an MRC DPFS award (MR/K015524/1) to HVC and CJAM

EFFECTS OF ACUTE Δ9-TETRAHYDROCANNABINOL ON POST-EXTINCTION RESTING-STATE DYNAMICS WITHIN FEAR-EXTINCTION NEURAL CIRCUITRY

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We have previously demonstrated that an acute dose of Δ9-tetrahydrocanninbinol (THC), administered prior to extinction learning, facilitates the later recall of extinction learning and modulates fear-extinction neural circuitry during recall, including the ventromedial prefrontal cortex (vmPFC), hippocampus (HPC), and amygdala (AMYG). It remains unknown whether THC-induced changes in fear-extinction neural circuitry can be detected following extinction learning, which may reflect ongoing processes involved in the strengthening of the extinction memory for longer-term storage. To address this gap, we used a randomized, double-blind, placebo-controlled, between-subjects design to compare acute pharmacological effects of THC (7.5mg) vs. placebo (PBO) on post-extinction resting-state functional connectivity (RS-FC) within fear-extinction circuitry in 77 healthy adult volunteers (THC = 40; PBO = 37). RS-FC was examined between vmPFC, HPC, and AMYG using two complementary approaches: 1) static RS-FC, computed as the average correlation in ROI-ROI pairs across the entire scan; and 2) dynamic (i.e., time-varying) RS-FC, computed as the sliding window correlation time series' variance. RS-FC was then linked to brain activation during recall of extinction learning, tested 24 hours later.

Compared to PBO, THC administration was associated with lower static RS-FC between AMYG and HPC, but higher dynamic RS-FC between AMYG and vmPFC. Lower static RS-FC between the AMYG and the vmPFC was associated with higher HPC activation to the previously extinguished cue during extinction recall – a pattern of activation previously associated with better extinction recall and shown to be augmented by pre-extinction administration of THC. Therefore, post-extinction RS-FC patterns may reflect sustained effects of THC on fear-extinction circuitry even in the absence of an overt task, and/or effects of ongoing processes that serve to strengthen the neural connections supporting the more permanent storage of the extinction memory.

Acknowledgements: Funded by the National Center for Research Resources (UL1RR024986 and UL1RR029879) and the National Institute of Mental Health (1R21MH093917).

A SYSTEMATIC REVIEW ON THE PHARMACOKINETIC PROFILE OF CANNABIDIOL IN HUMANS

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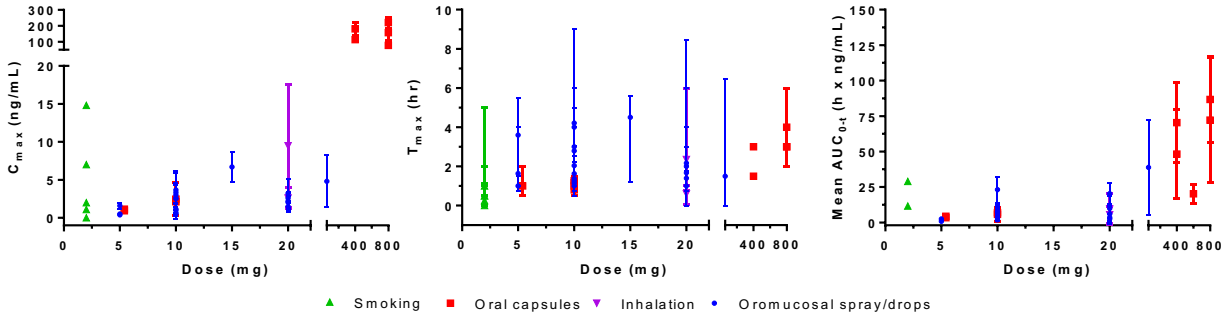
The aim of this systematic review was to collate data on the pharmacokinetics of cannabidiol (CBD) in humans. Databases (PubMed and EMBASE) were searched for original research articles measuring C_{max}, half-life, absorption, bioavailability, clearance, area under the curve, T_{max}, or volume of distribution published before October 2017.

Of the 788 articles retrieved, 21 included CBD pharmacokinetic parameters in humans. Routes of administration within the studies were intravenous (i.v.) (n=1), oromucosal spray (n=20), oral capsules (n=12), oral drops (n=2), nebuliser (n=1), aerosol (n=1) and smoking (n=7). CBD was administered on its own in 5 publications, while CBD was administered in combination with THC or within a cannabis extract in the remainder. The half-life of CBD was reported between 1.4 and 10.9 hr after oromucosal spray administration (n=3), 2-5 days after chronic oral administration (n=1), 24 hr after i.v. infusion (n=1), and 31 hr after smoking (n=1). Distribution volume was 2,520 L (n=1) following i.v. administration, and 23,866 L following oromucosal dosing (n=3). Plasma clearance (L/h) changes from 533 in a fed state to 2,546 in a fasted state, and is 3,252, 2,546, and 3,783 after 5, 10 and 20 mg CBD (n=2). A plasma clearance of 74.4 was reported following i.v. administration. Bioavailability following the smoking route was estimated at 31%, but was not reported in any other studies. The AUC and C_{max} of CBD is dose-dependent, and T_{max} is reached between 1 and 4 hours (Figure 1).

Bioavailability was increased during fed states compared to fasted states (n=2). Oral capsules



with piperine pro-nanolipospheres increased C_{max} by 4-fold and AUC by 2.2-fold compared to an oromucosal spray. None or minimal plasma accumulation of CBD was reported during repeated dosing experiments (n=7). CBD was consistently reported as safe and tolerable.

This review demonstrated there is a limited number of published studies investigating the PK of CBD in humans. Analysis and understanding of these pharmacokinetic properties of CBD is critical to its future application as a therapeutic compound. We suggest generating further PK data in humans, particularly following i.v. inhalation, infusion, rectal, intranasal, transdermal, subcutaneous or intraperitoneal routes.

A NATURALISTIC EXAMINATION OF THE PERCEIVED ACUTE EFFECTS OF CANNABIS ON NEGATIVE AFFECT

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Cannabis is commonly used to treat symptoms of depression, anxiety, and stress. However, few studies have examined the effects of cannabis, or its constituents (e.g., THC and CBD), on symptoms of negative affect. Moreover, the majority of previous studies have relied on oral or oromucosal methods of administration, whereas the predominant route of administration reported by medical cannabis patients is inhalation. Therefore, the present study was conducted to provide a naturalistic account of patients' perceived changes in symptoms of depression, anxiety, and stress as a function of inhaling different strains and doses of cannabis. Specifically, our objectives were to: 1) examine whether ratings of depression, anxiety, and stress are significantly reduced after using 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 symptom change, 4) investigate differences in symptom change across various doses, 5) explore potential tolerance effects across sessions, and 6) assess changes in baseline (i.e., pre-cannabis use) symptoms of negative affect across 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 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 THC and CBD content of the strain they are about to use, enter the method of ingestion and dose (number of puffs) of cannabis used, and then re-rate their symptom severity 20 minutes after cannabis use. The sample comprised 561 patients who used the app 3,153 times to track changes in depression, 770 patients who used it 5,085 times to track changes in anxiety, and 726 patients who used it 3,717 times to track changes in stress.

The results of a series of multilevel models revealed that patients perceived a 50% reduction in symptoms of depression and a 58% reduction in symptoms of anxiety and stress following inhalation of cannabis. Women reported significantly greater reductions in symptoms of anxiety than men, but men and women reported similar reductions in depression and stress. Low doses (2 puffs) appeared to be sufficient to reduce symptoms of depression and anxiety, while higher doses (10+ puffs) produced the greatest reductions in stress. High CBD/low THC strains produced the largest changes in ratings of depression, while high CBD/high THC strains produced the largest perceived changes in stress. THC/CBD content were unrelated to perceived changes in anxiety. Examinations of tracked sessions across time revealed no changes in the perceived efficacy of cannabis across sessions (i.e., no tolerance effects were detected). However, baseline symptoms of depression (but not anxiety or stress) appeared to be exacerbated across time/tracked sessions. The results indicate that acute cannabis use reduces perceived symptoms of negative affect in the short-term, but continued use may actually exacerbate baseline symptoms of depression over time.

Acknowledgements: Funded by Washington State University's Dedicated Marijuana Account

THE ANTI-INFLAMMATORY EFFECT OF CANNABINOIDS ADMINISTRATION IN GRAFT VERSUS HOST DISEASE MAY BE HAMPERED BY SUPPRESSIVE EFFECT ON LYMPHOCYTE RECONSTITUTION – COMPARISON OF Δ^9 -TETRAHYDROCANNABINOL (THC), CANNABIDIOL (CBD) AND CANNABIS EXTRACTS TREATMENT IN BONE MARROW TRANSPLANTATION MURINE MODELS

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Bone Marrow Transplantation (BMT) is a well-established treatment for malignant and non-malignant hematological diseases. Allogeneic transplantation comes with the risk of Graft versus Host Disease (GVHD), a major cause of morbidity and mortality in BMT patients. In addition, the toxicity of the conditioning protocol which precedes BMT impairs innate and adaptive immunity, making transplanted patients very susceptible to both common and unusual infections.

BMT patients, who often suffer from nausea and chronic pain, can benefit from treatment with Cannabis-based medicines. In addition to their effect on the nervous system, cannabinoids, the biologically active constituents of Cannabis, also have immunological effects. However, the influence of these drugs on rehabilitation of the hematological system after BMT and on GVHD is largely unknown.

In our study we tested the influence of THC, CBD or cannabis extracts on lymphocyte activation in vitro. We also administered these cannabis based drugs to BMT mice and examined their effects on rehabilitation of the hematological system. In addition, we compared the immune-modulatory effect of these drugs when used as a treatment for GVHD.

A better understanding of the effects of different cannabinoids on hematological recovery will allow the use of specific cannabinoid drug for each patient: as individualized medicines.

Acknowledgments: A part of this research was funded by the Israeli Ministry of Agriculture and Rural Development.

NEUROCOGNITION AND SUBJECTIVE EXPERIENCE FOLLOWING ACUTE DOSES OF THE SYNTHETIC CANNABINOID JWH-018: RESPONDERS VERSUS NON-RESPONDERS

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Synthetic cannabinoid mixtures have been easily accessible for years making people belief that these products were natural and harmless which contributed to its popularity. Nevertheless, there are many reports of users ending up in hospital due to side effects. Controlled studies on the effects of synthetic cannabinoids on human performance are lacking. In the current study, we looked at the effects of the synthetic cannabinoid JWH-018 after acute administration. 18 healthy cannabis-experienced participants took part in this placebo controlled, cross-over study. Participants received an average dose of 3,8 mg JWH-018, and were subsequently monitored for 12 hours, during which vital signs, cognitive performance and subjective experience were measured. Subjective high scores showed that there is a large variability in the subjective effects experienced by the participants. Therefore a split-plot repeated measures ANOVA, with Responder (i.e. subjective high score > 2) as a between subject factor, and Drug as a within subject factor was used. Serum concentrations of JWH-018 were significantly higher in the responders. Systolic blood pressure and heart rate was increased within the first hour after JWH-018 administration for all participants and JWH-018 significantly impaired critical tracking performance. Responders made more false alarms in the divided attention task, and had slower reaction times in the stop signal task and matching familiar figures test after JWH-018. Drug effects were also demonstrated on several subjective measures. JWH-018 administration caused large variability in drug concentrations and subjective experience. A suboptimal administration is probably responsible for this variation in response. JWH-018's impairing effects on cognition and subjective measures were mainly demonstrated in participant who experienced a subjective intoxication of the drug. An improved method of administrations is needed to obtain a more representative risk profile of JWH-018.

PHYSICOCHEMICAL PROPERTIES OF CANNABINOIDS PRODUCED BY BIOCATALYSIS

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The *Cannabis* THCA synthase and CBDA synthase enzymes were recombinantly produced in the yeast *Pichia*. The THCA synthase enzyme converted chemically synthesized CBGA into THCA and CBCA or CBGVA into THCVA and CBCVA in a bioreactor. The CBDA synthase enzyme converted chemically synthesized CBGA into CBDA, CBCA, and THCA or CBGVA into CBDVA, CBCVA, and THCVA. The bioreactor conditions required to produce more than 40 grams per liter of cannabinoids will be described. Each of the biocatalytically-produced cannabinoids was purified to greater than 99.5% purity. NMR analysis of the biocatalytically-produced cannabinoids shows they are structurally identical to their corresponding phytocannabinoids. The solubility of each of the biocatalytically-produced cannabinoids was determined after 1 hour in 0.1M phosphate buffered saline containing 1% DMSO at pH 7.4. The solubility of the cannabinoids ranged from 6.1 to 85 μM under these conditions. The implications of these findings for cannabinoid-based pharmaceutical development will be discussed.

IN VITRO AND *EX VIVO* EFFECTS OF NOVEL SEMI-SYNTHETIC CANNABINOID DERIVATIVES

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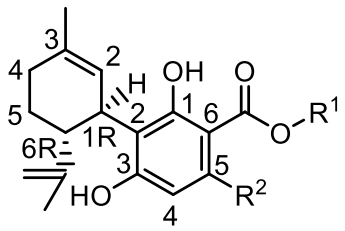
Background: Based on the work of Petrzilka et al.[1] and Crombie et al.[2] it is possible to produce cannabidiol (CBD), dronabinol ((-)-trans-Δ9-THC) and cannabidivarin (CBDV) efficiently by continuous synthesis.[3,4] Through modification of the side groups at position 6 (R^1) and position 5 (R^2) of the allyl benzene moiety, the synthesis route offers access to synthetic cannabinoids with a CBD or CBDV scaffold.[3,4] Nine new cannabinoids have been produced: 2-HEC, 2-HPC, GCBD, CHC, HC, NMSC, 2-HECBDV, CHCBDV, HCBDV.



Objectives: To study binding and intrinsic effects at CB1 and CB2 receptors, as well as the effects on inflammatory signal cascades in *in vitro* and *ex vivo* cell models.

Materials and methods: Binding affinity was studied in CB receptor-transfected HEK293EBNA cells. The intrinsic activity was studied in CHO cells. The induction of nuclear factor kappa B (NF-kappaB) and nuclear factor of activated T-cells (NFAT) was studied in Jurkat T cells. Induction of pro-inflammatory cytokines and chemokines (interleukin (IL)-6, IL-1beta, CC-chemokine ligand 2 (CCL2) and tumor necrosis factor (TNF) alpha) on the RNA level was studied in RAW264.7 macrophages. On the protein level, the expression of pro-inflammatory cytokines (IL-1beta, IL-6, IL-8, TNFalpha) and prostaglandin E2 (PGE_2) was studied in primary human monocytes.

Results: The CBD derivatives showed higher selectivity for CB2 receptors. The CBDV derivatives HCBDV and CHCBDV showed specific binding at CB1 and CB2 receptors in the nanomolar range. 2-HEC, 2-HPC, GCBD and NMSC acted as agonists at CB2 and as antagonists at the CB1 receptor. CHC binds CB1 and CB2 at the submicromolar range, and appears to be an agonist for both receptors. 2-HECBDV was demonstrated to be an agonist at CB2 and not active at CB1. In Jurkat T cells, NMSC inhibited both NF-kappaB and NFAT activity in a dose-dependent fashion. 2-HEC, 2-HPC and GCBD inhibited the expression of NFAT, also in a dose-dependent manner. CHC dose-dependently reduced the expression of IL-1beta and CCL2 mRNA in RAW264.7 macrophages. NMSC inhibited IL-1beta, CCL2, and TNFalpha and at higher doses induced a pronounced increase in IL-6 mRNA. In human primary monocytes 2-HEC and GCBD inhibited IL-1beta, IL-6 and TNFalpha synthesis in a concentration-dependent fashion. 2-HPC dose-dependently prevented TNFalpha and IL-6 in high concentrations. HC decreased TNFalpha, IL-1beta, and IL-6 release in a dose-dependent fashion. NMSC further increased LPS-elevated IL-1beta release but inhibited IL-8.

Conclusion: The CBD and CBDV derivatives studied here are suitable for targeting CB receptors. Some of the derivatives might be used as selective CB2 agonists. The length of the aliphatic rest at R^2 of CBD (pentyl) and CBDV (propyl) did not correlate with binding affinity. Higher polarity (2-HECBDV > NMSC > GCBD > 2-HEC) at R^1 appeared to favour the agonistic activity at CB2 receptors. To increase the significance of our results on structure-effect relationships, further synthetic derivatives and their testing would be necessary.

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ACTIVATION OF TRPM3 DECREASES CANNABIDIOL-INDUCED LARGE CATION UPTAKE VIA INTRACELLULAR TRPA1 IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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In rheumatoid arthritis (RA), synovial fibroblasts (SF) are one main contributor of joint destruction since they resist apoptosis and secrete pro-inflammatory cytokines and matrix degrading enzymes. Previous studies already demonstrated the functional expression of several transient receptor potential channels (TRP) such as TRPV1, TRPV2, TRPV4, TRPA1 and TRPM8 in SF. Upon ligation, these receptors increase intracellular calcium but they have also been linked to modulation of inflammation in several cell types. Additionally, activation of TRPA1, TRPV1 and TRPM8 permeates large cations such as the local anesthetic lidocaine through the plasma membrane. Similarly, cannabidiol (CBD) has been found to trigger the uptake of chemotherapeutic agents into cells via TRPV2. Until now, this mechanism has not been exploited in the treatment of RA and, therefore, we not only investigated the effect of CBD on calcium release but also on the uptake of the large cationic dye Po-Po3 iodide in SF.

In first experiments, we investigated calcium flux in SF and found that CBD dose-dependently increased intracellular calcium which was enhanced by TNF pre-treatment. Co-application of antagonists against several transient receptor channels (ruthenium red, 2-APB, capsazepine, 9-phenantrol, HC030031) revealed that TRPA1 was responsible for the observed effects. Interestingly, calcium flux was also increased when calcium-free extracellular solution was applied, suggesting an intracellular localization of TRPA1. By using the lysosomotropic agent glycyl-L-phenylalanine 2-naphthylamide, we found TRPA1 to be localized in lysosomal membranes.

Po-Po3 is a DNA binding dye whose uptake is dependent on leaky cell membranes. Although this is usually associated with necrosis, TRP channels are able to trigger the uptake of related compounds by pore dilation independent of cell death. Therefore, we investigated Po-Po3 uptake and found that CBD induced the uptake of this dye into SF. Similar to calcium mobilization this effect was enhanced by TNF-pretreatment. Since TRPA1 is located intracellularly, we looked for membrane receptors which might be activated in response to the increase in intracellular calcium by TRPA1 agonism. We found that activation of TRPM3 by pregnenolone sulfate (50µM) inhibited Po-Po3 uptake into TNF pre-treated SF, whereas the opposite was true for unstimulated cells. In conclusion, we propose the following mechanism of dye uptake in SF: i) activation of lysosomal TRPA1 leads to an increase in intracellular calcium; ii) lysosomal calcium release triggers the sensitization of cell membrane-bound TRPM3; iii) opening of TRPM3 triggers Po-Po3 uptake; iv) pregnenolone sulfate desensitizes TRPM3 and precludes dye uptake.

Clinical significance: This study might help to find a suitable delivery system to increase the concentration of anti-rheumatic drugs in cytokine-activated immune cells. In addition, the lysosomal localization of TRPA1 suggests an important role in autophagy and, therefore, TRPA1 might be an attractive therapeutic target to correct autophagy defects in e.g. lupus or RA.

EFFECT OF INHALED VAPORIZED CANNABIS ON PULMONARY FUNCTION IN PATIENTS WITH ADVANCED CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background: Chronic obstructive pulmonary disease is characterized by pathophysiological abnormalities in pulmonary function (e.g., expiratory flow limitation, lung hyperinflation) that contribute to the symptom of breathlessness, which is pervasive in this patient population. It follows, that improving airway function is essential to the symptomatic management of breathlessness in COPD. Available therapies, including inhaled bronchodilators and anti-inflammatory agents, appear to be sub-optimal at improving airway function in advanced COPD. Therefore, it is important to identify novel adjunct therapies that may improve airway function in COPD.

A series of studies conducted ~40 years ago demonstrated an acute bronchodilator effect of smoked cannabis in healthy and asthmatic adults that was comparable in magnitude and duration of effect to the short-acting β_2-adrenergic receptor agonist, isoproterenol. On the basis of these observations, the present study is the first to evaluate the acute effect of inhaled vaporized cannabis on airway function at rest in patients with advanced COPD.

Methods: Sixteen patients with advanced COPD (mean±SD forced expiratory volume in 1-sec (FEV_1) = 36±11 %predicted) completed spirometry and impulse oscillometry (iOS) before and immediately after inhalation of 35 mg of cannabis (Tilray House Blend-active, THC 18.2%, CBD <0.1%; Tilray, Nanaimo, BC, Canada) or 35 mg of a placebo control (CTRL, Tilray House Blend-control, THC 0.33%, CBD 0.99%), randomized to order. Cannabis and CTRL were administered in vaporized form using the Volcano Digit® vaporizer heated to 190°C (Storz and Bickel America, Inc., Oakland, CA).

Results: Compared with their respective pre-treatment conditions, neither CTRL nor cannabis had an effect on iOS or spirometric-derived pulmonary function measures. Compared with CTRL, inhaled vaporized cannabis had no significant effect on iOS (CTRL vs. cannabis: resistance at 5 Hz: 0.59±0.24 vs. 0.58±0.17 Hz; resistance at 20 Hz 0.34±0.13 vs. 0.33±0.06 Hz; reactance at 5 Hz: 24.0±5.1 vs. 23.3±4.2 Hz) or spiromteric-derived pulmonary function measures (CTRL vs. cannabis: FEV_1: 0.89±0.26 vs. 0.89±0.24 L; forced vital capacity (FVC): 2.93±0.85 vs. 2.90±0.90 L; FEV_1/FVC: 31±7 vs. 32±6 %; peak expiratory flow: 2.59±0.87 vs. 2.62±0.84 L · sec^{-1}).

Conclusion: Single-dose inhalation of vaporized cannabis vs. CTRL had no demonstrable effect on spirometric and iOS-derived measures of airway function at rest in patients with advanced COPD.

Acknowledgments: The study was supported by an Investigator Initiated Study grant from Tilray.

DISCOVERY OF CHIRAL ENDOCANNABINOID PROBES

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Ethanolamide and glyceride derivatives of polyunsaturated fatty acids are endogenously produced, metabolically labile substances involved in a number of (patho)physiological processes. Structural modifications to enhance the biological activities and target specificities of these lipids while increasing their metabolic stabilities, is a challenge. Here we report chiral 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. These novel endocannabinoid probes along with the recent determination of the crystal structures of the CB1 receptor will inspire the design of improved analogs for *in vitro*, *in vivo*, as well as computational studies aimed at exploring the physiological roles of AEA and 2-AG in cannabinoid receptor function.

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

A HIGH-RESOLUTION LC-MS/MS METHOD FOR PESTICIDES IN CANNABIS PRODUCTS

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The state of California has started to phase-in laboratory testing of cannabis products in 2018. One of the requirements is testing for residual pesticides. This requirement will be implemented in 2 steps. The first step started January 1, 2018 and all cannabis products currently must be tested for 21 different (Category I) pesticides. On July 1, 2018 and additional 45 (Category II) pesticides will be added to the testing requirement for a total of 66 pesticides. Currently there is no published method for these 66 pesticides in cannabis products.

In this presentation we will present a high resolution (QTOF) LC-MS/MS method for the quantification of 66 pesticides in various cannabis products. The use of high resolution MS/MS allows for unambiguous identification of pesticides without the need of an elaborate sample preparation procedure such as QUECHERS. Validation results for flower, concentrate and e-liquid for use in vaporizers will be presented.

RATIONAL MODULATION OF DRUG-TARGET RESIDENCE TIME FOR MONOACYLGLYCEROL LIPASE INHIBITORS

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For the past decade, growing amount of experimental data demonstrate that drugs with prolonged on-target residence time show higher efficacy. These data suggest that pharmacodynamic activity could be driven often by drug-target residence time, rather than thermodynamic affinity. Residence time (τ) can be recognized as a key optimization criterion for the design of new drugs. Currently, we lack the approaches for the experimental determination and practical implementation of τ values toward the design of potent and selective human monoacylglycerol lipase (hMGL) inhibitors with tunable and prolonged τ.

Here, we introduce a protein-based real-time ^{1}H NMR spectroscopy approach providing straightforward determination of τ for different types of covalent hMGL inhibitors. Using this approach, we observed that biochemical τ values for these inhibitors differ from hours to several days depending on the inhibitor structure. Specifically, we demonstrate that potent hMGL inhibitors being commonly used in pharmacological studies have relatively short biochemical τ values due to fast hydrolysis of covalent hMGL-inhibitor complex. The reversible nature of these covalent ligands may result in their weak or shortened activity *in vivo*.

The hydrolysis rate of hMGL-inhibitor complex is regulated by water accessibility to the binding pocket which is sensitive to the level of binding site occlusion from bulk water. The binding site occlusion level in turn depends on the scale of conformational changes triggered by high-affinity binding. Thus, in order to rationally modulate τ, structural changes and conformational dynamics as well as electronic and steric complementarity between ligand and hMGL binding site must be elucidated. All of these determinants and other specific factors influencing τ values will be presented and discussed. Results from these studies should provide a strategy for tuning the residence time of hMGL inhibitors.

Acknowledgments: Funded by the National Institute on Drug Abuse (Grants DA09158 and DA03801)

EFFECTS OF CB_2 RECEPTOR MODULATION IN ALZHEIMER'S DISEASE

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Alzheimer`s disease (AD) is the cause of the most common forms of dementia. Characteristics of AD include deposition of Aβ, microgliosis and attendant cytokine production, events believed to be important drivers of the pathogenic cascade. Accumulated evidence suggests a role for Cannabinoid CB_2 receptors in AD and indicates their potential as a therapeutic target against this neurodegenerative disease. We recently reported that the expression of cannabinoid CB_2 receptors are below the level of detection in a novel transgenic mouse model ($CB_2^{EGFP/f/f}$) that allows for identification of cells that are actively transcribing the *Cnr2* gene. However, EGFP expression is strongly up-regulated in microglial cells in areas of amyloid-triggered neuroinflammation in transgenic mice expressing the CB2 reporter and 5 familial AD genes ($CB_2^{EGFP/f/f}$/5xFAD).

To evaluate the role of the CB_2 receptor in AD, we analyzed the impact of *Cnr2* gene deletion on plaque formation, soluble amyloid levels and neuroinflammation, by generating a new strain of CB_2-deficient mice ($CB_2^{-/-}$/5xFAD mice). By means of immunofluorescence, we observed that CB_2 deficiency leads to decrease of neuritic plaques that was accompanied by a reduction of microgliosis. However, that observation was not paralleled by changes in levels of soluble $A\beta_{1\text{-}42}$, in the hippocampus. In addition, $A\beta_{1\text{-}42}$ exposed neonatal microglial cells obtained from $CB_2^{EGFP/f/f}$ mice, showed an increased EGFP expression. Finally, neonatal microglial cells obtained from $CB_2^{-/-}$ mice showed enhanced oxidative stress and impaired inflammatory response after exposure to $A\beta_{1\text{-}42}$.

We have now addressed the functional relevance of this selective expression of CB_2 receptors in activated microglia. To that end, 6-month old $CB_2^{EGFP/f/f}$/5xFAD mice were treated with a specific CB_2 agonist (HU308; 5mg/kg) or antagonist (SR144528; 1mg/kg), i.p., for 19 days. Both compounds produced a decrease in the density of hippocampal ß-amyloid plaques, and lower expression levels of the pro-inflammatory cytokine interleukin-1β (IL1β), without changes in microgliosis. We thus conclude that both compounds paradoxically led to similar changes, which could be explained by the specific properties of CB_2 receptors and the pharmacological profile of HU308 and SR144528. Taken together, these observations point to a relevant role of cannabinoid CB_2 receptors in Alzheimer's pathophysiology that warrants further research.

Acknowledgements: This work was supported by Ministerio de Economía y Competitividad (SAF2016/75959-R), Ministerio de Educación of Spain (PR2009-0169) and Comunidad de Madrid (S2010/BMD-2308), Universidad Francisco de Vitoria and the Research Component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin, the National Institute on Drug Abuse (DA041212).

HEPATIC ENDOCANNABINOID SYSTEM (ECS) IN COMMON MARMOSETS (*Callithrix jacchus)*

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Introduction: Common marmosets (*Callithrix jacchus*) are the new world non-human primates, used in toxicology research due to their small body size, lower feeding costs and high reproductive efficiency in captivity. Another advantage of this model is that some human antibodies react similarly to white blood cells of both marmosets and humans. Marmosets have been used as a model to the study CB1R related pathway of cocaine addiction. However, there are no data available regarding expression of components of endogenous cannabinoid system (ECS) in *Callithrix jacchus*. The aim of this study was to evaluate expression of ECS receptors and enzymes in hepatic tissue of common marmosets. **Materials and methods.** Banked hepatic tissues of *Callithrix jacchus* and *Papio spp.* and placental tissues of *Papio spp*. and human placenta, were used in western blot and IHC (Immunohistochemistry) analyses to check protein expression of endocannabinoid receptors (CB1R and CB2R), FAAH (Fatty Acid Amide Hydrolase), DAGLα (Diacylglycerol Lipase), MAGL (Monoacylglycerol Lipase) and COX-2 (Cyclooxygenase-2). Protein expression in western blot was normalized with β-actin. mRNA expression of CB1R was analyzed in marmoset using TaqMan Gene Expression Assay probes (Life Technology, USA) by qRT-PCR analysis. Additionally End Point PCR, molecular cloning, sequencing and sequence analyses were performed. **Results:** The hepatic protein expression of full length CB1R and COX-2 had lower expression in marmoset compared to baboon. There was no cross-reaction between marmoset and human antibodies for FAAH and MAGL, but CB2R and DAGLα were cross-reacting. (Table 1). **Conclusion:** The pharmacological and physiological responses to CB1R ligands and to FAAH inhibitors are documented in *Callithrix jacchus,* moreover these species along with *Papio spp.* have been described as a model of human fatty liver diseases, the main target of ECS pharmacology. Our data suggests, that *Callithrix jacchus* specific antibodies and probes should be used for evaluation of molecular biology of ECS.

Table 1: The protein and mRNA expression in marmoset liver, human placenta, baboon placenta and baboon liver by western blot, immunohistochemistry and qRT-PCR techniques, using human-specific antibodies in all three species and baboon and human specific primer-sets. (WB: Western blot, IHC: Immunohistochemistry)

	Marmoset Liver			Baboon Liver[1]			Baboon Placenta[1]			Human Placenta		
	WB	IHC	qRT-PCR	WB	IHC	qRT-PCR	WB	IHC	qRT-PCR	WB[2]	IHC[2]	qRT-PCR[3,4]
CB1R*	-	-	+	√	√	√	√	√	√	++	+	+
CB2R$^{\Delta}$	++	+	n/a	√	√	√	√	√	√	++	++	n/a
FAAH*	-	–	n/a	√	√	√	√	√	√	+	++	+++
DAGLα*	++	++	n/a	√	√	√	√	√	√	+	++	+++
MAGL*	+	+++	n/a	√	√	√	√	√	√	++	++	+
COX-2$^{\Delta}$	+	+++	n/a	√	√	√	√	√	√	++	+	n/a

[1]Am J Physiol Endocrinol Metab. 2017 Nov 14:ajpendo001192017.
[2] AGOG, January 2014Volume 210, Issue 1, Supplement, Page S94
[3]Placenta September 2013Volume 34, Issue 9, Page A80
[4] Placenta Volume 30, Issue 6, June 2009, Pages 516-522
*qRT-PCR analysis performed using TaqMan Gene Expression Assay Probes (Life Technology, USA)
$^{\Delta}$qRT-PCR analysis performed using Fast Start Essential DNA Probe Master Mix (Roche, USA)

EXCRETION AND PERSISTENCE OF MARIJUANA IN HUMAN BREAST MILK

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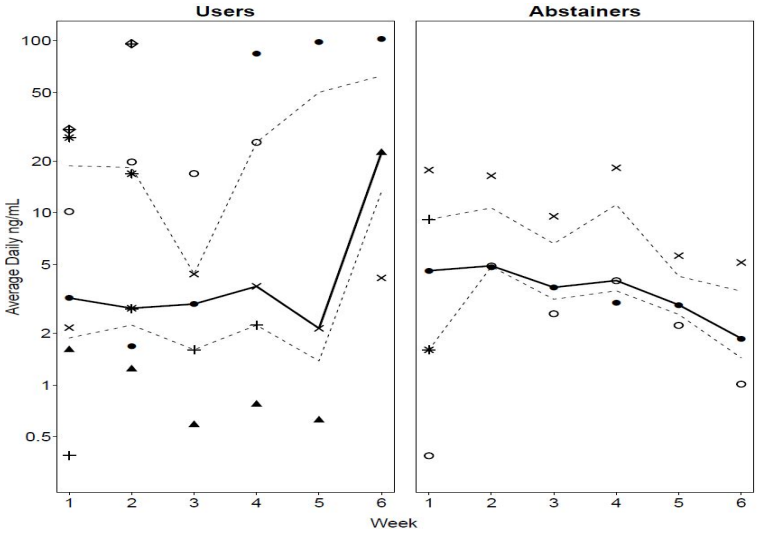
Background/Methods Marijuana (MJ) is the most commonly used illicit drug in pregnancy, with increasing legalization. Delta-9-tetrahydrocannabinol (THC), the psychoactive component of MJ, is highly lipophilic. There are a paucity of data regarding MJ concentration in breast milk. Our objective was to estimate the excretion and duration of THC and its metabolites in maternal plasma, urine and human breast milk after birth. We performed a prospective, observational pilot study enrolling women at a single tertiary center with a positive urine toxicology screen for isolated MJ use at delivery admission. Inclusion criteria were intent to breastfeed and abstain from MJ for 6 weeks postpartum. Self-reported surveys were administered at baseline, and weekly thereafter, to obtain substance use patterns during pregnancy and after delivery. Maternal plasma, urine and breast milk were obtained 2-3 times per week for 6 weeks. A published high-performance atmospheric pressure chemical ionization liquid chromatography tandem mass spectrometry based assay was used to quantify 11 cannabinoids and metabolites. The assay has been validated for urine and plasma and cross validated for breast milk. The lower limits of quantification for THC were 0.78, 0.39 and 0.39 ng/mL for breast milk, plasma and urine, respectively.

Results Of the 140 women screened, 13 women enrolled, 6 (46%) were Caucasians, mean age of 24 +/-5 years. Among the study cohort, 7 (54%) women reported abstinence for all study samples and 6 (46%) women completed the full 6-week study. There were 322 biological samples available for analysis. All women had detectable THC in breast milk throughout the 6-week study period. The median THC concentration at study end was 1.7 ng/mL (IQR 1.2-1.9) for those who abstained. Using the average 24-hour milk production in early postpartum period of 700ml, the median daily THC excretion among the abstainer group was 3.4 µg (IQR 3.4 - 7.5) at 2 weeks and 1.9 µg (IQR 1.6- 12.8) at study end. The milk:plasma partition coefficient for THC was approximately 7:1, where hydrophilic metabolites were not detectable in milk. Projected estimates of time to THC elimination from breast milk was 8 weeks. Urine THC-COOH glucuronide concentrations varied compared to plasma THC-COOH and breast milk THC.



Detectable THC in breast milk by weeks postpartum- Median and IQR

Conclusion Measurable quantities of THC were detected in breast milk for up to 6 weeks in all women, notably with <50% able to abstain from MJ use. Absence of detectable urine THC metabolites may not be an accurate marker for absence of THC in the plasma or breast milk. Ongoing pharmacokinetic modeling is needed to describe THC excretion in breast milk and urine. Inclusion of infants in future studies is essential to better understand their unique THC bioavailability, metabolism and neurodevelopmental effects with perinatal MJ exposure.

MATERNAL NUTRIENT RESTRICTION DOES NOT INFLUENCE FETAL CEREBRAL ENDOCANNABINOID AEA (ANANDAMIDE) PATHWAY

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Introduction: Poor nutrition during pregnancy is a major public health problem in the United States. The endogenous cannabinoid system (ECS) is a pharmacological target for the treatment of obesity, inflammation, cardiovascular and neuronal damage. The level of endogenous cannabinoid AEA in fetal brain is regulated by endocannabinoid receptor (CB1R) and ECS metabolic enzymes FAAH (Fatty acid amide hydrolase), DAGLα (Diaacylglycerol α) and COX-2 (Cyclooxygenase-2). The aim of this study was to evaluate temporal changes in CB1R and ECS metabolic enzymes (FAAH, DAGLα, and COX-2) in male and female offspring of maternal nutrient restricted baboon (*Papio spp.*) model.

Methods: Pregnant baboons underwent global dietary reduction by 30% (MNR group). Fetal brain tissue samples were collected at 165 dGA (days of gestational age) (CTR n=4 to 9; MNR n=4 to 5). Western blot analysis was performed to detect endocannabinoid and metabolic enzymes using commercially available antibodies. Protein expression was normalized using β-actin.

Results: Protein expression of CB1R, FAAH, DAGLα, and COX-2 did not differ between CTR and MNR group at 165 dGA in male and female fetuses.

Discussion: We previously reported increased fetal cerebral 2-AG related pathway in the baboon model of maternal nutrient restriction. Our data is in line with published differential responses of 2-AG and AEA pathways to nutrient restricted humans and pregnant rodents.

THE CANNABINOID 1 RECEPTOR ANTAGONIST O2050 POTENTIAL CARDIOPROTECTIVE EFFECTS IN CARDIOMYOBLAST CELLS

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Cannabinoid 1 (CB_1) receptor activation has been recognized in cardiac dysfunction and cell death associated with various forms of atherosclerosis, shock, and heart failure. The present study was designed to explore the effect of cannabinoid ligands on cell viability and oxidative stress in H9C2 cardiomyoblast cells. The tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay was carried out to determine the cell viability after treatment with cannabinoid agonists and antagonists in combination with the apoptosis inducer hydrogen peroxide (H_2O_2). After a 24 h treatment, the cells were incubated for 2 h with MTT at 37°C, and the absorbance was measured at 570 nm. Fluorescent microscopy was employed to evaluate the apoptosis by H_2O_2, in H9C2 cells after pretreatment with the CB_1 antagonist O2050. Effects of the CB_1 antagonist O2050 on mono-(2-ethylhexyl) phthalate (MEHP)-induced reactive oxygen species (ROS) production was evaluated using Guava EasyCyte™ a single laser-configuration flow cytometer. Data presented as Mean Fluorescence Intensity (MFI).

The viability of H9C2 cells treated alone with the cannabinoids agonists CP55940, HU210, or Win55212-2 and the cannabinoid antagonists SR141716 and O2050 (30 nM to 3 μM) were not significantly different from control (88±8, 95±4, 98±2, 90±6 and 102±3 % of control, respectively). Treating the cells with 400 μM H_2O_2 significantly reduced the cell viability in a dose-dependent manner (61% ±3 of Control at 400 μM), while co-incubation with cannabinoid ligands Win55212-2, SR141716 (30nM-3μM) was not able to prevent the H_2O_2-induced decrease in cell viability (61±3, 65±4% of control). However, co-incubation with 3 μM O2050 was able to significantly reverse (74±4 % of control). In order to further investigate the effect of pretreatment with O2050 on H_2O_2-induced apoptosis, cells were pre-treated 6 h with O2050 prior to incubation with H_2O_2 for 18 h before staining with acridine orange and 4',6-diamidino-2-phenylindole (DAPI) to detect the cell apoptosis using fluorescent microscopy. Data showed 400 μM H_2O_2 caused the majority of cells to be apoptotic, while pretreatment with O2050 (10 μM and 30 μM) decreased the H_2O_2-induced cell apoptosis and ameliorated cell morphology changes. Effects of O2050 on ROS production was determined using flow cytometry. MEHP (100 μM) significantly stimulated the ROS production in H9C2 cells (123±3 MFI), while pretreatment with 30μM O2050 was able to significantly inhibit ROS generation (90±3 MFI). These results suggest the protective effects of O2050 against H_2O_2-induced injury and the anti-oxidative capability of O2050 in H9C2 cells. The results also encourage more investigations to better understand the potential cardio-protective of CB_1 antagonists.

Acknowledgements: Support from NIH grant R01-DA04215 is appreciated.

FABP5 CONTROLS LIPID SIGNALING THAT PROMOTES PROSTATE CANCER METASTASIS

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The 5-year relative survival rate of men in the United States diagnosed with a distantly metastasized prostate carcinoma is 29%. Fatty acid binding proteins (FABPs) bind to long-chain fatty acids and facilitate their transport to diverse cellular compartments, including the nucleus. Upregulation of FABP5 increases the migratory and invasive capacity (metastatic potential) of prostate carcinomas and is associated with a shorter survival time of prostate cancer patients. Previous work indicates that overexpression of monoacylglycerol lipase (MAGL) and fatty acid synthase (FASN), enzymes that produce endogenous free fatty acids, increases the metastatic potential of prostate cancer. FABP5 translocates fatty acids to nuclear receptors, whose activation promotes tumor growth and metastasis. Here we test the central hypothesis that FABP5 is required to facilitate the transport of MAGL/FASN-generated lipids to increase the metastatic potential of human prostate carcinomas.

The expression of FABP5, MAGL, and/or FASN was modulated in the weakly metastatic LNCaP and the highly metastatic PC3 human prostate cancer cell-lines. Migration and invasion assays were employed to examine *in vitro* metastatic potential. The metastatic potential of PC3 cells stably-expressing luciferase was quantified using the *In Vivo* Imaging System following orthotopic implantation of cells into the ventral lobe of the prostate of mice.

Expression of FABP5 in the poorly metastatic LNCaP cells (which do not natively express FABP5) significantly increased their migratory and invasive potential *in vitro*. Pharmacological or genetic inhibition of FASN attenuated this increased metastatic behavior in LNCaP cells expressing FABP5. Overexpression of MAGL in LNCaP cells (which possess a low level of MAGL expression) failed to increase the migratory or invasive potential of LNCaP cells *in vitro*; however, concomitant overexpression of FABP5 and MAGL significantly increases metastatic potential (greater than overexpression of FABP5 alone). To assess whether the interplay between MAGL and FABP5 extends to tumors growing *in vivo*, mice were implanted orthotopically with PC3 cells (which natively express FABP5), and imaged weekly for ten weeks. Knockdown of FABP5 significantly reduced tumor growth and metastasis. PC3 cells overexpressing MAGL exhibited a significant increase in both the rate and magnitude of metastasis to bone, liver, and lungs. Conversely, knockdown of FABP5 in PC3 cells overexpressing MAGL significantly decreased their metastatic potential to a level comparable to wild-type PC3 cells.

The ability of MAGL and FASN to increase the metastatic potential of prostate cancer cells is dependent upon the simultaneous co-expression of FABP5. This positions FABP5 as an integral component of a lipid signaling network and a promising target for the development of novel therapeutics to treat aggressive prostate cancer.

Acknowledgements: This work was funded by NIH grants DA035949 and 4T32GM008468-15

ANALYSIS OF REGULATORY IMPROVEMENTS AND SETBACKS FOR MEDICAL CANNABIS PROGRAMS AND PRODUCT SAFETY STANDARDS

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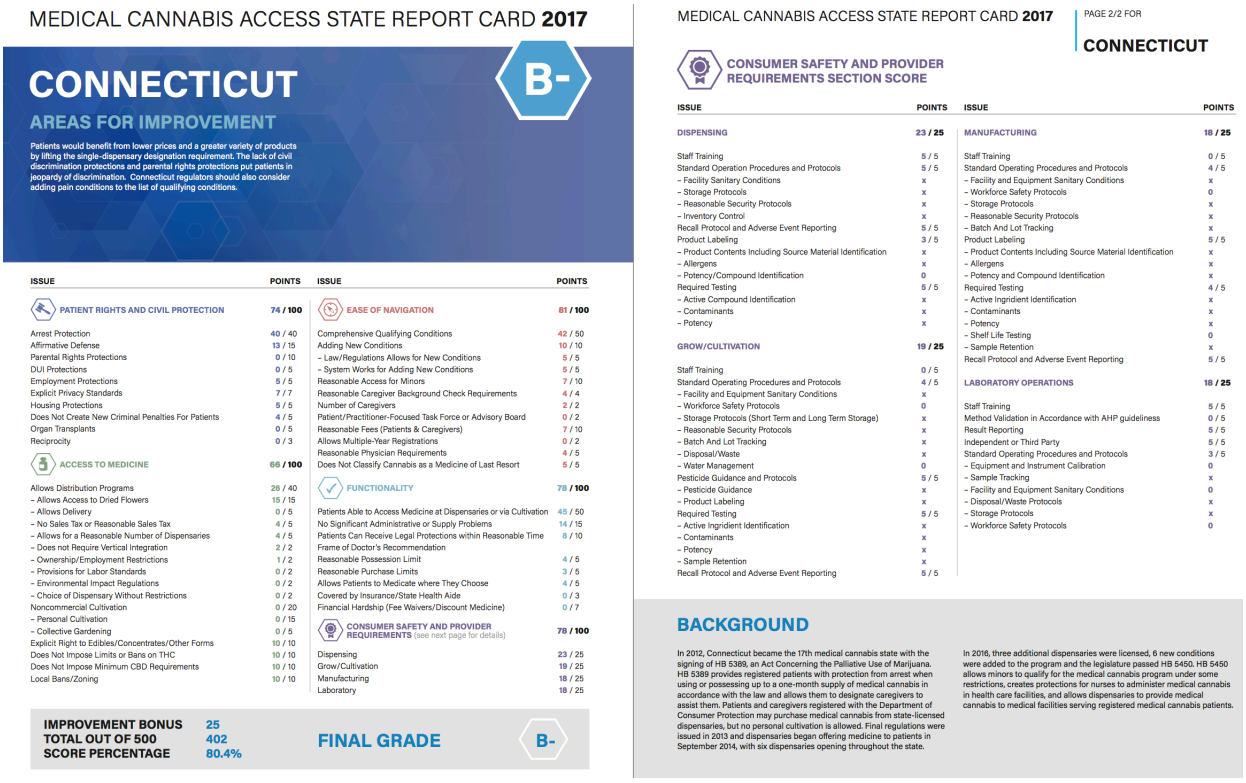
Patient Focused Certification (PFC) is a 3rd party certification program focused on medical cannabis product safety. PFC was developed by Americans for Safe Access (ASA), a medical cannabis advocacy organization. ASA set the standards in the U.S. for medical cannabis product safety and best practices for manufacturing, dispensing, cultivation, and laboratory operations. The PFC program is working around the world to implement existing standards and best practices for patients, providers of medical care, companies, regulatory bodies and legislators.

This presentation will provide an update on cannabis regulations in the U.S., data on cannabis dispensary staff training in the U.S., as well as a set of data grading regions in the U.S. with a medical cannabis program, under a 400 point assessment in 5 general categories: Patient Rights and Civil Protections, Access to Medicine, Ease of Navigation, Functionality, and Consumer Safety and Provider Requirements (see example of the analysis below).



MEDICAL CANNABIS ACCESS STATE REPORT CARD **2017**

CONNECTICUT — **B-**

AREAS FOR IMPROVEMENT

Patients would benefit from lower prices and a greater variety of products by lifting the single-dispensary designation requirement. The lack of civil discrimination protections and parental rights protections put patients in jeopardy of discrimination. Connecticut regulators should also consider adding pain conditions to the list of qualifying conditions.

ISSUE	POINTS
PATIENT RIGHTS AND CIVIL PROTECTION	**74 / 100**
Arrest Protection	40 / 40
Affirmative Defense	13 / 15
Parental Rights Protections	0 / 10
DUI Protections	0 / 5
Employment Protections	5 / 5
Explicit Privacy Standards	7 / 7
Housing Protections	5 / 5
Does Not Create New Criminal Penalties For Patients	4 / 5
Organ Transplants	0 / 5
Reciprocity	0 / 3
ACCESS TO MEDICINE	**66 / 100**
Allows Distribution Programs	26 / 40
– Allows Access to Dried Flowers	15 / 15
– Allows Delivery	0 / 5
– No Sales Tax or Reasonable Sales Tax	4 / 5
– Allows for a Reasonable Number of Dispensaries	4 / 5
– Does not Require Vertical Integration	2 / 2
– Ownership/Employment Restrictions	1 / 2
– Provisions for Labor Standards	0 / 2
– Environmental Impact Regulations	0 / 2
– Choice of Dispensary Without Restrictions	0 / 2
Noncommercial Cultivation	0 / 20
– Personal Cultivation	0 / 15
– Collective Gardening	0 / 5
Explicit Right to Edibles/Concentrates/Other Forms	10 / 10
Does Not Impose Limits or Bans on THC	10 / 10
Does Not Impose Minimum CBD Requirements	10 / 10
Local Bans/Zoning	10 / 10

ISSUE	POINTS
EASE OF NAVIGATION	**81 / 100**
Comprehensive Qualifying Conditions	42 / 50
Adding New Conditions	10 / 10
– Law/Regulations Allows for New Conditions	5 / 5
– System Works for Adding New Conditions	5 / 5
Reasonable Access for Minors	7 / 10
Reasonable Caregiver Background Check Requirements	4 / 4
Number of Caregivers	2 / 2
Patient/Practitioner-Focused Task Force or Advisory Board	0 / 2
Reasonable Fees (Patients & Caregivers)	7 / 10
Allows Multiple-Year Registrations	0 / 2
Reasonable Physician Requirements	4 / 5
Does Not Classify Cannabis as a Medicine of Last Resort	5 / 5
FUNCTIONALITY	**78 / 100**
Patients Able to Access Medicine at Dispensaries or via Cultivation	45 / 50
No Significant Administrative or Supply Problems	14 / 15
Patients Can Receive Legal Protections within Reasonable Time Frame of Doctor's Recommendation	8 / 10
Reasonable Possession Limit	4 / 5
Reasonable Purchase Limits	3 / 5
Allows Patients to Medicate where They Choose	4 / 5
Covered by Insurance/State Health Aide	0 / 3
Financial Hardship (Fee Waivers/Discount Medicine)	0 / 7
CONSUMER SAFETY AND PROVIDER REQUIREMENTS (see next page for details)	**78 / 100**
Dispensing	23 / 25
Grow/Cultivation	19 / 25
Manufacturing	18 / 25
Laboratory	18 / 25

IMPROVEMENT BONUS	25
TOTAL OUT OF 500	402
SCORE PERCENTAGE	80.4%

FINAL GRADE **B-**

MEDICAL CANNABIS ACCESS STATE REPORT CARD **2017** PAGE 2/2 FOR **CONNECTICUT**

CONSUMER SAFETY AND PROVIDER REQUIREMENTS SECTION SCORE

ISSUE	POINTS
DISPENSING	**23 / 25**
Staff Training	5 / 5
Standard Operation Procedures and Protocols	5 / 5
– Facility Sanitary Conditions	x
– Storage Protocols	x
– Reasonable Security Protocols	x
– Inventory Control	x
Recall Protocol and Adverse Event Reporting	5 / 5
Product Labeling	3 / 5
– Product Contents Including Source Material Identification	x
– Allergens	x
– Potency/Compound Identification	0
Required Testing	5 / 5
– Active Compound Identification	x
– Contaminants	x
– Potency	x
GROW/CULTIVATION	**19 / 25**
Staff Training	0 / 5
Standard Operating Procedures and Protocols	4 / 5
– Facility and Equipment Sanitary Conditions	x
– Workforce Safety Protocols	0
– Storage Protocols (Short Term and Long Term Storage)	x
– Reasonable Security Protocols	x
– Batch And Lot Tracking	x
– Disposal/Waste	x
– Water Management	0
Pesticide Guidance and Protocols	5 / 5
– Pesticide Guidance	x
– Product Labeling	x
Required Testing	5 / 5
– Active Ingridient Identification	x
– Contaminants	x
– Potency	x
– Sample Retention	x
Recall Protocol and Adverse Event Reporting	5 / 5

ISSUE	POINTS
MANUFACTURING	**18 / 25**
Staff Training	0 / 5
Standard Operating Procedures and Protocols	4 / 5
– Facility and Equipment Sanitary Conditions	x
– Workforce Safety Protocols	0
– Storage Protocols	x
– Reasonable Security Protocols	x
– Batch And Lot Tracking	x
Product Labeling	5 / 5
– Product Contents Including Source Material Identification	x
– Allergens	x
– Potency and Compound Identification	x
Required Testing	4 / 5
– Active Ingridient Identification	x
– Contaminants	x
– Potency	x
– Shelf Life Testing	0
– Sample Retention	x
Recall Protocol and Adverse Event Reporting	5 / 5
LABORATORY OPERATIONS	**18 / 25**
Staff Training	5 / 5
Method Validation in Accordance with AHP guidelines	0 / 5
Result Reporting	5 / 5
Independent or Third Party	5 / 5
Standard Operating Procedures and Protocols	3 / 5
– Equipment and Instrument Calibration	0
– Sample Tracking	x
– Facility and Equipment Sanitary Conditions	0
– Disposal/Waste Protocols	x
– Storage Protocols	x
– Workforce Safety Protocols	0

BACKGROUND

In 2012, Connecticut became the 17th medical cannabis state with the signing of HB 5389, an Act Concerning the Palliative Use of Marijuana. HB 5389 provides registered patients with protection from arrest when using or possessing up to a one-month supply of medical cannabis in accordance with the law and allows them to designate caregivers to assist them. Patients and caregivers registered with the Department of Consumer Protection may purchase medical cannabis from state-licensed dispensaries, but no personal cultivation is allowed. Final regulations were issued in 2013 and dispensaries began offering medicine to patients in September 2014, with six dispensaries opening throughout the state.

In 2016, three additional dispensaries were licensed, 6 new conditions were added to the program and the legislature passed HB 5450. HB 5450 allows minors to qualify for the medical cannabis program under some restrictions, creates protections for nurses to administer medical cannabis in health care facilities, and allows dispensaries to provide medical cannabis to medical facilities serving registered medical cannabis patients.

CANNABINOIDS DECARBOXYLATION AND METABOLOMIC TRANSFORMATION OF ENTOURAGE EFFECT BIOACTIVE COMPLEXES

Jana Hajslova[1*], Frantisek Benes[1], Jiri Hricko[1], Jahan Marcu[1], Pavel Kubu[2], Steph Sherer[2] and Ethan Russo[2]

[1]University of Chemistry and Technology Prague, Czech Republic
[2]International Cannabis and Cannabinoids Institute (ICCI), Prague, Czech Republic

Introduction: Cannabis is now available in many pharmacies in Europe. Doctors are not only recommending *cannabis flos* (flowers), but additional formulations including capsules, extracts and creams. However, various preparations show a much greater change in cannabinoid transformation than decarboxylation of THCA-A to THC and CBDA to CBD. Exposure to 120°C for 30 minutes in a controlled environment is considered sufficient to decarboxylate THC and CBD, however little evidence is available concerning the precise conversion rates of cannabis compounds, which varies with temperature and time of exposure. This affects the metabolomic fingerprint of the plant.

Methods: Using chemovars available in EU pharmacies, ICCI performed tests on two temperatures, 60°C and 120°C, on different chemovars in three forms of dispensed medication – cannabis flos, capsules with flos, and topical cream with flos mixed in. Target analysis included 12 cannabinoids, 30 terpenes, and non-targeted metabolomic fingerprinting with all 3 preparations of Bedrocan's chemovars.

Results: Heat exposure to the preparations at 120°C for 30 minutes produces a THCA-A/THC conversion rate up to 88% and net increases in decarboxylated cannabinoids. The 60°C treatment for 30 minutes resulted in changes in both acidic and decarboxylated cannabinoids. THCA-A concentrations increased by 20% under the lower temperature and there were net increases in non-acidic cannabinoids and decreases in other samples.

TEST RESULTS:	Bedrocan flos	Bedrocan enterosolvent capsules		Bedrocan cream 15%	Bedrobinol flos	Bedrobinol enterosolvent capsules		Bedrobinol cream 15%
compouding details:	1g of flos	with 0,0625g flos activated by 120°C for 30min	recount for 1g of activated flos	15g flos in 100g cream	1g of flos	with 0,333 g flos activated by 60°C for 30min	recount for 1g of activated flos	15g flos in 100g cream
Analyt	**Concentration [mg/kg]**	**Concentration [mg/kg]**	**Concentration [mg/kg]**	**Concentration [mg/kg]**	**Concentration [mg/kg]**	**Concentration [mg/kg]**	**Concentration [mg/kg]**	**Concentration [mg/kg]**
CBD (cannabidiol)	68.00	35.00	560.00	16.00	63.00	12.00	36.00	6.40
CBDA (cannabidiolic acid)	322.00	4.20	67.20	4.70	215.00	64.00	192.00	10.00
Δ^9-THC (delta-9-	57784.00	11592.00	185472.00	9806.00	17652.00	5247.00	15741.00	1918.00
Δ^8-THC (delta-8-	0.50	110.00	1760.00	117.00	0.50	0.50	1.50	0.25
Δ^9-THCA-A (delta-9-tetrahydrocannabinolic acid - A)	156231.00	111.00	1776.00	0.48	113507.00	45066.00	135198.00	6556.00
CBN (cannabinol)	1023.00	232.00	3712.00	167.00	741.00	230.00	690.00	63.00
CBG (cannabigerol)	2105.00	374.00	5984.00	291.00	381.00	130.00	390.00	27.00
CBGA (cannabigerolic acid)	6186.00	94.00	1504.00	61.00	1541.00	519.00	1557.00	70.00
CBDV (cannabidivarine)	1.80	0.03	0.48	60.00	1.50	0.50	1.50	0.25
CBC (cannabichromene)	628.00	0.07	1.12	0.46	308.00	85.00	255.00	27.00
THCV (tetrahydrocannabivarine)	NA	NA	NA	NA	115	39	117.00	11
CBDVA (cannabidivarinic acid)	NA	NA	NA	NA	3.6	0.82	2.46	0.25
Cannabinoids Total	**224349.30**	**12552.30**	**200836.80**	**10523.64**	**134528.60**	**51393.82**	154181.46	**8689.15**
CBD Total	***390.00***	***39.20***	***627.20***	***20.70***	***278.00***	***76.00***	228.00	***16.40***
THC Total	***214015.50***	***11813.00***	***189008.00***	***9923.48***	***131159.50***	***50313.50***	150940.50	***8474.25***

Conclusion: Additional investigation is required to ascertain the optimal decarboxylation temperature and time of procedure to maximize neutral cannabinoid yield and preserve total concentrations more consistent with herbal material. Also, different chemovars show different transformation rates. It is also important to control for moisture loss. Thus, chemovar-specific activation charts will improve product information for clinical use to achieve optimal concentration of neutral cannabinoid compounds and prevent loss of cannabinoid and terpene total concentrations in the dispensed medication.

CHRONIC ADMINISTRATION OF ACEA, A CB1 AGONIST, FAILED TO PREVENT TUMOR GROWTH IN A XENOGRAFT ECTOPIC OVARIAN CANCER MODEL

Henry Blanton[1], Kelsey Donckels[1], Isabel Castro[2], Kevin Pruitt[2] and Josée Guindon[1*]

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The scourge of affliction for chronic pain (Gaskin and Richard, 2012; Nahin, 2015) and cancer (Ferlay et al., 2013) continues unabated despite recent breakthrough discoveries. Preclinical (Guindon and Hohmann, 2011; Romero-Sandoval et al., 2017) and clinical (Blake et al., 2017) evidence show that cannabinoid-based therapies have been used increasingly by cancer patients for their analgesic and antiemetic properties. Studies evaluating the impact of long term cannabinoid therapy on tumor growth and cell proliferation in the context of chemotherapy treatment and/or cancer are mandatory. This translational project will investigate the role of cannabinoid agonist 1 (ACEA) therapy in alleviation of pain, estrous cycle and estradiol changes and tumor growth in a xenograft ectopic ovarian cancer model. Preliminary results from our laboratory suggest that xenograft ectopic inoculation of OVCAR-5 ($1x10^6$ cells per 1 ml subcutaneous) cancer cells grow (from 0 to 32 days) steadily and exponentially better in SCID relative to NuNu mice without development of mechanical (digital von Frey) and cold(acetone) allodynia. After 32 days of ectopic OVCAR-5 cancer cells growth, we administered daily a CB_1 agonist (ACEA 0.5 mg/kg i.p.) in SCID mice shows increase in tumor volume (mm^3) similar to vehicle treated animals. ACEA failed to prevent tumor growth in our ectopic ovarian cancer model in both SCID and NuNu mice. We also observed following chronic administration of ACEA changes in estrous cycle to the metestrus phase and decrease in estradiol levels in chemotherapy-induced pain. Further studies are needed to evaluate a potential time and/or dose-dependent effect in ACEA failing to prevent tumor growth. This study provides direct evidence to pursue *in vivo* preclinical exploration of cannabinoid agonists to improve our understanding of their role in tumor growth and changes in estrous cycle in ovarian cancer.

Acknowledgements: Funded by The CH Foundation (Grant 241865 (JG)), the CPRIT Foundation (Grant RR140008 (KP)), the NCI (Grant CA155223 (KP)) and Texas Tech University Health Sciences Center School of Medicine (Grant 121035 (JG)).

PRESCRIPTION CANNABINOID USE IN A CANADIAN PROVINCE: A POPULATION-BASED STUDY (2004-2014)

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[1] College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada,
[2] Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

Objectives: We aimed to assess the temporal trends of prescription cannabinoid utilization in Manitoba, Canada. Cannabinoids (nabilone, dronabinol, nabiximols) have been available as therapeutic options for indications including pain, spasticity, and vomiting. The extent of their utilization from a population perspective is unknown. The timing of this study provides baseline estimates before the expected legalization of marijuana.

Methods: We conducted a retrospective, population-based cohort study using administrative (health claims) data from 2004 to 2014. Data were obtained from the administrative databases from the Manitoba Centre for Health Policy. We estimated the annual prevalence and incidence of cannabinoid use over the study period as well as demographics, and morbidities of users. Ordinary least square regression analysis was used to test for temporal trends in use.

Results: The annual incidence of cannabinoid use increased from 1.21 users/10000 person-year in 2004 to 6.22 users/10000 person-year in 2014, an average increase of 0.39 users/10000 per year. There was a linear upward trend in the use of nabilone ($P<0.0001$), while dronabinol use dropped, ($P<0.001$) and nabiximols use remained unchanged over time. The mean (SD) age of new users was 50.6 (14.7) years, 58% were female. We identified that 50.6% of users were being treated for chronic pain in the year before their first cannabinoid prescription.

Conclusion: Among the cannabinoids, only the use of nabilone increased among Manitobans over the study period. The expected introduction of recreational marijuana and its greater legal availability may affect this trend.

Acknowledgements: Results and conclusions are those of the authors; no official endorsement by Manitoba Health, Seniors and Healthy Living or MCHP is intended or should be inferred.

THE USAGE OF AN INHALATION DEVICE FOR PULMONARY ADMINISTRATION OF CANNABIS ('BEDROMEDIC') AMONG MEDICINAL CANNABIS PATIENTS

Mikael A. Kowal*, Wouter Dijkstra and Femke Starrenburg

Bedrocan International, Veendam, The Netherlands

Medicinal cannabis patients in the Netherlands are able to obtain cannabis from the Office of Medicinal Cannabis (Ministry of Health, Welfare and Sport) based on a doctor's prescription. One of the approved methods of administration of the herbal material is through inhalation using a device called a vaporizer. This technique is aimed at heating cannabis to a temperature high enough to evaporate the active components, but without burning the plant material so that no toxic pyrolytic compounds are formed. At the moment, there are a number of vaporizers available on the market. However, in terms of scientific research, most studies investigating the effects of inhaled cannabinoids on various medical indications have used the Volcano® vaporizer in their study designs. Nevertheless, aside of the tested reliability of the device, the Volcano® has frequently been reported by medicinal cannabis patients as overly expensive and not sufficiently portable. Consequently, due to the need for an affordable, portable, and reliable device Bedrocan International has developed a new vaporizer device – the Bedromedic.

We studied user experience with the Bedromedic among 20 medicinal cannabis patients in the Netherlands who use cannabis based on a doctor's prescription. This was done in the form of an online questionnaire. The goal was to obtain feedback from medicinal cannabis patients that will allow to improve aspects of the device in order to make it more user-friendly and reliable to use.

The presentation will provide an overview of the results of the questionnaire study. Specifically, data will be presented on the inhalation process, side-effects of the device, handling of its different elements, cannabis usage with the device and overall patient satisfaction.

IDENTIFICATION OF NOVEL MOUSE AND RAT CB1R ISOFORMS AND *IN SILICO* MODELING OF HUMAN CB1R FOR PERIPHERAL CANNABINOID THERAPEUTICS

Qing-Rong Liu[1*], Nicholas S. Huang[1], Hong Qu[3], Jennifer F. O'Connell[1], Isabel Gonzalez-Mariscal[1], Sara Santa-Cruz Calvo[1], Maire E. Doyle[1], Zheng-Xiong Xi[2], Yun Wang[4] and Emmanuel S. Onaivi[5]

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Unravelling the underlying physiology of the peripheral cannabinoid receptor 1(CB1R) is necessary to separate any peripheral effects of endocannabinoids from central actions. We previously reported a human hCB1b isoform that is selectively enriched in pancreatic beta-cells and hepatocytes, providing a potential peripheral therapeutic hCB1R target. It is unknown if any mouse or rat CB1R (mCB1 and rCB1) isoforms are peripherally enriched. In this study we found no evidence of peripherally enriched rodent CB1 isoforms; however, some mCB1R isoforms are absent in peripheral tissues. We show that the mouse *Cnr1* gene contains six exons that are transcribed from a single promoter. The first of these, mCB1A, is a spliced variant of extended exon-1 and protein coding exon-6; mCB1B is a novel spliced variant containing un-spliced exon-1, intron-1, exon-2, and then spliced to exon-6; and mCB1C is a spliced variant including all 6 exons. Using RNAscope *in situ* hybridization we show that the isoforms mCB1A and mCB1B are colocalized in GABAergic and glutamatergic neurons in the ventral tegmental area (VTA) outside of dopaminergic neurons. RT-qPCR reveals that mCB1A and mCB1B are enriched in the brain while mCB1B is not expressed in the pancreas or liver. mCB1A and mCB1B are differentially regulated by hyperinsulinemia induced by beta-cell specific knockout mCB1R and S961 insulin antagonist treatment in peripheral and brain tissues. Rat rCB1A and rCB1B isoforms have different tissue expression patterns in comparison with the counterparts of the mCB1Rs and differentially expressed in primary cultured neurons, astrocytes, and microglia. We also investigated modulation of *Cnr1* expression by insulin *in vivo* and we carried out *in silico* modeling of CB1R with JD5037, a peripherally restricted CB1R inverse agonist, using the published crystal structure of hCB1R. The results provide models for future CB1R peripheral targeting.

Acknowledgements: This research was supported in part by the Intramural Research Program of the NIA/NIH and NIDA/NIH; NIH grant DA032890; National Health Research Institutes and Central Government S & T Grant, Taiwan (106-1901-01-10-02), The National Science Foundation of China (31671375).

TREATMENT WITH PHYTOCANNABINOID CANNABIDIOLIC ACID (CBDA) ON PERICYTES UNDER HYPOXIC CONDITIONS *IN VITRO*

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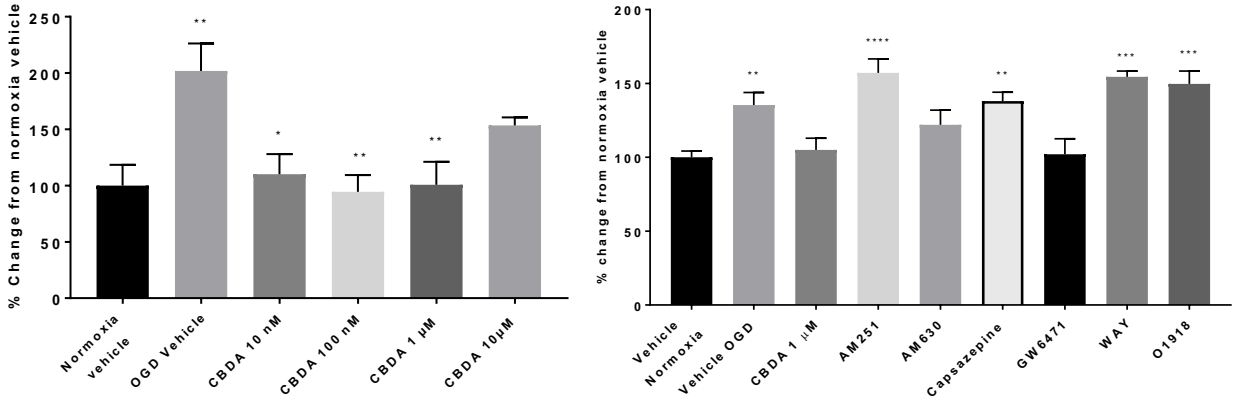
Cannabidiol is well known for its neuroprotective effects, however little is known about the action of its precursor cannabidiolic acid (CBDA). CBDA acts through similar mechanisms as CBD ($5HT_{1A}$ activation), which led us to hypothesise that CBDA would be protective in an *in vitro* model of stroke, as previously shown for CBD. To test this, the aim of this study was to investigate the effects of CBDA on pericytes in hypoxia as they have been shown to increase blood brain barrier (BBB) permeability after ischaemic insult.

Experiments were performed on confluent perciytes (passages 3-5) in 24 well plates (n=9, 3 separate experiments). In oxygen-glucose deprivation conditions (OGD), cells were treated with CBDA (1 nM-10 μM, placed in glucose free medium, 0% O_2 environment (BD GasPak™ pouch) for 4hrs. Reperfusion was established by returning cells to normal pericyte medium in normoxia (20% O_2) in increasing concentrations of CBDA. In normoxia, cells were maintained in normal pericyte medium plus CBDA 1 nM-10 μM and a vehicle control. At 24h, cells were lysed with RIPA buffer containing protease and phosphatase inhibitors. To probe receptor involvement, cells were treated with CBDA (1μM) in the presence of antagonists; AM251 (100 nM), AM630 (100 nM), capsazepine (1 μM), GW6471 (100 nM), (S)-WAY-100,630 (300 nM) and O1918 (1 μM) and a vehicle control. Media samples were analysed for IL-6 (Affymetrix) and ICAM-1 (R&D systems) by ELISA, and normalised to total protein (bicinchoninic acid assay-BCA). Statistical analysis was conducted using one-way ANOVA.

After OGD, IL-6 levels rose significantly ($p \leq 0.01$) compared to the normoxia vehicle and IL-6 secretion was attenuated by CBDA at 10 nM, 100 nM and 1 μM ($p \leq 0.01$, $p \leq 0.001$ and $p \leq 0.01$ respectively; in 24hrs post-OGD samples, one-way ANOVA). CBDA 1 μM still attenuated IL-6 secretion when applied alongside antagonists AM251, AM630, capsazepine, GW6471 and O1918, displaying no significant difference to the normoxia vehicle (ns, one-way ANOVA). S-WAY-100,630 inhibited CBDA mediated reduction in IL-6 secretion in 24hr post OGD samples ($p<0.0001$ normoxia vehicle vs WAY+CBDA, one-way ANOVA). ICAM-1 levels also rose significantly after OGD ($p \leq 0.001$), but CBDA had no effect on the secretion of this adhesion molecule.



Figures 1 and 2: A) Measured IL-6 secretion in pg/ mL /mg of protein in pericytes treated with CBDA in a range of concentrations (n=9) 24 h after 4hr OGD, expressed as a % change from the normoxia vehicle. CBDA vs OGD vehicle **B)** Measured IL-6 secretion in pg /mL/mg of protein in pericytes treated with 1 μM CBDA alongside antagonists 24h after 4hr OGD. Antagonist treated + 1 μM CBDA vs vehicle normoxia. Data is expressed as mean ± SEM. Statistical analysis conducted using one-way ANOVA; ****P<0.0001, ***P<0.001, **P<0.01,* P<0.05.

CBDA attenuates the inflammatory response to 4hr OGD in pericyte monolayers *in vitro*. The 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. These data suggest that like CBD, CBDA is effective in a cellular model of stroke.

METABOLISM OF CANNABINOIDS BY FUNGAL SKIN COMMENSALS OF THE SCALP

Christian Giroud[1*], Candice Galé[1+2], Mattia Furer[1+2],
Michel Monod[3], Marina Fratti Ducreux[3] and Frank Sporkert[1]

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Aims*: Malassezia* fungi are abundant lipophilic skin commensals that can be pathogenic causing various health disorders including dandruff. THCCOOH in hair is generally considered as a biomarker of active cannabis use. However, this assumption could be questioned if scalp and hair microorganisms are able to metabolize contaminating THC into THCCOOH. We tested this hypothesis by incubating cannabinoids *in vitro* with *Malassezia sympodialis* molds.

Materials and methods: Using 6-well plates, *Malassezia* molds were grown *in-vitro* on Dixon medium prepared with low temperature melting agar, in the absence or presence of THC or THCCOOH (1, 10 µg/mL) for one week at 30°C and 100% humidity. Different control conditions were tested: a first negative control assay containing only the growth medium, a second negative control assay made with the growth medium and *Malassezia* fungus, a positive control containing the growth medium and the substrate (THC or THCCOOH), and the test condition containing the growth medium, either cannabinoid substrate and fungus. THC, 11-OH-THC, THCCOOH and CBN were determined by GC-MS operated in the SIM mode using deuterated internal standards after sample harvesting, heat liquefaction at 80°C of the Dixon medium, sample dilution followed by liquid-liquid extraction with hexane:dichloromethane (9:1) and silylation with MSTFA. Screenings operated in the SCAN mode were also performed in order to detect other putative metabolites.

Results and Discussion: We did not observe any trace of cannabinoids in the two negative control conditions. A significant decrease in THC levels was observed after 7 days of incubation in both the positive control and test condition, respectively without and with *M. sympodialis*. However, the magnitude of the decrease was significantly larger in the test condition than in the positive control assay. After 2 days incubation, the kinetic profile of the THC concentration decrease revealed a significant difference that increases over time between the test condition and the positive control. For THCCOOH, the decrease was not significant. Neither 11-OH-THC, nor THCCOOH were detected, suggesting that THC could be degraded into other, not yet, identified metabolites. High-resolution accurate mass spectrometry and metabolomics was used for tentative identification of possible THC metabolites. However, results interpretation was made difficult by the complex composition of the lipid-rich growth medium.

Conclusion: Our findings indicate that THC in scalp hair is metabolized by *Malassezia* molds. Therefore, if hair THC is used as a marker of cannabis exposure, the risk of false negative results cannot be excluded. Our results also indicate that THC is not oxydated into THCCOOH. For that very reason, the hypothesis that THCCOOH is a specific marker of active cannabis consumption cannot be disproved.

A SYSTEMATIC REVIEW OF CANNABIDIOL DOSING IN PATIENT STUDIES

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Aims: Cannabidiol (CBD) is a popular food-supplement and drug with potential applications in a wide-variety of medical contexts. A wide-range of doses ranging from the micro-(<50 mg) to the macro-scale-(>2000 mgs) have been used in published and anecdotal reports, however the effective therapeutic doses of CBD remains unclear. The purpose of this systematic review was to investigate what doses of CBD have been used and which have been effective in published literature.

Methods: Publications involving administration of CBD alone to patients were collected by searching online databases (PubMed, EMBASE and ClinicalTrials.Gov) using the key-terms 'Cannabidiol' and 'CBD', and filtered to include 'Clinical Trials and Case Reports' on 'Humans', resulting in a total of 768 articles. Included in this analysis are 30 publications (19 clinical studies and 11 case reports) after excluding those investigating herbal forms of cannabis and un-specified quantities of cannabinoids.

Results: Of the 19 clinical trials with CBD, the doses investigated ranged from <1 mg/kg up to 50 mg/kg per day, and for the case reports, doses ranged from 2 mg/kg to 25 mg/kg per day. 29 studies administered CBD orally (p.o. 11 in solution and 18 in capsules) and 1 via inhalation. Of the 19 clinical trials, 12 reported significant improvement in primary outcomes (reduction of convulsive seizure frequency, anxiety and improved well-being) with effective doses ranging from 2.5 mg/kg to 50 mg/kg per day (~500 mg/day). Seven trials were unsuccessful in the primary outcomes (no significant reduction of motor symptoms in Parkinson's and Huntington's disease, ocular hypertension, diabetic High-density lipoproteins (HDL) levels or Crohn's disease activity index) using doses from <1 mg/kg to 25 mg/kg per day (~300 mg/day). Of the 11 case reports, 8 reported positive symptom relief with doses from 2 mg/kg to 25 mg/kg per day (~800 mg) and 3 negative outcomes of no symptom relief with 2.5 mg/kg to 25 mg/kg per day (~1,300 mg/day). CBD doses between 3 and 50 mg/kg/day are effective in treatment of convulsive seizure frequency in epilepsy (354 participants, 9 publications). CBD doses between 75 and 600 mg/day may be beneficial for Parkinson's disease patients (anxiolysis and improved sense-of-wellbeing and cognition but not motor-symptoms; 31 participants, 3 publications). Results are more variable for schizophrenic and psychiatric patients (167 participants, 10 publications), with a therapeutic effect observed in some but not others. Doses from <1 mg/kg and to 10 mg/kg (~650 mg/day) are reported to improve insomnia and anxiety (in 62 participants, 5 publications). CBD was not effective in increasing HDL diabetes (200 mg/day, 13 participants, one publication) or Crohn's disease activity (20 mg per day, 10 participants, one publication).

Conclusion: From the wide range of investigated doses in heterogeneous studies, it appears that doses of ~500 mg/day (~6.25 mg/kg/day for adults) may be effective in reducing convulsive seizures and improving psychological symptoms such as anxiety. Negative studies tended to use lower doses of CBD (~300 mg/day), but it is not clear whether this is due to the dosing or the indications/primary endpoints being pursued. More research is needed to understand the therapeutic threshold of CBD and how this varies depending on patient characteristics (e.g. variations in pharmacokinetics), routes of administration of the drug and the pathology requiring treatment.

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL IMAGING PROBES FOR THE VISUALIZATION OF THE CANNABINOID TYPE 2 RECEPTOR (CB_2R)

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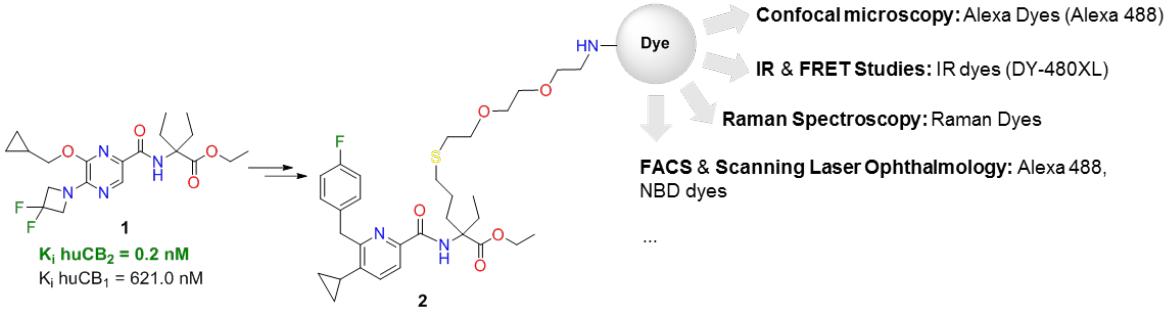
Fluorescent probes have emerged as powerful tools to study G-protein-coupled receptors (GPCRs) structure and function in living systems. In drug discovery processes fluorescently labelled pharmacophores enable the visualization and investigation of GPCR targets within a wide range of applications i.e. study of GPCRs dynamic processes (as internalization and trafficking), binding assays and *in vivo* drug-target engagement.[1] The cannabinoid type 2 receptor (CB_2R) is a GPCR with the potential to treat various pathologies, including chronic and inflammatory pain, neuroprotection and inflammation.[2] However, to further elucidate the role of CB_2R as a drug target in several pathophysiological conditions and to foster clinical development appropriate tools for CB_2R study *in vitro* and *in vivo* are a current need. In this work, the design, synthesis and biological validation of CB_2R-Agonist fluorescent probes will be presented.



There are several challenges in the synthesis course of a CB_2R fluorescent probe: the linker length, composition and placement, as well as the dye size, nature and charge can strongly influence the biosensor overall properties. For our probe design, the CB_2R-selective 2,5,6-trisubstituted pyrazine **1**[3] was chosen as starting point (recognition element). Herein we will disclose a high affinity and selective fluorescent probe (**2**) containing a very robust attachment end for dye conjugation. The structure-activity relationship (SAR) analysis and *in silico* modelling used to guide probe optimization efforts and to identify appropriate linker attachment points at the recognition element will be reported. Moreover, we will present physicochemical and ADME properties, as well as initial selectivity results of these derivatives. First successful applications such as FACS and confocal microscopy of compound **2** will be shown. We believe that these new and highly CB_2R-selective probes will help to further understand the manifold roles of the cannabinoid receptors and unravel the biological significance of the endocannabinoid system.

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BENEFICIAL EFFECTS OF CANNABIDIOL IN SEVERE LUNG DAMAGE FOLLOWING MECONIUM ASPIRATION IN NEWBORN PIGLETS

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Background: Neonatal Meconium Aspiration Syndrome (MAS) is a devastating complication occurring during delivery with high mortality and morbidity, due to the combination of airway obstruction (impairing oxygenation) and extensive inflammation-related lung damage (impairing ventilation, that is CO_2 elimination). There is no current specific treatment other than supportive management. Cannabidiol (CBD) 1 mg/kg i.v. has demonstrated beneficial effects in newborn piglet model of mild inflammation-mediated brain hypoxi-ischemia-induced lung damage. Thus, we aim to study whether CBD could reduce lung damage secondary to MAS in newborn piglets.

Methods: 30 min after intratraqueal instillation of 3 mL of meconium 20% in saline to 2-3 day-old mechanically ventilated piglets, animals received i.v. vehicle (VEH, n=9)or CBD 5 mg/kg (CBD, n=6) (both supplied by GW Research Ltd, Cambridge, UK). Then, hemodynamic (including mean arterial blood pressure -MABP) as well as respiratory parameters (blood oxygen saturation -SO_2, arterial pH, pO_2 and pCO_2,inspired air oxygen fraction -FiO_2, mean airway pressure -MAP, tidal volume -Vt, and oxygenation index -OI [$MAPxFiO_2/pO_2$]) were continuously monitorized for six hours. Non-SAM similarly managed piglets served as controls (SHM, n=6).

Results: MAS led to severe oxygenation impairment with progressive SO2 decline despite of increasing OI. CBD has no effect in this regard, confirming that in this group MAS severity, in terms of secondary airway obstruction, was similar to that of the VEH group. MAS also led to progressive impairing on ventilation markers (Table 1). In this case, CBD prevented the deleterious effects of MAS, reducing ventilation impairment even with a lower Vt needed (that is the ventilator parameter influencing CO_2 elimination) than for VEH or SHM. Besides, MAS led to haemodynamic impairment with progressive MABP decline, prevented by CBD (Table 1).

	SHAM	MAS+VEH	MAS+CBD
Ventilation			
pH	7.38 (7.36,7.39)	7.25 (7.19,7.30)*	7.38 (7.35,7.42)$^{\#}$
pCO_2	40.0(38.5,48.0)	55.0 (54.1,62.0)*	46.0 (44.0,51.0)$^{\#}$
Vt (mL/kg)	9.0 (8.5,9.5)	9.5 (8.4,11.6)	7.9 (6.2,8.6) *$^{\#}$
Haemodynamics			
MABP (mmHg)	81.0 (79.0,84.5)	63.0 (49.0,78.0)*	80.5 (72.5, 85.5)$^{\#}$

Results expressed as median(IQR) of data obtained at the end of the experiment. (*) p<0.05 vs SHM; (#) p<0.05 vs MAS+VEH, all by 2-way ANOVA carried out on the data obtained hourly.pH: Time: F 2.9, p=0.008; Group: F 38.26, p=0.0000001; Interact: F 0.08, p=0.99. pCO2: Time: F 3.25, p=0.003; Group: F 30.5, p=0.0000002; Interact: F 0.46, p=0.86. Vt: Time: F 0.22, p=0.97; Group: F 12.2, p=0.0007; Interact: F 1.4, p=0.21. MABP: Time: F 3.56, p=0.002; Group: F 17.7, p=0.00005; Interact: F 1.06, p=0.39)

Conclusions: In a model of a severe lung disease as MAS, CBD prevented lung compliance deterioration, as showed by the lower Vt needed, and preserved normal ventilation. Both results pointed to a reduction by CBD of MAS-induced inflammatory lung damage. As in other previous experimental paradigms, CBD was showing a remarkable haemodynamic stabilizing effect.

Supported by grants from iNO Therapeutics and GW Research Ltd.

THE (IR)REVERSIBILITY OF DAGL-α INHIBITORS

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Diacylglycerol lipase α is the predominant source of 2-arachidonoylglycerol in the brain. This endocannabinoid plays an important signalling role in numerous physiological processes. To study the role of DAGL-α a growing number of inhibitors have emerged. DH376 is one of the highly potent inhibitors published recently and binds to the enzyme in a covalent fashion. The activated ureum of DH376 functions as the electrophile and is attacked by the catalytic serine of DAGL-α, resulting in permanent inhibition. Covalent irreversible inhibitors bind to proteins in a two-step manner, leading to time-dependent inhibition. Two parameters determine binding: the K_i and the k_{inact}. To investigate the kinetics of binding of this inhibitor we synthesised 5 variants of the heterocyclic leaving group and coupled them to enantiospecifically synthesised (S)-2-benzylpiperidine. We analysed the kinetics of enzyme inhibition in a modified surrogate substrate assay. Our data shows the importance of the azole lies mostly in the binding affinity of the inhibitors. This is most likely due to strong hydrogens bond(s) formed. No correlation was found between enzyme reactivity and the acidity of leaving group.

SINGLE AND COMBINATIVE EFFECTS OF CANNABIDIOL AND β-CARYOPHYLLENE ON THE LIEBER-DECARLI DIET

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Rationale: The Lieber-Decarli diet is an NIAAA-approved protocol for inducing alcohol-associated liver damage, as well as generating a pro-inflammatory response in rodents. Cannabidiol (CBD), a non-psychoactive cannabinoid, and β-caryophyllene (BCP), a bicyclic sesquiterpene found in the essential oil of the *Cannabis* plant, have been previously characterized to possess neuroprotective and anti-inflammatory properties. The aim of this study was to investigate the effects of CBD and BCP alone and in combination on the pro-inflammatory effects of the Lieber-DeCarli diet. It was hypothesized that both individual and dual cannabinoid treatment would alleviate the pro-inflammatory effects induced by the Lieber-DeCarli diet.

Methods: Female C57/BL6 mice were treated with a 5% ethanol liquid diet *ad libitum*, and received daily CBD, BCP, or CBD+BCP (30 mg/kg IP) injections for 10 days. Additional groups of mice receiving no cannabinoid treatment were used to assess withdrawal behaviors 24h post gavage of a single ethanol dose (31%, 5 g/kg). The effects of the Lieber-DeCarli diet and cannabinoid treatment on pro-inflammatory response were determined using plasma borne markers. Hippocampal and cerebellar microglial activation was analyzed using immunohistochemistry.

Results: Ethanol intake increased concentrations of pro-inflammatory cytokines IL-1β and IL-6. CBD decreased IL-1β plasma levels, while BCP significantly decreased IL-1β, IL-6, and CCL22 levels. Ethanol consumption alone increases cerebellar microglial cell number, but not in the hippocampus. Cannabinoid treatment was ineffective in attenuating microglial activation in either brain region. Ethanol withdrawal post binge feed increased handling-induced convulsion (HIC) scores and decreased open field center times.

Conclusion: Our findings suggest that the Lieber-DeCarli diet is capable of producing withdrawal behaviors in mice, but it's ability to induce pro-inflammatory responses and increased microglial activity appears inconsistent. BCP and CBD treatment may attenuate plasma markers of inflammation, while neither appear to have any influence on microglial count.

Acknowledgements: Funded by Department of Defense Award W81XWH-14-1-0389 (SJW)

COMPARISON OF VAPORIZED AND NON-VAPORIZED *CANNABIS SATIVA* L. CHEMICAL COMPLEXITY USING HPLC-DAD AND MS-BASED METABOLOMICS

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Cannabis sativa L. is an annual plant containing over 400 known chemical compounds from a diverse range of phytochemicals such as sugars, hydrocarbons, steroids, flavonoids and cannabinoids. Cannabis has been used in Canada for a range of medical purposes such as chronic pain and insomnia, among other symptoms and conditions, and a Canadian survey in 2015 showed that vaping was the most popular mode of delivery for medical cannabis. Vaping is a method of ingestion that can extract active principles of cannabis with heated gas while minimizing the inhalation toxic compounds associated with combustion. In this study, we applied a metabolomic approach to qualitatively and quantitatively compare the chemistry of different strains of dry cannabis flowers and oils to the chemistry of corresponding vapour extracts. Subsamples of each strain of dried flowers were extracted with solvents for a broad spectrum, "*in planta*" extract, and by supercritical fluid CO_2 extraction to yield oils comparable to commercial products. Subsamples of each dry flower and oil were then vaporized using a Volcano© vaporizing system (Storz & Bickel) at 410°F and the collected vapour was drawn through a cold trap using an optimized solvent mixture with methanol, chloroform, hexane and ethyl acetate to capture metabolites.

HPLC analysis revealed different chemical spectra between vaporized and non-vaporized cannabis. This was further supported by multivariate statistical analysis of the High Resolution Mass Spectrometry metabolomics dataset which showed differences between the dry and vaporized cannabis in terms of both chemical classes and individual metabolites, which were putatively identified. It was seen that vaporization can alter cannabis chemistry in dried flowers qualitatively and quantitatively leading to different chemical compositions in vaporized and non-vaporized cannabis extracts. In addition, similar results were seen with the oil extracts between vaporized and non-vaporized samples as well as qualitative and quantitative differences between dry flower vapour and oil vapour.

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

DESIGN OF NOVEL GPR18 ANTAGONISTS USING A FRAGMENT REPLACEMENT SCAFFOLD HOPPING APPROACH

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GPR18 is an orphan Class A G-Protein Coupled Receptor (GPCR) that has been postulated to belong to the cannabinoid family. This receptor has been shown to be a possible target for the treatment of pathological conditions such as inflammation, intraocular pressure, cancer, or metabolic disorders. A variety of endogenous, phytogenic, and synthetic cannabinoid ligands have been reported to target GPR18. However, controversy remains as pharmacological inconsistencies and the lack of potent GPR18 ligands are precluding its appropriate characterization.

Recent studies reported the identification of the imidazothiazine PSBCB5 (CID-85469571, Figure 1) as a sub-micromolar selective GPR18 antagonist.[1,2] By using a fragment replacement core hopping approach on this scaffold we aim to design novel potent GPR18 ligands.

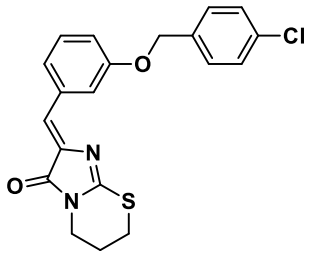

Figure 1. Structure of **PSBCB-5**

For this purpose, we used our recently developed GPR18 inactive state model. This homology model has been constructed using the crystallized delta opioid receptor structure as a template.[3] Sequence divergences in transmembrane helixes were explored using the Monte Carlo/simulated annealing program, Conformational Memories.[4] Docking studies of PSBCB5 and related analogs in the R state GPR18 model helped us to identify key receptor-ligand interactions. Furthermore, using a fragment-based replacing scaffold hopping approach on the imidazo[2,1-*b*]thiazine moiety (Figure 1), we have identified novel potential GPR18 chemotypes. Among the over thousand outputs obtained using the core hopping fragment database screening [1,2,4]triazolo[4,3-*b*]pyridazine and benzoisoxazole derivatives were selected to be developed. These novel chemotypes present better ligand-receptor interactions and may allow to define pharmacophoric structural features involved in antagonist binding at GPR18. *In silico* ADME properties and the absence of promiscuous moieties (the so-called Pan Assay Interference Compounds or PAINS) were also considered in the drug design process.

The identified molecules may serve as basis for the development of potent pharmacological tools for further characterization of GPR18. [Support: NIDA KO5 DA021358 PHR]

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THE EFFECTS OF SR144528 ANALOGUES ON GPR3 AND GPR6

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GPR3 and GPR6 are constitutively active G protein-coupled receptors. Even though they have no confirmed endogenous ligands, GPR3 and GPR6 are phylogenetically related to CB1 and CB2 cannabinoid receptors. We began the current study by investigating the effects of SR144528, a CB2-selective antagonist, on GPR3 and GPR6 signaling, using both the cAMP accumulation and the β–arrestin2 recruitment assays. In GPR3- and GPR6-transfected HEK293 cells, SR144528 did not significantly affect cAMP accumulation at concentrations up to 10 μM. By contrast, SR144528 inhibited β–arrestin2 recruitment to GPR3 and GPR6 in a concentration dependent manner, with SR144528 showing higher potency on GPR6 compared to GPR3. Taken together, these results identify SR144528 as a biased inverse agonist at both GPR3 and GPR6.

In order to better understand the SR144528 activity, we next conducted a structure-activity relationship analysis using five analogues of SR144528. These analogues were chosen based on modifications to the pyrazole central ring, the amide group, and the para-methylphenyl ring of SR144528. None of the five analogues of SR144528 had any significant effects on either GPR3- or GPR6-mediated cAMP accumulation. In contrast, significant effects were observed with the SR144528 analogues in β–arrestin2 recruitment assays. In GPR3, increased potency was seen with Compound 6, which retains the pyrazole and amide functionalities, but alters the para-methylphenyl ring, making the compound more compact. Also in GPR3, loss of the N-2 pyrazole nitrogen as seen in the pyrrole analogs Compounds 3 and 4 resulted in significantly increased potency. However, while the efficacy of Compound 3 remained comparable to SR144528, Compound 4, which also lacks the amide group (implicated in an H-bond interaction with the receptor), displayed significantly reduced efficacy. In GPR6, loss of the amide functionality resulted in significantly decreased efficacy with Compound 2 and to a lesser extent, with Compound 4. However, none of the other modifications in SR144528 changed its potency or efficacy on GPR6.

In summary, our results suggest the potential importance of H-bonding and steric interactions in the observed SR144528-induced GPR3 and GPR6 inverse agonism in β–arrestin2 recruitment. We have identified SR144528 as a scaffold for acting on GPR3 and GPR6 and provided preliminary structure activity data from which more potent and efficacious ligands for GPR3 and GPR6 may be designed. Further research is required to better understand the signaling bias on GPR3 and GPR6 and its potential therapeutic applications.

CANNABICHROMENE IS A CB2-SELECTIVE PHYTOCANNABINOID

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Cannabichromene (CBC) is one of the most abundant phytocannabinoids (Turner et al. 2017). In contrast to the wealth of information about Δ^9-tetrahydrocannabinol (THC), we only know about CBC affinity for the cannabinoid receptors (CBR) (Rosenthaler et al. 2014). There is also little information on CBR-mediated bioactivity, although CBC alone has been shown to be analgesic in mice and to have synergistic effects with THC (Davis & Hatoum 1983). CBR induce cellular hyperpolarization through the opening of G-protein coupled inwardly-rectifying potassium (GIRK) channels, which have been shown to play a role in antinociception in rodents (Blednov et al. 2003). We investigated the activities of CBC and other phytocannabinoids at the GIRK pathway, as it may be involved in the therapeutic activity of *Cannabis spp*.

We examine the actions of CBC on CBR activation of GIRK in ATt-20 cells stably transfected with human HA tagged-CB1 and CB2 receptors. Channel activation was measured using a FLIPR membrane potential assay in a Flexstation 3. Modulation of receptor internalization was also studied using live AtT20 antibody feeding technique and quantified with a BMG-Labtech Pherastar plate reader.

CP55940 (CB1, EC_{50} 19 nM; CB2 EC50 74nM) and THC (CB1 EC_{50} 280 nM, CB2 Emax 20±3), hyperpolarized AtT20-CBR cells, with THC having a lower maximal effect than CP55940, consistent with it being a lower efficacy CBR agonist. CBC did not activate CB1 receptors but caused a dose-dependent hyperpolarization in AtT20 -CB2 cells (EC_{50}: 10μM). There was no response to any drug in wild-type AtT20 cells. CBC response in CB2 cells was blocked by pertussis toxin and 10μM CBC response was attenuated by 96±3% (p=0.0067, n=5) in the presence of AM630 (3μM), a CB2-specific antagonist. 10μM CBC caused a 30% reduction in the response to 300nM CP55940-induced hyperpolarization of AtT-20 CB2 cells (p=0.0031, n=4). Preliminary results show that 10μM CBC internalises CB2 receptors and this effect is also antagonised by AM630

CBC is a low potency and low efficacy Gαi protein-dependent CB2-specific agonist. These results are important because it is the first report of a cannabinoid receptor selective phytocannabinoid exclusively found in *Cannabis spp*. This result, in addition to reports on the key role of CB2 receptors in neuropathic pain, suggest CBC analgesia could be CB2-receptor mediated. CBC could also be further investigated as a potential treatment for neuropathic pain without concern for CB1-mediated psychoactivity seen with THC.

Acknowledgement: Phytocannabinoids supplied by the Lambert Initiative for Cannabis Therapeutics and research supported by the Macquarie University International Research Scholarship.

PHARMACOLOGY, TOXICOLOGY, AND THERMAL STABILITY OF EMERGENT SYNTHETIC CANNABINOID 4-CYANO CUMYL-BUTINACA

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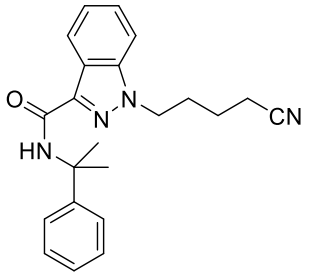
Synthetic cannabinoid receptor agonists (SCRAs) are used as intoxicants despite numerous associated health risks. SCRAs are the fastest growing class of novel psychoactive substance (UNODC), but in most cases, newly detected SCRAs are uncharacterised in terms of their pharmacology and toxicology. Moreover, SCRAs are typically used by smoking and inhalation, but often contain thermally labile chemical moieties. One such compound, 4-cyano CUMYL-BUTINACA (also known as CUMYL-CYBINACA, SGT-78), has been detected in recreational products since 2015. It has been associated with eleven fatalities in the EU, and was detected in 85 post-mortem samples in Turkey in the latter half of 2016. Despite clear harms associated with this compound, no pharmacological or toxicological evaluations have been performed.

In this study, 4-cyano CUMYL-BUTINACA was first evaluated in terms of its basic *in vitro* pharmacology via functional cellular assays. In mice, we utilise radio biotelemetry, behavioural testing, and post-mortem histological measures to evaluate *in vivo* pharmacology and toxicology. Additionally, the thermal stability of 4-cyano CUMYL-BUTINACA was investigated using an automated pyrolysis unit coupled to gas and liquid chromatograph-mass spectrometers, enabling identification of specific thermal degradants.

In vitro, 4-cyano CUMYL-BUTINACA was a highly potent, CB_1 receptor preferring agonist with a hCB_1 EC_{50} of 580 pM and hCB2 EC_{50} of 6 nM, and nanomolar binding affinity (hCB_1 K_i: 2.6 nM; hCB_2 K_i: 14.7 nM). *In vivo* pharmacological and toxicological assessment is ongoing, and preliminary thermal stability tests reveal that cyanide can be liberated from the terminal cyano group and/or carboxamide moiety when heated.



4-cyano CUMYL-BUTINACA

These data emphasise that the latest wave of SCRAs are not only ultra-potent cannabinoid receptor agonists, but also sources of a range of potentially cannabimimetic and/or toxic thermal degradants. Continued evaluation of the toxicology of novel SCRAs is essential, particularly for compounds that have been implicated in numerous poisonings and fatalities worldwide.

THE EFFECTS OF CANNABIDIOLIC ACID (CBDA) COMPARED WITH CANNABIDIOL (CBD) ON HUMAN COLON CANCER CELL PROLIFERATION

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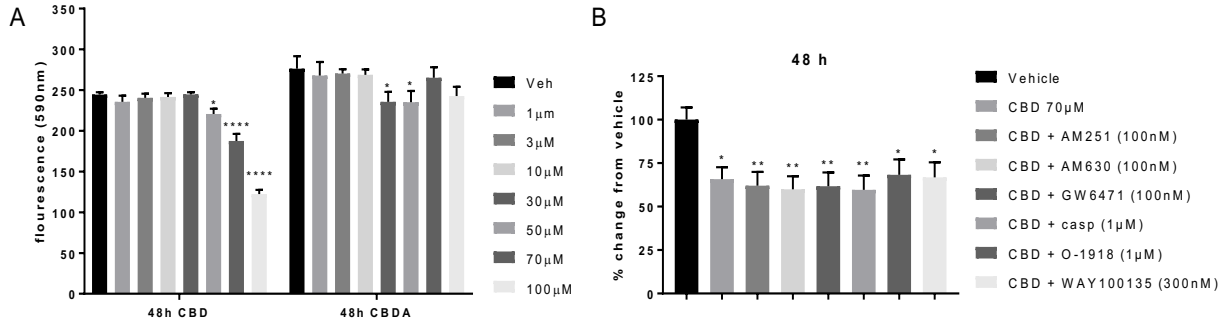
Cannabidiol is well known for its anti-proliferative effects in numerous cancer cell lines including colon cancer, however little is known about the action of its precursor cannabidiolic acid (CBDA). Some studies have shown that that CBDA is beneficial in breast cancer by down-regulating COX (Takeda et al, 2014; 2017), but no work to date has looked in colon cancer where downregulating COX would also be of benefit (Wang & DuBois, 2010). We hypothesised that CBDA would be anti-proliferative in human colon cancer (CaCo-2) cells.

CaCo-2 cells (passage 21-23) were serum starved (1% FBS) for 24 h, then seeded at a density of 10,000 cells/well in 96 well plate. 4 h after seeding (to allow cells settle before addition of the drug), the medium was changed to 10% FBS medium containing CBD or CBDA at concentrations from 1 to 100 µM. Resazurin was added at the recommended concentration immediately after the medium was changed (time 0). At 24, 48 and 72h, fluorescence was measured at 560 nm excitation and 590 nm emission for the conversion of resazurin to resorufin by viable cells as a marker of cell proliferation. Absorbance values were analysed by 2-way repeated measures ANOVA with post-hoc analysis comparing against a vehicle control (0.1% ethanol for CBD and 0.1% acetylnitrile for CBDA) at each time-point. Preliminary studies examined the effects of receptor antagonists on the response to CBD.

In control condition (i.e. drug free), there was a significant increase in absorbance over time showing cell growth. In the presence of CBD, cell proliferation was inhibited at 24 h (50 µM $P<0.05$; 70 µM $P<0.0001$; 100 µM $P<0.0001$), 48h (50 µM $P<0.05$; 70 µM $P<0.0001$; 100 µM $P<0.0001$) and 72 h (70 µM $P<0.0001$; 100 µM $P<0.0001$). By contrast, in the presence of CBDA, proliferation was inhibited by 30 and 50 µM only ($P<0.05$) at 48h and 72h with a smaller effect size than seen with CBD (see Figure 1A). Preliminary studies with a range of receptor antagonists found that the effects of CBD were not affected by blocking CB_1, CB_2, TRPV1, PPARα, the O-1918-senstive CB receptor or 5HT1A (Figure 1 B).



Figures 1 A) Mean fluorescence levels of resorufin in CaCo-2 cells 48 h after application of CBD or CBDA at various concentrations. **B)**. % change from the vehicle control 48 h after application of CBD alone or in combination with various receptor antagonists. Data is expressed as mean ± SEM. Statistical analysis conducted using ANOVA; ****$P<0.0001$, **$P<0.01$,* $P<0.05$.

As previously published, CBD inhibits human colon cancer cell proliferation *in vitro* in the micromolar range (Sreevalsan et al., 2011; Macpherson et al., 2014). However, CBDA did not affect colon cancer cell proliferation. Further experiments will establish the mechanisms of action of CBD in human colon cancer cells and also in human tumour tissue explants.

ANALGESIC EFFECTS OF NEW PYRAZOLYL-PYRIDINE BASED SCAFFOLDS TARGATING CB2 RECEPTORS IN OSTEOATHRITIS-RELATED PAIN

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Osteoarthritis (OA) is the most common form of arthritis, a mutual cause of pain and movement limitation among people over the age of 60. This slowly progressive disease leads to degradation of joint structure, especially articular cartilage, synovial membrane and subchondral bone. As a consequence, patients experience joint stiffness (most perceptible in the morning) and chronic, dull pain. There is no specific medical treatment to slow down cartilage degradation or to repair damaged cartilage in osteoarthritis. The goal of treatment in OA is to reduce joint pain and inflammation while improving and maintaining joint function.

The analgesic effects of cannabinoids have been well documented. Following the recent evidence for cannabinoid receptors type 2 (CB_2) up-regulation in chronic pain states much research is now aimed as thudding light on the role of the CB_2 receptor in OA. The analgesic and anti-nociceptive action of CB_2 is present in both neuropathic and inflammatory pain. However, the exact mechanism through which CB_2 receptors' agonists act as analgesics is still debated. Further studies are required to clarify the molecular mechanism by which this receptor exerts anti-inflammatory and analgesic actions, the development of selective molecules that can differently functionally activate CB_2 receptor is a promising and attractive prospective to challenge such disabling pathologies. Therefore in the present study, we focused on the role of novel CB_2 receptor modulators in treatment of pain, resulting from cartilage degradation in an animal OA model.
We aimed to test new pyrazolyl-pyridyne scaffolds – COR1114 (full agonist of CB_2), and COR1073, COR1119 (both inverse agonists of CB_2) and its potential role in osteoarthritis-related pain treatment. In the animal OA model (induced by 1 mg MIA i.a.), efficacy of all compounds depended on the dose used. We proved, that all derivatives triggered the analgesic effect in lower dose (1 mg/kg), rather than 5mg/kg. The effect was the highest after 1 hour and persisted up to 120 minutes after drug administration.

In the *in vitro* study, both COR1114 and COR11073 differently regulated *cnr2* and *MIF* (macrophage migration inhibitory factor) mRNA level in human osteoarthritic chondrocytes (HC-OA). Mechanism of action of direct agonist of CB_2 receptor might be related with its direct interaction with CB_2 whereas the inverse agonists of CB_2 can inhibit the pro-inflammatory cytokines produced at the periphery by activated monocytes and macrophages.

In summary, all compounds triggered analgesic effect, but they possibly act through different endogenous pathways. Further studies are needed to verify this hypothesis.

Acknowledgements: supported by the National Science Centre, Poland, grant OPUS UMO-2014/13/B/NZ7/02311 and IF PAN statutory funds. Assistance provided by M. Kostrzewa is greatly appreciated.

ANALGESIC EFFECT OF (*E*)-β-CARYOPHYLLENE IN RAT MODEL OF OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common chronic condition of the joints. OA is most frequent cause of disability in human. People mostly suffer due to impaired motor functions and pain. Available pharmacological therapies are based on Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), their mechanism of action is only symptomatic and does not stop progressive cartilage destruction. Moreover patients under NSAIDs therapy may suffer from many unwanted side effects. Yet we still classify OA as untreatable disease there is a genuine need to search for therapies that may reverse cartilage destruction.

Cannabinoid receptors CB_1 and CB_2 agonists have well documented role in the modulation of pain, especially of chronic origin, condition that is often refractory to therapy. A major limitation for the therapeutic development of compounds that directly activate CB_1 receptors is unwanted psychotropic effect, while agonists targeting CB_2 receptors have been proposed as therapies for the treatment or management of a range of painful conditions, including OA-related pain. Recently it was shown that (E)-β-caryophyllene (BCP), sesquiterpene majorly found in essential oils derived from *Cannabis sativa,* is a natural agonist of endogenous CB_2 with well-established anti-inflammatory properties. Therefore in the present study we aim to test therapeutic effects of natural and safe compound BCP on arthritis.

Intrarticular (i.a.) injection of monosodium iodoacetate (MIA, 1mg) has been used to induce OA in Wistar rats. Pain symptoms were assessed by behavioral tests: knee joint hypersensitivity test (PAM test) and tactile allodynia test (von Frey test). BCP (50 mg/kg, i.p.) was administered i.p. at late stage of OA (on day 28 after model induction).

We proved that BCP showed significant; however short lasting (up to 60 min in a 240 min time-course) effect when tested for knee hypersensitivity. An interesting analgesic profile was noted for BCP in von Frey test, we observed a biphasic elevation of tactile allodynia in response to CB_2 activation. Phase I lasted up to 60 min after BCP administration while phase II started 240 min after its injection. This effect may be mediated through indirect molecular mechanisms, pointing out that CB_2 activation may mediate anti-nociception indirectly, inhibiting the release of pro-inflammatory factors or/and engaging other systems involved in analgesia, such as endogenous opioid system. This will be a subject of our subsequent *in vitro* studies.

A recent study has provided support on the antinociceptive role of CB_2 receptor in a model of OA pain. Thus, BCP may be an excellent and safe therapeutic agent to prevent OA-related pain.

Acknowledgements: supported by the National Science Centre, Poland, grant OPUS UMO-2014/13/B/NZ7/02311 and IF PAN statutory funds.
We wish to acknowledge dr Jürg Gertsch (Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland) for generously providing us with BCP.

THE INFLUENCE OF MEDIA PREFERENCES ON ADOLESCENT CANNABIS USE: A DUAL-PROCESSING APPROACH

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Several studies have demonstrated that media, particularly film and music, have an impact on adolescent substance use (Dal Cin et al., 2009; Oberle & Garcia, 2015). Dual-process theories posit that 'fast' (System 1) and 'slow' (System 2) cognitions affect our choices and actions (Stanovich & West, 2000). The current study examines the role of media and its effects on Systems 1 and 2. Based on prior research, we hypothesized that a preference for media that includes mentions of cannabis would increase later cannabis use and that System 1 cognitions would mediate the role of music on cannabis use while System 2 cognitions would mediate the role of film (Rustad et al., 2003). Our participants were a group of students in Western Canada (grades 7 – 9 at Time 1). Participants were assessed twice, two years apart. Participants listed their preferred musical artist and film and both types of media were coded dichotomously (Yes/No) for mentions of cannabis. We measured System 1 cognitions through word association tasks (WATs) and System 2 cognitions through the Outcome Expectancy Liking task (OEL; Fulton, Krank, & Stewart, 2012). Preference for music with cannabis mentions was significantly correlated with past year THC use ($\varphi = 0.15$, $p < 0.001$), while film preference was not. Logistic regressions demonstrated that a model with OEL, WAT, and music preferences was statistically significant ($X^2 = 120.52$, $p < 0.001$, $df = 4$). The Wald criterion demonstrated that music preference, but not film preference, provided a significant contribution to prediction (Wald = 4.14, $p < 0.05$). A structural equation model demonstrated adequate model fit (CFI = 0.99, $X^2 = 9.51$, $df = 7$, $p = 0.22$) for a mediation model where latent variable Cognition (as measured by WATs and OELs) mediated the relationship between Media (Film and Music) and Past Year Cannabis Use. Therefore, a preference for music with mentions of cannabis predicts use of cannabis two years later. However, film preferences do not predict later cannabis use. These results provide a potential explanation of the how music preferences affect later cannabis use through substance use cognition. Moreover, music preferences may be an important, and heretofore, ignored, target for substance use prevention programs.

MEDICAL CANNABIS FOR ADULT ADHD (ATTENTION DEFICIT HYPERACTIVITY DISORDER): MEDICAL SOCIOLOGICAL CASE-STUDY OF CANNABINOID THERAPEUTICS (CT) IN FINLAND

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This poster presents a detailed patient case study of a male patient who was diagnosed in adulthood (aged 33) with Attention Deficit Hyperactivity Disorder (ADHD) and treated initially with immediate-release methylphenidate (Ritalin® 10mg x 2 times daily). After experiencing adverse effects from prolonged use of this medication, and afterwards other medications that were prescribed as alternatives, the patient discovered that cannabinoid therapeutics (CT) had been experimented inside the EU area to treat patients with ADHD. Subsequently, the patient was evaluated by a physician in Germany (June 2010) who prescribed cannabinoid therapeutics (Bedrocan®, Bediol®). A Finnish neurologist later confirmed the two prescribed medicines (Bedrocan®, Oct 2010; Bediol®, May 2011) in the patient's own country of permanent residence, Finland.

During a five-year period of access, Bedrocan® was found to be helpful in alleviating the patient's ADHD symptoms, in particular poor tolerance to frustration, outbursts of anger, boredom and problems related to concentration. The second CT medication, Bediol®, which contains the non-psychoactive cannabinoid cannabidiol (CBD), was found to neutralize excessive dronabinol effects of Bedrocan®, as well as offering other medical benefits (relief from chronic neuropathic joint pain, and improved sleep). The poster also offers an overview of the current status of CT in Finland. The laws governing the authorization of cannabinoids, and the practice within individual EU Member States, need to be considered when evaluating successful cannabinoid therapeutics.

Acknowledgements: The author is funded by the Finnish Academy of Science and Letters / Eino Jutikkala Fund

SYSTEMIC INJECTIONS OF CANNABIDIOL ENHANCE ACETYLCHOLINE LEVELS FROM BASAL FOREBRAIN IN RATS

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Cannabis sativa contains more than 500 components, of which the most studied are Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD). Several studies have indicated that CBD displays neurobiological effects, including wake promotion. In this regard, experimental evidence shows that injections of CBD enhance wake-related compounds, such as monoamines (dopamine, serotonin, epinephrine, and norepinephrine). Despite that CBD modulates several neurochemicals linked with wakefulness promotion, no clear evidence is available regarding the effects of CBD on additional wake-related compound such as acetylcholine (ACh). Here, we determine that systemic injections of CBD (0, 5, 10 or 30mg/Kg, i.p.) at the beginning of the lights-on period, increased the extracellular levels of ACh collected from basal forebrain by using microdialysis and HPLC means. These findings indicate that CBD promotes ACh accumulation in the basal forebrain, a brain region related to wake control.

Acknowledgements. Funded by UC MEXUS-CONACyT (Grant no. CN-17-19) and Escuela de Medicina, Universidad Anáhuac Mayab (Mérida, Yucatán. México; Grant no. PresInvEMR2017) given to E. M.-R.

DETERMINATION OF CANNABINOIDS IN HAIR AFTER CONSUMPTION OF CBD-RICH CANNABIS EXTRACTS

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Background. Medical cannabis is becoming increasingly popular for many different ailments and general well-being. Especially CBD-rich extracts are easily available over online pharmacies, health stores or directly from the producers. However, almost all of the extracts contain small amounts of THC. The question arises if, in case of continuous use, THC concentrations in hair may rise above accepted limits for driving licence restitution or workplace testing for example.
In our study, we investigated CBD, CBN and THC in samples from regular CBD users. The goals were to (1) determine levels of these cannabinoids in hair and (2) to evaluate a possible relationship between the daily CBD intake and CBD hair levels.

Methods. All participants in the study (4 women and 6 men) consumed cannabis extracts from the same producer. It contained CBD at a concentration of 8, 16 or 32% and small amount of THC with a CBD/THC concentration ratio of 30. The self-declared CBD dosage ranged from 4 to 128 mg CBD/day, corresponding to 0.1 to 4.3 mg THC/day.
Briefly, hair samples were washed and pulverized. Then they were treated with NaOH 1 M at 60°C during 1.5 h. After solid phase extraction, the residues were derivatizated with MSTFA.
The extracts were analysed using gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) with deuterated internal standards. Data acquisitions were performed in the multiple reaction-monitoring (MRM) mode. The method validation included linearity, accuracy, precision, sensitivity limits (LLOQ, LOD) and extraction yield. All analyses were done in duplicate.

Results. CBD concentrations ranged from 10 to 325 pg/mg of hair. No significant linear correlation was observed between CBD concentrations in hair and the daily dose ($r^2 < 0.98$). THC was detected in only one sample at a concentration of 7 pg/mg of hair. This is well below 50 pg/mg of hair, the cut-off level recommended by the "Society of Hair Testing". The THC degradation compound CBN was not detected in any sample.

Conclusion. In this study, we showed that after chronic consumption of CBD-rich cannabis extracts in medium to high doses, consumers are tested negative for THC in hair.

Acknowledgment. Master student Anaïs Rodrigues is grateful to "Cannad'Our Sàrl" for her scholarship.

EFFECTS OF NOVEL SYNTHETIC (+)-ENANTIOMERS OF NATURAL OCCURING CANNABINOIDS AND THEIR DERIVATIVES ON CB1 AND CB2 SIGNALLING

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Background: The biological effects of nature identical cannabinoids and their derivatives on CB1 and CB2 receptors are widely known with (-)-trans-Cannabidiol as one of the most common representatives. However, the effects of (+)-enantiomers of natural occuring cannabinoids are quite uncharted.[3] Synthetic modifications of the cannabinoid core structure offers access to these to date insufficient researched compounds. Based on a Symrise owned process, we were able to produce synthetic (+)-enantiomers of known cannabinoids and novel (+)-cannabinoid derivatives.

Objectives: Five (+)-cannabinoids were tested for their binding and signalling effects at CB1 and CB2 receptors. Furthermore, their potencies in several cellular tests were studied.

Materials and methods: Binding affinity was studied using human CB1 and CB2 receptor-transfected cell membranes (RBHCB1M400UA and RBXCB2M400UA, respectively). The effects on CB1 and CB2 signalling were done using functional assays in HEK293T cells expressing CB1 or CB2. The following cell models were used to study the biological activity: primary human monocytes, primary human dermal fibroblasts and HaCAT keratinocytes.

Results: We found that the tested (+)-cannabinoids revealed anti-inflammatory effects in different cell types. Furthermore their binding and signalling effects at CB1 and CB2 receptors differ strongly compared to their natural (-)-counterparts.

CANNABIDIOL AFFECTS THE BEZOLD-JARISCH REFLEX VIA TRPV1 AND 5-HT$_3$ RECEPTORS AND HAS PERIPHERAL SYMPATHOMIMETIC EFFECTS IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS

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Cannabidiol (CBD) is a non-psychotropic constituent of *Cannabis sativa L.*. It is suggested to be useful in hypertension (*Pisanti et al., Pharmacol Ther. 2017;175:133*). Under in vitro conditions it activates vanilloid TRPV1 (*Iannotti et al., ACS Chem Neurosci. 2014;5:1131*) and inhibits serotonin 5-HT$_3$ receptors (*Yang et al., J Pharmacol Exp Ther. 2010;333:547*), i.e. receptors involved in Bezold-Jarisch reflex stimulation. The aim of our study was to compare the cardiovascular effects of CBD in spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats.

Experiments were performed on conscious, anaesthetized or pithed rats anaesthetized with urethane. Basal heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure were higher in *conscious, anaesthetized and pithed* SHR than in WKY. Bolus i.v. injection of single doses of CBD (3, 10 and 30 mg/kg [≈ 100 μmol/kg]) to *anaesthetized* rats induced biphasic depressor responses. The initial rapid and short-lasting decreases in HR, SBP and DBP were stronger in SHR than in WKY. Maximally they amounted to about 220 and 160 beats/min (HR); 25 and 20 mmHg (SBP) and 45 and 30 mmHg (DBP) in SHR vs WKY, respectively. All above responses were not dependent on basal parameters and were prevented by bilateral vagotomy. The CBD (10 mg/kg)-induced decreases in HR but not in BP were diminished by the TRPV1 receptor antagonist capsazepine 1 μmol/kg and almost completely abolished if CBD 10 mg/kg was re-injected 15 min after CBD 30 mg/kg. The delayed decreases in HR were longer than the initial ones, were comparable in SHR and WKY and were not modified by capsazepine and vagotomy. In SHR (but not in WKY) CBD induced subsequent increases in SBP and DBP. Both in SHR and WKY, CBD 10 mg/kg reduced the reflex bradycardia elicited by i.v. injection of the 5-HT$_3$ receptor agonist phenylbiguanide, but not that evoked by the TRPV1 agonist capsaicin. In *pithed* SHR and WKY CBD (1, 3 and 10 mg/kg) caused comparable increases in HR and SBP by about 90 beats/min and 20 mmHg and decreases in DBP by about 15 mmHg. Propranolol 1 μmol/kg completely abolished the CBD-induced increases in HR and SBP without affecting the decreases in DBP.

Conclusions: Cannabidiol (1) induces the Bezold-Jarisch reflex via TRPV1 receptors (which undergo tachyphylaxis) more markedly in SHR than in WKY; (2) inhibits the Bezold-Jarisch reflex induced by activation of 5-HT$_3$ but not TRPV1 receptors; (3) has peripheral sympathomimetic and vasodilatory effects, which are masked by its central influence.

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

CHALLENGES TO SIMPLE GENETIC MARKERS OF CANNABIS CHEMOTYPE: EVIDENCE FROM SEQUENCE DIVERSITY OF PUTATIVE CBDAS GENES IN A CANADIAN SAMPLE SET

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Cannabis strains have been historically classified into one of five chemotypes based on the phenotypic presentation of ratios of THCA, CBDA, and CBGA in the trichomes of mature inflorescences. Published literature contains simple genetic marker tests such as the B1080/B1192 system for classification of chemotypes I/II/III. Such systems, if accurate, have potential for use in marker assisted selection and strain breeding programs. We report here on the analysis of a set of 18 cannabis samples obtained on the Canadian market and demonstrate discordant results between the B1080/B1192 marker and reported chemotype on 4 of 18 samples examined. Sequence analysis of the putative CBDAS genes from the sample set was informative. Two genetically distinct strains, phenotypically high CBD (indicative of Chemotype II or III) but reported as Chemotype I by the B1080/B1192 system, were found to have CBDAS genes clustering separately from the bulk of reported sequences but closely associated to each other and with extant Genbank records. Two other strains (a putative Chemotype III and Chemotype I or II) yielded no results in the B1080/B1192 system, and were found to have CBDAS sequences divergent from the majority of Genbank CBDAS accessions. For the putative Chemotype I/II sample, this sequence appears both significantly divergent from and without closely clustering sequences in public databases, showing roughly equal divergence from commonly observed CBDA and THCA gene types. Overall, our preliminary results suggest a wide spectrum of sequence diversity for this locus, blurring the distinction between CBDAS and THCAS enzyme types and further suggesting that until specific active site catalytic residues can be elucidated as providing THCA or CBDA product specificity, simple genetic marker systems such as the B1080/B1192 system will have limited reliability as markers for mature plant chemotype.

EDUCATIONAL AND EMPLOYMENT DEMOGRAPHICS OF MEDICAL CANNABIS PATIENTS IN THE CALIFORNIA BAY AREA

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The effects of cannabis use have been widely reported to negatively impact cognitive processes and stunt educational abilities. However, several studies employing self-reports among cannabis patients/users from diverse cultures have demonstrated that cannabis users' rate of attaining education beyond high school, at a college or university level, equals that of the general population. Equal levels of educational attainment are reported between cannabis users and non-users in Swedish men (Danielsson, Falkstedt, Hemmingsson, Allebeck, & Agardh, 2015) in the United States (Barnwell, Earleywine, & Wilcox, 2006) and national samples from Spain (Hernández-Serrano, Gras, & Font-Mayolas, 2018). This rate of educational attainment among cannabis users is supported by a recent analysis from the United Kingdom (Passarotti, Crane, Hedeker, & Mermelstein, 2015), and in the United States (Williams & Hagger-Johnson, 2017) which indicates that students scoring higher on standardized tests necessary for college entrance self-report as occasional to persistent users of cannabis. Although the literature supports equal educational attainment and work status for cannabis users (Barnwell, Earleywine, & Wilcox, 2006), there is a paucity of information regarding the type of degree attained and job industry of work.

To address this issue, we distributed a self-report questionnaire to a sample of medicinal cannabis users in the Bay Area of California. We found that educational attainment among the sample revealed that all reporting participants had a high school diploma. Beyond high school, 39.7% reported "some college," and a majority (55.1%) reported receiving a university or college degree. The distribution of participants reporting degree attainment level is: (1) associate degrees (18.6%); (2) bachelor degrees (48.8%); (3) master degrees (20.9%); (4) professional degrees (7.0%) or Ph.D. (4.6%). Of the degrees received, 40% of those awarded were in science, technology, engineering, and math (STEM) related fields.

The majority of working or work-seeking participants reported cannabis use for both physical and psychological conditions (80.4%) in comparison to physical only (7.1%) or psychological only (12.5%).

Most working participants reported restricting cannabis use to weekends (75.6%), and 19.8% of those retired, unemployed, not looking for work, or disabled restricted cannabis use to weekends. Of those employed 79.7% reported employment status with no job drug testing, 13.6% reported pre-employment testing and testing with cause, 1.7% reported testing with notice and random testing, and 5.1 % reported unknown job drug testing criteria.

HYPERTENSION MODIFIES THE CANNABIDIOL-MEDIATED VASCULAR RESPONSE IN ISOLATED HUMAN PULMONARY AND RAT SMALL MESENTERIC ARTERIES

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The non-psychoactive phytocannabinoid cannabidiol (CBD) has been pointed to possess favorable properties in the cardiovascular system, since it decreased blood pressure in rats (Walsh et al., *Br J Pharmacol.* 160 (2010) 1234–1242) and humans (Jadoon et al., *JCI Insight.* 2 (2017) e93760). To date, there is a lack of data on its potential role in the vasculature in the hypertension. The aim of our study was to investigate the vasorelaxant potential of CBD in isolated human pulmonary arteries (hPAs) and small mesenteric arteries (sMAs) of rats with primary and secondary hypertension.

Functional studies were performed in isolated lobar and segmental hPAs obtained from 21 patients during resection of lung carcinoma, as well as in the sMAs isolated from (1) spontaneously hypertensive (SHR); (2) 11-deoxycorticosterone acetate, high salt-diet treated and uninephrectomized (DOCA-salt) hypertensive rats or their appropriate normotensive controls: (3) Wistar-Kyoto (WKY) or (4) uninephrectomized (SHAM) animals. Isolated hPAs were subjected to organ bath and sMAs to wire myograph. After incubation with one of the following receptors antagonists (1 μM): AM251 (CB_1 antagonist); AM630 (CB_2 antagonist); capsazepine (vanilloid TRPV1 antagonist), the concentration response curves (CRCs) for CBD were constructed in hPAs and sMAs preconstricted with the agonist of the prostanoid TP receptor U46619 or α_1-adrenergic receptor phenylephrine, respectively. In some experiments endothelium was mechanically removed. The results are expressed as a percentage of the contraction obtained at the beginning of each experiment.

CBD (0.1 - 30 μM) induced almost full concentration-dependent vasorelaxation in each experimental group. Relaxation was attenuated in hPAs from hypertensive patients (pEC_{50}=4.0±0.3, $p<0.05$, n=8) compared to normotensive (pEC_{50}=4.9±0.1, n=13). In rats, CBD-induced relaxation was dependent on hypertension model. The potency of this reaction was enhanced in DOCA-salt (pEC_{50}=5.9±0.1, $p<0.01$, n=12 vs SHAM: pEC_{50}=5.5±0.1, n=12) and attenuated in SHR (pEC_{50}=5.6±0.03, $p<0.01$, n=11 vs WKY: pEC_{50}=6.0±0.1, n=11). The CBD-induced relaxation was inhibited in the presence of: (1) AM251 in SHR (pEC_{50}=5.1±0.04, $p<0.001$, n=6) and DOCA-salt (pEC_{50}=5.3±0.1, $p<0.05$, n=5); (2) AM630 and endothelium denudation only in DOCA-salt (pEC_{50}=5.2±0.2, $p<0.05$, n=5; pEC_{50}=4.6±0.3, $p<0.001$, R_{max}=67.0±6.4, $p<0.001$, n=6, respectively), compared to respective control groups. Capsazepine did not influence CRCs for CBD. None of antagonists and endothelium denudation changed the potency and efficacy of CBD in normotensive controls.

Our study reveals that hypertension modulates CBD-mediated relaxation in human and rat isolated arteries. In sMAs from hypertensive rats, CB_1 receptors can play protective role in the relaxant effect of CBD. However, more experimental and clinical studies are still needed to clarify the role of CBD in different types of hypertension.

Acknowledgements: Funded by the National Science Centre (Poland) (Grant No 2015/19/B/NZ7/02270) and the Medical University of Białystok (N/ST/ZB/17/003/2213)

CANNABIDIOL, BUT NOT URB597, ENHANCES BEHAVIORAL EFFECTIVENESS OF ESCITALOPRAM IN MICE SUBMITTED TO CHRONIC STRESS

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Despite their widespread use, antidepressant drugs show some limitations in the clinical practice, including a delayed onset of action. Preclinical studies suggest that cannabinoid compounds, such as cannabidiol (CBD) and the anandamide degradation inhibitor URB597 (URB), induces behavioral effects similar to those induced by antidepressants. In the present study, we evaluate the behavioral and neuroplastic effects of the one-week of treatment of the combination of sub-effective doses of CBD and URB with 10mg/Kg of escitalopram (ESC)- dose that prevented-stressed induced behavioral changes only after 3 weeks of treatment. Male C57Bl6 mice were subjected to a 10-day protocol of either social defeat stress (SDS) or chronic unpredictable stress (CUS). Animals were divided in 7 experimental groups (n=8-13; Control (Non stressed); Vehicle (Veh/Veh); ESC 10mg/kg (Esc/Veh); CBD 7,5mg/kg (Veh/CBD); URB 0,1mg/kg (Veh/CBD); ESC/CBD; ESC/URB). On the 4th day of the stress protocols mice received the first of 7 daily injections of treatments. In the end of the protocols, hippocampus (HPC) and prefrontal cortex (PFC) were isolated for protein expression determination. Our results suggest that the combination of ESC and CBD produced responses predictive of antidepressant-like effects in the tail suspension test and of anxiolytic-like effects in the novelty suppressed feeding in both the homotypic stress (SDS) and the heterotypic stress (CUS) models. In parallel, WB analysis revealed that the ESC/CBD group showed a higher relative expression of synaptophysin in the PFC, suggesting a pro-synaptogenic effect of the combination. We also observed increased expression of synaptotagmin, synapsin 1a/b and β-actin in the HPC and PFC. Results presented in this study provide evidence that CBD can optimize the action of the antidepressant escitalopram.

THE BIOCATALYTIC PRODUCTION OF CANNABINOIDS IN HIGH VOLUMETRIC EFFICIENCIES

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Biocatalysis provides a very attractive alternative route to chemical synthesis and plant extraction for manufacturing cannabinoids. Utilizing two recombinantly produced cannabinoid synthase enzymes, namely THCA synthase and CBDA synthase, we demonstrate the synthesis of a large number of cannabinoids including the rarer Varin compounds. Biocatalysis is carried out using a biphasic solvent system. The substrate for the cannabinoid synthase enzymes is chemically synthesized CBGA or CBGVA. The substrate is dissolved in a water immiscible organic solvent or in oil and the enzyme is dissolved in aqueous buffer. Biocatalysis is initiated by overlaying the organic or oil layer containing CBGA or CBGVA on to the aqueous buffer containing the enzyme and then agitating the system to promote enzyme-substrate contact. The bioreactor conditions required to produce more than 40 grams per liter of cannabinoids will be described. Biocatalysis using dipentene as a water-immiscible organic solvent was better than soybean oil and data comparing biocatalysis using dipentene and oil will be presented. Different ratios of cannabinoids including the rarer Varins are obtained by modulating pH, or by altering the composition of the biphasic solvent system through the addition of co-solvents. The biocatalytically produced cannabinoids, including the rarer Varins, were purified to greater than 99.5% purity and their structures were confirmed by NMR analysis. Preliminary biological data including aqueous solubility studies and stability studies in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) will be presented and the implications of these findings for cannabinoid-based pharmaceutical development will be discussed.

Δ^9-TETRAHYDROCANNABINOL (THC), CANNABIDIOL (CBD) OR A THC/CBD COMBINATION DRIVE DIFFERENTIAL CHANGES IN ENDOCANNABINOIDS, PROSTAGLANDINS, AND RELATED LIPIDS IN MICROGLIA, ASTROCYTES, AND NEURONS

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Recent data from our lab shows that acute THC drives broad changes in the CNS lipidome. With the expanding focus of medical cannabis on neural disease the question of how THC, CBD, and combinations of THC/CBD effect different cell types in the CNS arose. Here, we test the hypothesis that THC, CBD, or a THC/CBD combination drive specific changes in the lipidome of microglia, astrocytes, and neurons.

Methods: BV2 microglia, C6 glioma astrocytes, and N18 neurons were grown to 90% confluence, then serum starved for 24 hours. Cell were than challenged with 1μM THC, CBD, or CBD+THC or vehicle for 2 hours. Cells were then harvested, and lipids extracted using 100% methanol then partial purification on C18 solid-phase extraction columns. ~70 different lipids were analyzed using MRM mode for specific lipid species using HPLC/MS/MS. A minimum of 6 flasks per treatment group were used for each cell type in each condition.

Results and Conclusions: BV2 microglia activation by THC drives significant increases in the endocannabinoid, *N*-arachidonoyl glycine (NAGly) and many of its lipoamine endogenous congeners as well as increases in prostaglandins, whereas, 2-AG and Anandamide were unaffected. C6 astrocytes, showed a significant decrease in 2-acyl glycerols and Anandamide levels, yet no change in NAGly with only small changes in additional lipoamine congeners. This result more closely mirrors the effects in whole brain. N18 neurons mirrored the increase in NAGly and its congeners seen in microglial cells. The only change that was consistent across all cell types was an increase in N-acyl ethanolamines with CBD stimulation. This was not accompanied by the characteristic decrease in lipids like NAGly and *N*-arachidonoyl taurine that is measured in the FAAH KO and with FAAH inhibition. Therefore, it is likely that this effect is not specifically a FAAH inhibition phenomenon. The combination of THC/CBD generated a third lipidomic phenotype in each cell type. Some results mimicked those of THC and some of CBD stimulation; however, the majority were different than either individual molecule

ACUTE EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON RESTING STATE BRAIN FUNCTION AND THEIR MODULATION BY COMT GENOTYPE

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Cannabis produces a broad range of acute psychotropic effects such as 'feeling high' and perceptual alterations. Strikingly, there is significant inter-individual variability in the susceptibility to these effects, which may be partially explained by genetic differences in dopamine turnover (Henquet et al., Neuropsychopharmacology. 31 (2006) 2748-57). A limited number of neuroimaging studies have examined the acute impact of cannabis on resting state brain neurophysiology, thereby mapping its psychotropic effects (van Hell et al., Int. J. Neuropsychopharmacol. (2011) 1377-88). Moreover, how genetic variation influences acute effects of cannabis on resting state brain function is unknown. Here we investigated acute effects of Δ9-tetrahydrocannabinol (THC), the main psychoactive ingredient of cannabis, on resting state brain neurophysiology, and examined the impact of catechol-methyl-transferase (COMT) Val158Met genotype, associated with prefrontal dopamine turnover, on these effects. Thirty-nine healthy males participated in a double-blind, randomised, placebo-controlled crossover pharmacological MRI study. THC (6 mg) was administered using a Volcano vaporizer. Acute effects of THC were assessed on resting state perfusion measured with Arterial Spin Labelling (ASL) and resting state connectivity measured with functional MRI. Subjective THC effects were assessed with composite visual analogue scales measuring perception, dysphoria and relaxation.

Compared to placebo, THC increased perfusion in bilateral insula, medial superior frontal cortex, and left middle orbital frontal gyrus. This latter brain area showed significantly decreased connectivity with the precuneus after THC administration. THC effects on perfusion in the left insula were significantly related to alterations in perception and relaxation. Resting state perfusion was modulated by COMT genotype. This was indicated by a significant interaction effect between drug and genotype in the executive network, consisting of the bilateral prefrontal and parietal cortex, with increased perfusion after THC in Val/Met allele carriers only. These findings indicate that THC enhances metabolism and thus neural activity in the salience network, and reduces connectivity within the default mode network. This is in line with previous resting state studies, and suggests a THC-induced increase in awareness of both internal and external salient stimuli. This is further supported by the demonstrated significant correlations between perfusion and subjective effects. Finally, variation in COMT genotype modulated acute THC effects on resting state brain activity. This is consistent with previous studies that demonstrated a modulating role of COMT genotype in the effects of cannabis. However, whereas other studies showed strongest cannabis effects in Val allele carriers, our findings indicate an augmenting effect on activity in the executive network of Val/Met carriers. This suggests that prefrontal dopamine levels are involved in the susceptibility to acute effects of cannabis.

Acknowledgements: Funded by TI-Pharma and the Netherlands Organisation for Scientific Research.

USE OF SUBLINGUAL ADMINISTRATION OF CANNABIDIOL AND PALMITOYLETHANOLAMIDE FOR THE TREATMENT OF MILD TO MODERATE MUSCULO-SKELETAL PAIN

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Pure Green, a USA cannabis pharmaceutical manufacturing company formulating cannabinoid-based medicines and I, have 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 modality to treat mild to moderate pain. There has been no novel treatment for mild to moderate pain since the release of prescription ibuprofen in 1974, naproxen in 1976, and naproxen sodium in 1980. Additional NSAIDs are not new formulations, including over the counter ibuprofen, but are iterations of the aforementioned medicines. Off label uses of medications for the treatment of pain include SSRIs, SNRIs, and anti-seizure medications. These medications have proven to be inconsistent in their pain-relieving efficacy. Opioids should be reserved for treating severe pain, but current evidence of widespread opioid abuse and opioid related deaths reveal that opioids are being used for other painful conditions. Failure to adequately manage pain may lead to widespread multisystem deleterious effects involving the immune, endocrine, cardiovascular, neurologic, psychologic, gastro-intestinal and musculo-skeletal systems and a diminished quality of life.

Adverse side effects with the use of over-the-counter pain relievers are well described. Acetaminophen can cause liver damage and may contribute to dementia. Chronic use of NSAIDs can lead to dyspepsia, gastritis, ulcers, renal failure, hypertension, stroke, arrhythmia, and heart attack. Palmitoylethanolamide (PEA) and Cannabidiol (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.

Cannabis and cannabinoids, and specifically CBD and PEA have been reported in the literature to be useful for the treatment of chronic pain as well to reduce or replace opioids for pain management. We propose that a non-psychoactive formulation of whole plant extract of cannabidiol (CBD) and palmitoylamide (PEA) and terpenes administered sublingually can be an effective, low risk, low side effect alternative to the present medicinal pain-relieving options.

Methods: We trialed 16 patients ages ranging from 20 to 87, both male and female. Pain scale scores ranged from a 3-8 prior to taking our tablets. Inclusion criteria were mild to moderate pain, not currently treating it (so no overlap of medications). Any patient taking concomitant pain medication were disqualified from the trial. Patients were provided with instructions and a diary. They were instructed to assess and document their pain on a pain scale score of 0-10 using VAS (visual analog scale) and Wong-Baker Faces Pain Rating Scale to help with the VAS scoring. At the onset of pain, initially take ½ cannabis tablet and place it under your tongue. The tablet will dissolve rapidly in about 60 seconds or less. The patient may swallow normally, but do not eat or drink anything for 3-5 minutes post dose.

1. Record the date, time, dose, and initial pain score using the patient diary form.
2. After 20 minutes, note the pain score and record in the patient diary.
3. Repeat the pain assessment and record score at 40, 60, and 120 minutes.
4. If after 20 minutes, there is not pain relief or not completely relieved, take the second ½ tablet and repeat instructions in Step 1.
5. If the pain is persistent, you may continue to dose, as needed, but not to exceed 3 whole tablets in a 24 hour period. If additional dosing is required, follow the sequence of steps above and record in the patient diary

Measurement parameters: VAS scores, patient reports of pre and post treatment pain. Patients were encouraged to take notes to describe pre and post treatment pain and how it would compare with their usual treatment.

Outcomes:

Average age 53.2	Average pain score 20 mins= 2.0	Average pain score 120 mins=0.25
10 females	Average pain score 40 mins = 1.25	
6 males	Average pain score 60 mins=0.43	

Conclusions:

The results of this proof of concept test were nothing short of remarkable. Patients uniformly responded with significantly less pain and onset to pain relief was faster than the expected 20 minutes. Although not formally measured, most patients felt relief for a prolonged period after only one dose before the pain returned- the shortest time was 6 hours and the longest time was over 24 hours. All of the patients reported that they did not experience intoxication, the pain remitted quickly, and many had an overall sense of well-being. No adverse side effects were reported. The combination of ingredients seemed to behave synergistically where the pain-relieving response of the tablet was greater than what would be expected from the same ingredients if taken separately at the same dose, as evidenced in the literature. We conclude that this combination of ingredients in a rapidly dissolvable tablet would be a safe, effective, non-intoxicating and easy to use method to treat mild to moderate pain. The idea that it is a cannabinoid medicine may limit the acceptability of this option by physicians, patients, and geography. However, as more states and countries onboard medical cannabis programs the acceptability of this medicine will be expanded. We note that the product contains no THC and is exempt from concerns about intoxicating or recreational cannabis issues. Further studies will be conducted.

DEVELOPMENT OF A DATA-DRIVEN TOOL TO EDUCATE PATIENTS ON EFFICACY AND TOLERABILITY OF CANNABIS-BASED PRODUCTS

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Introduction – With the abundant presence of non-pharmaceutical cannabis-based products (CBP), their use for medicinal purposes has increased. Whereas it is difficult to find, there is a need for objective information on the efficacy and tolerability of CBP that is easy to understand for patients. Our aim was to improve patient education by building an online tool for finding scientific information on the potential efficacy and tolerability of CBP based on patient symptoms and personal characteristics, in the form of a questionnaire that results in a personalized report.

Methods – Building the tool: First, we conducted a literature review of several research areas: pharmacokinetics, efficacy, and tolerability for various patient populations and co-variates. Then we filtered these results for relevance and quality. Secondly, we compiled and analyzed raw data from clinical studies. All were translated to algorithms for consistent tool output based on: administration method, dose, efficacy, and tolerability. Testing the tool: Prototype and technical feasibility was tested internally with 7 and 12 subjects respectively. After optimizations an external testing round (n=17) studied the interpretation of the questions, answers, and generated reports.

Results – Building the tool: Literature and raw data provided enough information to build algorithms for basic information on administration method, dose, efficacy, and tolerability. Several parameters could not be addressed due to limited or no availability of data. Testing the tool: 4/17 had difficulties with interpreting questions and/or answers from the questionnaire and 16/17 participants reported that the tool's report provided clear insight into available scientific literature. Although aimed at patients, the Rocky Mountain Poison & Drug Center, a professional organization of health care providers, expressed interest and is currently using the tool as well.

Discussion – Patients reported that the tool provided clear insight into available scientific literature. As opposed to our assumptions, the references to primary research were very much appreciated and thoroughly studied. We expected our report texts to require major adaptations, however, adaptations were needed in the questionnaire. Improvements have been inplemented and new tests are ongoing of which new data will be presented during the ICRS conference.

Future Directions – Due to a knowledge gap, we are building a HIPAA-compliant data gathering platform with validated questionnaires to facilitate observational and other clinical studies. Also, we aim to continuously improve the online tool and to serve health care organizations.

Acknowledgements: Tomori Pharmacology: Adam Truesdell (internal and external testing), Danica Boncales, R.Ph. and Paula Ramos, R.Ph. (literature review). Shireen Banerji, Pharm.D. and Christopher Hoyte, M.D., Rocky Mountain Poison & Drug Center, Denver, CO, USA; Centre for Human Drug Research, Leiden, The Netherlands; Joshua A. Lile, Ph.D., University of Kentucky College of Medicine, Lexington, KY, USA; Ryan Vandrey, Ph.D., Johns Hopkins University School of Medicine, Baltimore, MD, USA for sharing their data.

CB1 AND CB2 RESPONSES TO MEDICAL CANNABIS EXTRACTS

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Medical cannabis contains as many as 568 phytochemicals among which over 120 are phytocannabinoids. Depending on the genetics of medical cannabis, the concentrations of Δ^9-THC and CBD could vary significantly, and patient experiences differ as well. Cannabinoids exert their functional responses via cannabinoid receptors, CB1 and CB2, as well as a number of other central and peripheral receptor systems. CB1 receptor is generally associated with psychotropic effects of cannabinoids, while the CB2 receptors are linked to inflammatory and neuronal signalling systems. Canada has had medical cannabis available to registered patients since 1999, and the country is poised to legalize cannabis for recreational purposes. Our group has been interested in understanding the cannabinoid receptors responses when exposed to different varieties of medical cannabis.

We obtained four varieties of medical cannabis from the Canadian licensed producers, and these were extracted at low temperatures (25 °C) to capture the natural composition of the chemicals (native extracts). We also subjected the extracts to heat to completely decarboxylate the cannabinoid acids, and obtain the activated resin. These native and activated resins were then evaluated against CB1 and CB2 receptors, and dose-responses were recorded. The pure cannabinoids, Δ^9-THC and CBD were also evaluated along with other controls to compare the receptor responses. In the presence of native extracts, CB1 receptors exhibited response when Δ^9-THCA was present, however CB2 receptor did not exhibit any comparable response for all native extracts tested. When fully activated extracts were used, both CB1 and CB2 receptors exhibited response, and the presence of CBD attenuated the response due to Δ^9-THC in these extracts. All extracts were evaluated to understand their agonistic and/or antagonistic behavior, as a mixture, at CB1 and CB2 receptors using the synthetic agonist, CP-55,940.

Acknowledgements: Funded by CannScience Innovations, Inc. (#410003527), Ontario Innovation Trust and Canada Foundation for Innovations (#CFI 32350).

CBD DOES NOT POTENTIATE THE ANTICONVULSANT ACTION OF CLOBAZAM ON HYPERTHERMIA-INDUCED SEIZURES DESPITE INCREASING PLASMA CLOBAZAM CONCENTRATIONS IN A MOUSE MODEL OF DRAVET SYNDROME

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A recent placebo-controlled trial of cannabidiol (CBD) showed a reduction in seizure frequency in children with refractory epilepsy, including the severe epileptic encephalopathy Dravet syndrome. However, since many of these children received multiple antiepileptic drugs (AEDs) in addition to CBD, it remains a possibility that CBD might simply enhance the efficacy of the AEDs via a drug-drug interaction rather than CBD having a direct anticonvulsant action. CBD is metabolized by the cytochrome (CYP) P450 pathway, specifically isoforms CYP2C19 and CYP3A4 and has been shown to be a potent inhibitor of both. Clobazam and its active metabolite, *N*-desmethylclobazam, share this same metabolizing pathway. A metabolic drug-drug interaction, which resulted in a significantly increased plasma concentration of *N*-desmethylclobazam, was observed in patients with refractory epilepsy receiving CBD and clobazam concurrently. Here we used the *Scn1a*$^{+/-}$ mouse model of Dravet syndrome to investigate whether the anticonvulsant effect of clobazam is potentiated by concomitant CBD treatment. The *Scn1a*$^{+/-}$ mouse model exhibits the hallmark features of Dravet syndrome, including spontaneous seizures, hyperthermia-induced seizures and reduced lifespan. Consistent with previous studies, clobazam treatment significantly increased the temperature threshold for thermally-induced seizures. CBD comedication, however, did not potentiate this effect despite the presence of a pharmacokinetic interaction resulting in increased plasma clobazam levels. Results from these experiments suggest that the pharmacokinetic drug-drug interaction between CBD and clobazam does not necessarily mediate the success of CBD in the treatment of Dravet syndrome. Our research is currently investigating whether oral co-administration of CBD and clobazam promotes supra-additive reductions in spontaneous seizures and the prolongation of lifespan in *Scn1a*$^{+/-}$ mice.

PILOT AND DEFINITIVE RANDOMISED DOUBLE-BLIND PLACEBO CONTROLLED TRIALS OF EVALUATING AN ORAL CANNABINOID-RICH THC/CBD CANNABIS EXTRACT FOR SECONDARY PREVENTION OF CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING

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Background: Up to half of patients receiving chemotherapy of moderate or high emetic risk experience CINV despite optimal anti-emetic prophylaxis[1]. Limited evidence suggests cannabinoid medicine in the form of tetrahydrocannabinol (THC) may reduce CINV, and addition of cannabidiol (CBD) may improve efficacy and tolerance. The aim of this multi-centre, randomised, placebo-controlled, phase II/III trial is to determine efficacy and cost-effectiveness of addition of an oral cannabinoid-rich THC/CBD cannabis extract for control of CINV.

Methods: Target population is adult patients experiencing CINV during moderate and highly emetogenic chemotherapy regimens despite appropriate anti-emetic therapy, who are scheduled to receive at least 2 more consecutive cycles (A, B and, where applicable, C). Treatment consists of oral THC 2.5mg/CBD 2.5mg (Tilray TN-TC11M) capsules or placebo TDS days -1 to 5, in addition to guideline-consistent anti-emetics, including rescue medications. Patients will start with 1 tablet PO TDS and can dose-titrate to a maximum of 4 tables PO TDS based on nausea control and side-effects. In the pilot trial (N=80), subjects are randomised for cycle A, cross-over for cycle B, and nominate preferred treatment for cycle C. The planned definitive trial (N=250) will randomise subjects to investigational product or placebo for cycles A, B and C in a parallel design. The primary end-point is the proportion of patients gaining a complete response (no emesis and no use of rescue medications) (0 – 120h), with additional end-points of (i) complete response, (ii) no emesis, (iii) no significant nausea and (iv) no use of rescue medication during the a) acute, b) delayed, and c) overall phases of cycle A, B and C, (iv) adverse events, (v) quality of life, and (vi) cost-effectiveness.

As of 30/03/2018, 48 of 80 patients have been recruited to the pilot study, with expected recruitment completion in 4th quarter 2018.

Funding: NSW Department of Health.

Acknowledgements: Trial participants, investigators and research staff. Drug supply by Tilray. ACTRN: 12616001036404

FREE-SOLUTION, INTERFEROMETRIC ASSAYS ENABLE HIGH-SENSITIVITY, AFFINITY QUANTIFICATION OF MEMBRANE ASSOCIATED CB1 AND CB2 LABEL-FREE

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Free-solution, tether-free, matrix-independent molecular interaction assays, enabled by interferometric transduction will be described. Because the free-solution assay (FSA) signal can be attributed to conformation and hydration changes upon ligand-receptor binding, it has no need for labels, exhibits no relative mass sensitivity and is matrix independent (1,2). Thus, this new method enables robust binding assays on wide range of ligand and target pairs including membrane-proteins with small molecules, antibodies, ions, proteins, DNA, aptamers and protein complexes. Previous studies with the mix-and-read FSA interaction determination have shown that it is quantitative with the zeptomole sensitivity facilitating determination of pM-mM equilibrium binding affinities (K_D) on femtomoles of membrane proteins with numerous ligands. We have also demonstrated the capability to use FSA to study allosteric interactions and measure multi-step equilibria. This presentation will focus on binding investigations of various ligands with the membrane-associated proteins, CB1 and CB2, performed in *native membrane* environments. First we describe the interferometric reader that enables these unique, free-solution interaction assays in nanoliter volumes. Then we will describe how FSA permits mix-and-read, free-solution interaction assays on a wide array of matrices such as serum, cells, unaltered human erythrocytes and tissues. Next, we will report on our CB1 and CB2 binding determinations performed on full-length membrane proteins in cells. Finally, we will discuss the prospects for investigating allosteric interactions in the cannabinoid system and the potential for quantifying CB1 and CB2 in a variety of matrices such as serum, cells and tissue without using antibodies.

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CONSISTENCY IN THIRD PARTY QUALITY CONTROL TESTING IN THE UNITED STATES

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An important part of the cannabis market is quality control and testing. In the U.S., there are different regulations and mandated tests between states where cannabis is legal for either medical or recreational purposes. Most states that allow cannabis (medical or recreational) require some type of potency and/or quality control testing. The regulations on what the allowable limits and variance for finished products also differ from state to state. Potency testing on flowers, oils, and distillates are relatively straightforward, however, infused products can be much more difficult. There are a wide range of different types of infused products currently on the market, including everything from a simple body lotion, to transdermal patches, ice cream, suppositories to infused sexual lube. Additionally, failed tests result in products that are not allowed for sale, presenting a significant road block to production facilities. These different matrices present problems and complicate extraction and quantification. The objective of this project is comparing and contrasting the different regulations from state to state and comparing potency results of complex matrices from certified labs in the state of Colorado.

For the purpose of this research we sent 4 different products to 9 testing facilities in the state of Colorado. The 4 different products included 2 sublingual tinctures; one containing only CBD, and one containing a 1:1 ratio of THC to CBD; a transdermal product, Gel Pen high in CBN; and a topical product, Muscle Freeze containing CBD. Each product was produced into a batch of 20 units total, except the Gel Pen batch contained 22 units. All testing facilities were licensed for both Medical and Recreational Cannabis testing through the Marijuana Enforcement Division in the state of Colorado and each have been audited by the Colorado Department of Public Health Environment, (CDPHE) for potency testing. Additionally, 4 of the 9 testing facilities are ISO 17025 accredited for potency. All samples were tested for potency on an internally on a Thermo Fisher Scientific Dionex Ultimate 3000 UHPLC.

THERAPEUTIC USE OF CANNABIS AND SUBSTITUTION FOR PHARMACEUTICAL MEDICATIONS IN A PRENATAL SAMPLE

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Cannabis is the most frequently used non-pharmaceutical drug throughout the prenatal period. While anecdotal reports have indicated therapeutic use and substitution for pharmaceuticals as primary motives for prenatal cannabis use, this is the first study with the intent to clarify motives and patterns of use in a pregnant population. Over one hundred (N = 103) women who indicated that they were or had been pregnant were recruited via social media ads. Participants answered questions pertaining to perceptions of prenatal cannabis use, personal prenatal history of pharmaceutical and non-pharmaceutical drug use, as well as vignettes of comparative substance use during pregnancy.

Use of cannabis during the prenatal period was reported by 34% (n = 35) of participants. Approval of use during pregnancy depended on motive, as women approved of medical use substantially more than recreational use $F(2,91) = 113.12$, $p < 0.01$. Over two thirds of women who indicated prenatal cannabis use (n = 24; 68.57%), reported substituting cannabis for pharmaceuticals. Vignette ratings indicated that cannabis use was perceived to be more acceptable than benzodiazepine use to treat anxiety during pregnancy $F(1,94)=22.47$, $p <0.01$. Approval of cannabis use to treat morning sickness was equal to the approval rate of pharmaceuticals $F(1,92)=0.15$, $p =.70$. Morning sickness was reported by 92% (n = 31) of women who had used cannabis, and all reported that cannabis use was at least slightly effective in relieving symptoms, while almost half (48%, n =15) indicated that it was almost always effective. All nine women who reported experiencing hyperemesis gravidarum, a severe, potentially dangerous, and notoriously treatment-resistant form of morning sickness indicated that cannabis use was effective in managing their symptoms. Stigma was prominent in this population as 40.7% (n = 42) of all women surveyed indicated that they did not feel comfortable discussing prenatal cannabis use with their doctor, and 34.9% (n = 36) indicated they would not tell their doctor if they used cannabis during pregnancy.

The results of this study indicate that prenatal therapeutic cannabis use was primarily as a substitute for pharmaceutical medications. Respondents reported perceptions of effective symptom relief and greater approval of cannabis relative to other medications. A substantial proportion of respondents reported discomfort and reluctance in discussing cannabis use during pregnancy with their physician. These results highlight the need for improved health care provider communication surrounding cannabis use and pregnancy. Results also elucidate attitudes toward cannabis use and pregnancy among Canadian women.

GMP PRODUCTION OF HIGH CBD CANNABIS EXTRACT FORMULATION: STABILITY STUDY

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The aim of this work is to prepare Cannabis extract from high CBD variety grown at the University of Mississippi and to prepare an oil formulation for clinical trials. The preparation was performed following GMP regulations. The stability of this extract at different temperatures (Freezer, 4 ^{0}C and room) was evaluated as well as the stability of the oil formulation at room temperature.

Cannabis plant material (high in CBD/low in THC) was used to prepare the extract. The extract was analyzed for solvent residues, cannabinoids contents, microbial contamination, heavy metals and aflatoxins. The extract was then used to prepare an oil formulation for clinical studies at 50 mg/mL equivalent of CBD. Stability of the extract was performed at three different temperatures (room temperature, refrigerator and freezer) and analyzed by GC/FID for the CBD and THC contents. The formulation stability was only performed at room temperature.

The CBD and THC contents of the extract did not change after 18 months of storage at all the tested temperatures. At the 2 years' time point, CBD was stable at all temperatures while THC dropped below 90% (89%) of time zero level only at room temperature. The formulation was stable at room temperature after 6 months storage (last data point).

We conclude from these findings, that the GMP high CBD cannabis extract and the formulation prepared thereof are therefore compliant with the USP specifications for microbial, aflatoxin and heavy metals contents. Both preparations have long shelf life, even at room temperature storage.

Acknowledgements: This research was funded in part by the National Institute on Drug Abuse (Contract # N01DA-15-7793).

PILOT DATA FROM A PROSPECTIVE OBSERVATIONAL NATURALISTIC STUDY OF CANNABIDIOL OIL IN THE TREATMENT OF ANXIETY

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The current report follows up on our group's prior research regarding the therapeutic value of cannabinoid medicines in non-psychotic mental health conditions (Moller, 2017). In the present naturalistic observational pilot study, we focus more specifically on the intervention of cannabidiol oil (10%CBD/ 0%THC, titrating from a dosage 0.25 bid. to 1ml bid over the first four weeks) in the treatment of anxiety disorder, employing the Beck Anxiety Inventory (BAI) and the Global Quality of Life Scale (GQLS) over a prospective repeated measures design.

Our methodology employs a standardized electronic questionnaire and data visualization instrument providing standardized severity self-ratings (5-point Likert Scale) of key common symptoms of pain, sleep quality, mood, relaxation and stress-perception, as well as overall Composite Wellness Score (CWS, a mean of our 5 core indicators). We are investigating a cohort of 20 fully informed, consenting patients specifically reporting clinically significant anxiety *(*BAI>10 study entry criterion*)* over a 12-week period. Use of concomitant medicines was allowed and noted. Data was collected at weekly intervals by standardized telephone interview.

We are reporting the findings from our first 8 subjects (6M, 2F, mean age= 42+/-9.7) who have completed the therapeutic intervention and provided complete data at the 9-week time-marker. Changes-over-time within-group comparison of means was used to compare metrics gathered at t=0, t=3wks, t=6wks and t=9wks.

	Relax	*Stress*	*Mood*	*Pain*	*Sleep*	*CWS*	*BAI*	*GQLS*
At t=0	1.8 (0.7)	1.3 (0.5)	2.3 (1.5)	2.6 (1.3)	1.6 (0.7)	1.9	26.5 (10.4)	45.4 (18.6)
At t=3wks	2.6 (1.1)	2.4 (1.2)	3.1 (0.8)	3.3 (1.4)	2.8 (1.0)	2.8	12.1 (6.9)	59.1 (17.4)
At t=6wks	3.3 (0.7)	3.0 (1.1)	3.5 (0.8)	3.8 (1.0)	3.3 (1.0)	3.4	11.6 (8.6)	69.0 (16.7)
At t=9wks	3.0 (0.8)	3.0 (1.3)	3.6 (0.7)	3.4 (1.5)	3.1 (1.0)	3.2	9.6 (6.8)	70.0 (14.1)

In summary, our initial findings for this pilot study suggest general symptomatic improvements across several dimensions of wellbeing in patients with clinical anxiety who have received continuous therapy with CBD oil, with most prominent improvements noted in the initial treatment phase. BAI as well as GQLS scores demonstrated marked improvements from baseline. These preliminary findings in our still ongoing study demonstrate a promising role of a CBD-oil-weighted cannabinoid regimen, congruent with emerging research (Esther et al, 2015) to specifically address clinical anxiety. The study will continue to recruit patients until the sample size target has been reached and more robust data has been collected.

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SYNTHETIC CANNABINOIDS IN MOUSE THC DISCRIMINATION: EFFECTS OF SEX AND ROUTE OF ADMINISTRATION

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The structural diversity of abused synthetic cannabinoids has continued to increase since JWH-018 ("Spice") was identified in a confiscated product in the mid-1990s. Determination of the abuse potential of novel synthetic cannabinoids in preclinical models such as drug discrimination is crucial for scheduling these compounds. However, the parenteral injection method that is used in these models is not the typical smoke/vapor route of administration in human abusers. To this end, we have developed a procedure to expose mice to vapor generated by an electronic cigarette device. We examined the discriminative stimulus profiles of three synthetic cannabinoids (CP55,940, AB-CHMINACA and MMB-FUBINACA) in male and female C57BL/6J mice trained to discriminate 5.6 mg/kg intraperitoneal (i.p.) delta-9-THC (THC) in a standard two nose-poke aperture procedure. Dose-effect curves were determined with THC and the three synthetic cannabinoids after i.p. injection followed by concentration-effect curves with the synthetic cannabinoids via aerosol exposure.

THC i.p. fully and dose-dependently increased responding on the THC-associated aperture in both sexes, with dose-effect curves almost completely overlapping in male and female mice. When injected i.p., all three synthetic cannabinoids also produced dose-dependent increases in THC-associated responding and were more potent than THC. In males, the three synthetic cannabinoids fully substituted for THC, with relative potencies of CP55,940=MMB-FUBINACA>AB-CHMINACA. In females, AB-CHMINACA showed similar potency as in the males; however, CP55,940 and MMB-FUBINACA were less potent in females. When the compounds were vaporized, the rank order potencies for males shifted such that MMB-FUBINACA>AB-CHMINACA>CP55,940. Shifts were also noted for females, such that the three compounds were no longer equipotent. Rank order potencies for the females were the same as for males when the compounds were vaporized, but with lack of full substitution for MMB-FUBINACA and AB-CHMINACA. Despite similarity in rank order potencies, sex differences also were evident: MMB-FUBINACA and AB-CHMINACA were more potent in males than in females. In fact, the highest concentration of MMB-FUBINACA tested (2.4 mg/mL) fully substituted in males but severely suppressed rates whereas this same concentration in females failed to reach full substitution but also had little to no effect on rate of responding. In contrast, vaporized CP55,940 was approximately equipotent in both sexes. These results demonstrate the feasibility of evaluating synthetic cannabinoids via a more translational route of administration. In addition, the observed sex differences highlight the importance of inclusion of both sexes in preclinical research.

Acknowledgements: Funded by DA-003672, DA-016644 and DA-040460.

BIOAVAILABILITY OF Δ^9-TETRAHYDROCANNABINOL (Δ^9-THC) FROM DIFFERENT DOSAGE FORMS CONTAINING ITS PRODRUG Δ^9-THC-VAL-HS (NB1111)

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A prodrug of Δ^9-THC (Δ^9-THC-Val-HS; NB1111) was prepared and formulated in three different pharmaceutical dosage forms, namely; suppositories, transmucosal delivery systems, and eye drops. The suppository dosage form was tested in rats at 3.67 and 7.9 mg/kg doses, the transmucosal delivery system was tested in a swine model at 5,10 and 20 mg d0ses, and the eye drops were tested in the rabbit in a 0.6% solution in Tocrisolve. All animal studies were performed using University of Mississippi IACUC approved protocols.

Blood levels of Δ^9-THC, 11-OH-Δ^9-THC, and 11-Nor-9-Carboxy-Δ^9-THC were measured after suppository and transmucosal delivery system administration while only Δ^9-THC was measured in the different eye tissues after the eye drops administration.

The data suggest that NB1111 is a good candidate for development in these formulations to deliver Δ^9-THC both systemically and topically.

Acknowledgements: Supported in part by Nemus Bioscience and NIH Grant #2R42GM067304-02.

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.

HEALTH OUTCOME COMPARISONS BETWEEN EPILEPSY PATIENTS WHO USE VERSUS DON'T USE CANNABINOID PRODUCTS

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Introduction. Epilepsy is a class of serious neurologic disorders that is difficult to treat. Increasingly, individuals with epilepsy are turning to the use of cannabis, cannabinoid extracts, or other cannabinoid medications for symptom relief. Little systematic data has been collected about patient-level outcomes for those who obtain cannabis products for medicinal purposes outside of traditional clinical trials. Here we report data obtained from a cohort of epilepsy patients from a large observational research registry in the U.S.

Methods. Patients or adult caregivers registered with the Realm of Caring Foundation were invited to participate in a web-based survey study that assessed demographic and health-related outcomes. The Realm of Caring Foundation is a non-profit company focused on education related to the use of cannabinoid products for a variety of health conditions generally, but historically has focused on the use of CBD-rich products for the treatment of seizure disorders. Patients included those using cannabinoids and those not using cannabinoids, but interested in education about these products. Participants completed a baseline assessment and were then asked to complete follow-up surveys every 3 months. Analyses compared patients using cannabinoids versus those who were not at baseline, and to evaluate changes from baseline among patients who were not using cannabinoids at baseline, but initiated use during the follow-up assessments. T-tests and Chi-Square tests were used and significance was determined at $p<0.05$.

Results. Of 1337 patients registered for the observational study, 362 listed some form of epilepsy as their primary health condition for which they were or were considering the use of cannabinoids. At baseline, 227 patients were using cannabinoids and 135 were contemplating initiating use, but had not yet done so. On average, cannabinoid users were significantly older than non-users, but users and non-users did not differ on other basic demographic variables, age of epilepsy diagnosis, presence of generalized versus focal seizure types, or use of ketogenic diet, vagal nerve stimulation, or having had brain surgery to alleviate epilepsy symptoms. Products high in cannabidiol (CBD), versus those high in THC, were predominant among those who had initiated cannabinoid use. Self-report ratings on the World Health Organization Quality of Life (Brief) questionnaire showed no differences between cannabinoid users versus non-users on composite outcome scores, but cannabinoid users reported greater health satisfaction. Cannabinoid users reported significantly better sleep compared with non-users on validated sleep assessments for both adults (Pittsburgh Sleep Quality Index) and children (Children's Sleep Habits Questionnaire). On the Hospital Anxiety and Depression Scale (HADS), cannabinoid users had qualitatively lower anxiety and depression scores, but differences were not statistically significant ($p= 0.064$ and $p= 0.058$ respectively). Non-users were more likely to report past month outpatient hospital, inpatient hospital, or emergency room visits, and were more likely to have had a sick day from work/school in the past month compared with cannabinoid users. There were no differences between groups on the number of prescription or OTC medications used.

Of the patients not using cannabinoids at baseline, 35 initiated use prior to completing a follow-up assessment. Compared with baseline, the individuals who initiated cannabinoid use had improved self-reported health satisfaction, reduced the number of prescription medications taken, and reduced depression after initiating cannabinoid use. Children in this cohort also had improved sleep following initiation of cannabis or cannabinoid products. One patient stopped use of cannabinoids due to a self-reported increase in seizure activity after initiating use.

Conclusions. In a convenience sample of epilepsy patients enrolled in an observational research registry, current users of cannabinoids reported better outcomes across a number of health-related measures compared with a demographically similar group of epilepsy patients not using cannabinoids. Among non-users at baseline, initiation of use was associated with improvements in health and a reduction in prescription medications used. These data indicate an overall clinical benefit of cannabinoid use among epilepsy patients who obtain products outside of a pharmacy and typically high in cannabidiol. This compliments the outcome of recent clinical trials in which cannabidiol was associated with a significant reduction in seizures among those with epilepsy.

DIFFERENTIAL EFFECT OF AEA AND 2-AG IN INFLAMMATION

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The endocannabinoid (eCB) system is one of the emerging key players in immunoregulation. For example, immune stimulation have been shown to increase the eCB activity and endogenous, and synthetic cannabinoids have been shown to induce an immunosuppressant effect (ADD REF). However, prospective roles of peripheral endocannabinoids (eCBs), anandamide (AEA) and 2-arachidonoylglycerol (2-AG), following immunotherapy of interferon-alpha in a clinical sample have never been explored.

In this study, we investigated whether circulating eCBs are involved in the immune response following the inflammatory challenge of interferon-alpha treatment in patients with chronic hepatitis C (CHC).

We measured serum concentrations of AEA and 2-AG using High Performance Liquid Chromatography with Tandem Mass Spectrometry, and serum concentrations of cytokines using Meso Scale Discovery (MSD) electrochemiluminescence V-PLEX assay, in 75 patients and 42 healthy controls. The samples were collected at baseline, treatment weeks (TW) 4 and 24, end of treatment and six months follow up (6mFU).

Our results show that CHC patients had lower AEA levels (0.93±0.05) compared with controls (1.18±0.05) at baseline ($t = -4.040$, $p<.001$), whereas there was no difference in 2-AG levels (HCV: 6.28±0.55; controls: 5.88±0.81; $p = .22$). Both, AEA and 2-AG concentrations significantly increased during treatment in the whole sample (AEA: $F = 73.657$, $p<.001$; 2-AG: $F = 47.899$, $p<.001$). Interestingly, 2-AG levels normalized at 6mFU (controls: 5.88±0.81, 6mFU: 6.39±0.58; $t=.474$, $p=.64$), whereas AEA levels remained elevated in the whole sample (controls: 1.18±.05, 6mFU: 1.46±0.08; $t=3.109$, p.002). AEA levels were negatively correlated with IL-2 levels at TW24 ($r_s=-.312$, $p=.04$), end of treatment ($r_s=-.513$, $p=.001$) and 6mFU ($r_s=-.382$, $p=.02$), and with IL-6, and IL-17a levels at 6mFU ($r=-.412$, $p=.01$; $r_s=.-361$, $p=.03$). 2-AG levels were negatively associated with IL-2 and IL-4 levels at the end of treatment ($r_s=-.336$, $p=.03$; $r_s=-.331$, $p=.04$).

The increase in AEA and 2-AG at different stages of IFN-α treatment suggests that both eCBs participate in different phases of immunomodulation. The negative association between eCBs and cytokines suggests the potential immunosupressant effect of AEA and 2-AG.

This study represents independent work supported by the Medical Research Council (UK) MR/J002739/1 and the Commission of European Communities Seventh Framework Programme (Collaborative Project Grant Agreement no. 22963, Mood Inflame), and part funded by the NIHR/Wellcome Trust, King's Clinical Research Facility and the National Institute for Health Research (NIHR) Biomedical Research Centre [and Dementia Unit] at South London and Maudsley NHS Foundation Trust and King's College London.

CANNABIDIOL MODULATES MIGRATION AND OXIDATIVE METABOLISM IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Background: Polymorphonuclear leukocytes (PMN) are at the forefront of the body defence against harmful foreigners. However, they also contribute to ongoing inflammation in a plethora of pathological conditions, through inadequate production of inflammatory mediators such as reactive oxygen species (ROS) and pro-inflammatory cytokines. Therefore, PMN represent a valid druggable target for inflammatory disorders. Cannabidiol (CBD) is the major nonaddictive component of cannabis, and circumstantial evidence suggests that it may affect the immune response, however little information exists about its effects on immune cells.

Aim of the study: To evaluate the effects of CBD on PMN functions including ROS generation and cell migration.

Methods: Human PMN were isolated from buffy coat by Dextran-Ficoll Paque density gradient centrifugation. Intracellular ROS levels were assayed by use of the redox-sensitive dye C-DCFH-DA. In each experiment, CBD was added to PMN for 1 h. ROS production was then evaluated over 30 min by means of spectrofluorimetry in PMN in resting conditions and after stimulation with 0.1 μM N-formyl-Met-Leu-Phe (fMLP), measured as fluorescence intensity and expressed in arbitrary units (AU). Migration was investigated by the Boyden chamber assay in PMN alone and in the presence of IL-8 (10 ng/mL). PMN migration was quantified by light microscopy measuring the distance (in μm) from the surface of the filter to the leading front of cells.

Results: fMLP increased ROS production in PMN [from (mean±SEM) 34.4±4.3 AU to 236.7±62.5 AU, n=5, $P<0.05$]. CBD treatment attenuated fMLP-induced respiratory burst in a concentration-dependent manner with the maximum effect at $1x10^{-5}$ M [236.7±62.5 FI vs. 70.4±2.3 FI, n=5, $P<0.05$]. In contrast, CBD $1x10^{-5}$ M increased ROS production in resting PMN (62.5±10.2 AU vs. 203.5±40.5 AU, n=5, $P<0.01$). On the other hand, IL-8 increased PMN migration (30.17±0.93 μm vs. 13.17±1.17 μm in resting cells, n=3, $P<0.01$), and this effect was concentration-dependently reverted by CBD down to 16.33±1.09 μm with CBD $1x10^{-5}$ M (n=3, $P<0.01$ vs. IL-8 alone).

Conclusion: CBD reduces ROS generation and chemotaxis in human activated PMN, suggesting its usefulness in treating or preventing inflammatory disorders. The stimulatory effect of CBD on ROS production by resting cells warrants further evaluation.

Acknowledgements: The authors wish to thank Linnea SA for providing cannabidiol

WEIGHT LOSS AND MOOD IMPROVEMENT AFTER AEROBIC EXERCISE TRAINING IS RELATED TO CHANGES IN CIRCULATING ANANDAMIDE

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Ample data suggest the participation of endocannabinoids such as anandamide in energy metabolism homeostasis and neurobehavioral processes, with implications on metabolic and mental health. We aim at investigating the long-term effect of aerobic exercise training on resting plasma anandamide, and explore its relationship with changes in body weight, cardiorespiratory fitness, and mood status in healthy, previously physically inactive individuals. Participants were randomly allocated into a 12-weeks supervised walking/jogging program (EXE group), performed at the ventilatory threshold (VO_{2VT}), or into waitlist, control condition (CT group). Plasma AEA was measured by LC/MS/MS, aerobic fitness (VO_{2peak}) was assessed by open-circuit computerized spirometry during maximal cardiopulmonary exercise test, and mood profile was assessed by POMS questionnaire.

Thirty participants (age = 38±11.5, BMI = 26.6±3.6) concluded the study (EXE: n = 15; CT: n = 15). After intervention, there were significant decreases in AEA ($p < 0.01$), anger, anxiety, and body weight ($p < 0.05$), whereas aerobic fitness (both VO_{2peak} and VO_{2VT} increased ($p < 0.05$) in the EXE group. No significant changes were found in CT group. Adjusted R^2 of multiple regression analyses showed that 12.2 % of change in AEA was explained by changes in body weight ($\beta = 0.39$, $p = 0.033$), while 27 % of change in total mood disturbance (TMD; $\beta = 0.546$, $p = 0.003$), and 12 % of change in anger ($\beta = 0.404$, $p = 0.03$) was explained by changes in AEA. Our data suggest that the therapeutic effects of regular moderate exercise regarding weight loss and mood improvement may involve changes in the basal tone of peripheral anandamide signaling.

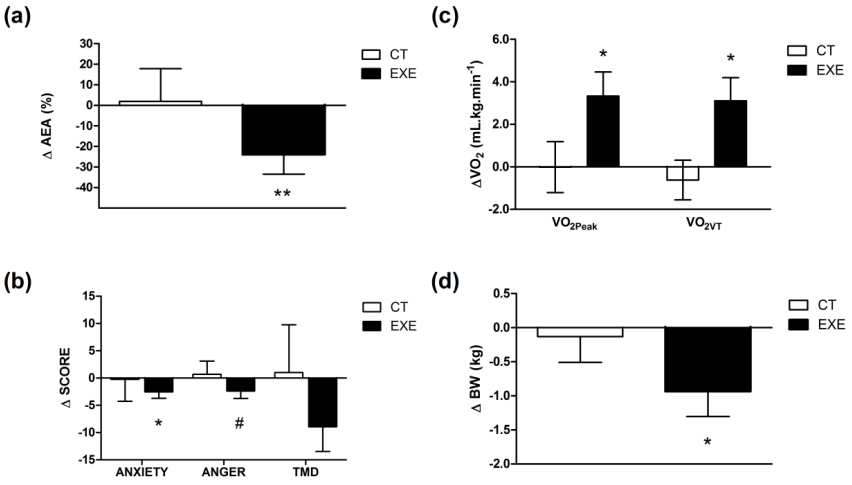

Figure 1. Changes in primary (a) and secondary (b-d) outcome variables after intervention. Variables are expressed as mean±SEM of either relative (a) or absolute (b-d) delta values. *: p < 0.05, r > 0.5 vs baseline; **: p < 0.01, r > 0.5 vs baseline; Paired t-test #: p < 0.05, r = -0.43 vs baseline; Wilcoxon Signed-Rank test.

Acknowledgements: This work was granted by the Brazilian government research funding agency CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

DELETION OF SGIP1 ALTERS ENDOCANNABINOID SIGNALING AND BEHAVIOR IN MICE

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The endocannabinoid system plays a pivotal modulatory role in synaptic transmission and plasticity. SH3 domain GRB2 like endophilin interacting protein 1 (SGIP1) interacts with cannabinoid receptor 1 (CB1R) in mammalian brain and modifies its signaling properties. SGIP1 is a novel CB1R interaction partner that had been previously characterized and connected to obesity in *Psammomys obesus* (Trevaskis et al., 2005). In transfected HEK293 SGIP1 hinders internalization of CB1R and promotes biased signaling of CB1R (Hajkova et al., 2016).

We further characterized the relationship between CB1R and SGIP1 in autaptic hippocampal neuronal cultures from SGIP1 knock out mice using electrophysiology. These neurons express an endogenous form of CB1- and 2-AG-mediated neuronal plasticity known as depolarization-induced suppression of excitation (DSE). We find that DSE and CB1 receptor desensitization are both impacted by SGIP1 deletion.

We also investigated the role of SGIP1 in vivo using several behavioral tests. Mice lacking SGIP1 have an altered profile on the 'tetrad' behavioral tests, consistent with an alteration of cannabinoid signaling. This included changes in responses to Δ^9-tetrahydrocannabinol (Δ^9-THC) in several behavioral paradigms. The results from 'cannabinoid tetrad' tests point to suppressed development of tolerance to Δ^9-THC in SGIP1 KO mice.

In summary, SGIP1 interferes with CB1R signaling in isolated autaptic neurons. The lack of SGIP1 also profoundly changes the behavior of mice including their response to Δ^9-THC. The precise mechanism by which this interaction is driven needs to be further studied.

Acknowledgements: This work was supported by GACR 16-24210S and J. W. Fulbright Scholarship

N-ACYL DOPAMINES INDUCE APOPTOSIS AND DIFFERENTIATION IN PC12 AND C6 CELLS VIA THE GPR55 AND CB1 RECEPTORS

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Bioactive lipids N-acyl dopamines (NADA)are conjugates of dopamine with fatty acids, which usually are unsaturated, and belong to the endocannabinoid/endovanilloid family. They are synthesized in mammals, as well as in some more primitive organisms. The known molecular targets of NADA are the GPCRs CB1, CB2 and several non-CB1/CB2 receptors, ion channel TRPV1 as well as some other proteins (dopamine receptors, potassium and calcium channels, etc.). The objective of the study was to evaluate the long-term effects of NADA on cell proliferation and death and to elucidate the underlying mechanisms.

As far as most of the known NADA effects occur within thenervous system, we used rat PC12 pheochromocytoma and C6 glioma cells as a model. For each cell line, the LC50 of a set of NADA was determined, and after that the cultures were incubated with various non-lethalconcentrations of NADA for two weeks. Cell death was assessed using MTT test, DNA fragmentation, caspase activity, cytochrome *c* ELISA and PS-annexin staining. The differentiation degree was determined by the count and length of the processes, mRNA expression of several stem (Oct-3/4), neural progenitor (nestin), astrocyte (GFAP) and mature neuron (NSE, beta-III tubulin) markers and by immunocytochemistry for these markers. The molecular target of NADA and signal transduction pathways were determined using inhibitor screening, siRNA knockdown, nitric oxide and ROS measurement, or using specificreporter systems.

NADA with arachidonic, oleic and docosahexaenoic fatty acid residues, as well as arachidonic acid amides of tyramine and norepinephrine were cytotoxic for both cell lines with LC50 in range 2-30 uM; cell death type was determined asapoptosis via the intrinsic induction pathway. The prolongation of cell culture treatment with non-lethal NADA concentrations induced neurite outgrowth both in PC12 and C6 cells with a shift towards neuronal and astrocytic marker expression, accordingly. However, only NADA with an intact catechol group and non-oxidized fatty acid residue were capable of such action; tyramine and 3- or 4-O-methyldopamine derivatives of arachidonic acid and dopamine amide of prostaglandin E2 failed to induce differentiation. The dopamine chrome derivative of arachidonic acid was only cytostatic.

In PC12 cells, the molecular target of the NADA action was found to be the non-CB1/CB2 receptor GPR55, which transduced signal through PLC, IP3R and calcium to the CaMKIV, and then to the CREB transcription factor with NO synthase expression induction; the differentiation signal also required Ras signaling. In the C6 cells, which lack the GPR55 receptor, the receptor target for both differentiation and apoptosis induction was CB1receptor, and thus in this model NADA acted as classical endocannabinoids.

Thus, on the model of the rat C6 glioma and the PC12 pheochromocytoma cells, we demonstrated for the first time the ability of N-acyl dopamines to induce depending on concentrationdifferentiation or apoptosis via specific receptor, CB1 for C6 and GPR55 for PC12 cells.

Acknowledgements: partially funded by the RFBR grant # 16-04-00729a.

RATS DEVOID OF SOCIAL INTERACTIONS EXHIBIT CHANGES IN CB1 RECEPTOR EXPRESSION AND INCREASE IN ALCOHOL INTAKE

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Drug addiction is considered a consequence of drug intake only; however, the majority of people who takes drugs does not develop addiction. Several brain mechanisms, among them, the endocannabinoid system is affected by drugs. However, these changes might be a consequence of life experiences that induce epigenetic changes that may induce a vulnerability in the subject to develop addiction. Among them, those induced by the quality of social interactions. In order to test the impact of having a good family- and good peer-interactions on drug intake, we studied it in rats. Four groups were developed. Gp1 (social group) was formed by rats that were with their mother at all times during the postnatal day 0 (PND0) to PND21 (non-maternal separation, NMS), and they were housed from PND21 to PND60 in a group of 10 rats (non-socially isolated, NSI). Gp2. was formed by rats that were NMS (PND0-PND21), but at PND21-PND60 they were housed individually (socially isolated, SI). Gp3. These rats were separated from their mother (maternal separation, MS) for 3h daily from PND2-PND16, then they were maintained with the mother at all times (PND17-PND21). They were NSI (PND21-PND60). Gp4. These rats were subjected to MS (PND2-PND16), then maintained with the mother (PND17-PND21). They were SI (PND21-PND60). Upon the completion of this period, all rats were housed individually to expose them to voluntary alcohol intake (PND61-PND71). Rats were exposed to two bottles of liquid, one with tap water and the other with an alcohol solution (10% of alcohol in water v/v). Liquid intake was measured every 24h and alcohol was replaced with a fresh preparation daily. Results indicated that rats in gp1. ingested a low amount of alcohol compared with those rats in the other three groups. Moreover, Western blot analysis of the expression of CB1R and D2R in the nucleus accumbens showed that those rats that underwent MS showed more CB1R regardless if in addition they underwent SI. SI by itself did not modify CB1R expression. Regarding D2R, both MS and SI increased its expression. Although gp4. also showed and increased in D2R, there were no additive or potentiating effects induced by MS+SI. Our results indicate that MS induces changes in both the CB1R and D2R expression, while SI only affects D2R. Either change in these receptors seems to foster alcohol intake in higher amount.

Acknowledgement: Funded by DGAPA-PAPIIT-UNAM (Grant IN218316)

ENDOCANNABINOID-MEDIATED REGULATION OF OSTEOARTHRITIC CHONDROCYTES: IMPLICATIONS FOR CB1 AND PPARγ RECEPTORS

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Purpose: A number of preclinical and clinical studies evaluated the therapeutic applications of cannabinoids as a treatment strategy for chronic pain. Together with broad anti-inflammatory potential of cannabinoids, endocannabinoid system (ECS) became a promising target to control both OA symptoms and disease progression. However, little is known about pathophysiological properties of endocannabinoids in osteoarthritic chondrocytes. Better understanding of cellular mechanisms underlying cartilage formation and degeneration in OA could imply novel disease modifying drugs targeting ECS for arthritis disorders' treatment. Nonetheless, endocannabinoids are involved in various, complex intercellular signalling systems beyond ECS eg. nuclear receptor superfamily PPARs (peroxisome-proliferator-activated receptors) have been suggested as the target. Results presented hereby evaluate the role of cannabinoid receptor 1 (CB_1) and PPARγ as a modulators of matrix metalloproteinase (MMPs) expression in human chondrocytes (HC) by the two best studied endocannabinoids: anandamide (AEA) and 2-Arachidonoylglycerol (2-AG).
Methods: HC and osteoarthritic-HC (HC-OA) cells from Cell Applications, Inc. were used in the present study. HC-OA were stimulated for 24h with 10μM concentration of AEA or 2-AG. BrdU assay was used to investigate their influence on cell proliferation capacity and LDH assay to determine cytotoxicity. Finally mRNA expression in response to AEA or 2-AG treatment was measured by means of RT-qPCR method. To further distinguish between CB_1- and PPARγ-mediated effects we used their respective antagonists, AM251 and GW9662.
Results: Gene expression analysis revealed strong mRNA upregulation of MMPs encoding genes, namely *MMP2, MMP3, MMP13* in HC-OA when compared to regular HC. HC-OA cells were also characterized by increased levels of *CNR1* and *PPARG* mRNA, which encode CB_1 and PPARγ, respectively. AEA treatment of HC-OA caused downregulation of *CNR1, MMP2* and *MMP3,* whereas, 2-AG caused *CNR1, PPARG* and *MMP13* downregulation but *MMP2* and *MMP3* upregulation. Further assays revealed *PTGS2* and *ERK* upregulation following 2-AG, but not AEA, treatment. Co-treatment with antagonist partially reversed endocannabinoid-mediated changes in mRNA expression. Moreover, we observed no changes in proliferation of HC-OA following AEA treatment, however 2-AG caused significant decrease in cells' proliferative capabilities.
Conclusions: This results show that endocannabinoids, AEA and 2-AG differentially regulate proliferation and expression of MMPs and COX2 in HC-OA cells. It has already been presented for the nervous tissue, that endocannabinoids can exert differential effects due to their distribution and off-target activity (i.e. triggering of TRPV1 by AEA or PPARy by 2-AG). Hereby presented results reveal significant discrepancies between AEA and 2-AG action represented by their off-target action or their metabolic products activity (Lee et al., 2007; Raman et al., 2011). In fact, both endocannabinoids are biosynthesized and inactivated independently of each other, providing flexibility in response to pathophysiological conditions. Recent studies showed marked increase in 2-AG, but not AEA, levels in synovial fluid of OA dogs (Valastro et al., 2017). Our unpublished *in vitro* results also showed an increase of thereof in HC-OA. We conclude that high cell and tissue levels of 2-AG suggests that its off-target activity observed in our *in vitro* study may be relevant to OA pathophysiology. This result implies that the apparent role of endocannabinoidome complexity should be taken under consideration in further research upon its role in osteoarthritis.

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Acknowledgments: *Supported by National Science Centre, Poland by grants: OPUS no. UMO-2016/23/B/NZ7/01143 and statutory funds.*

SIGNALLING AND FUNCTIONAL EFFECTS OF CANNABINOIDS ON PRIMARY HUMAN T LYMPHOCYTES

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An increasing body of evidence suggests an involvement of human cannabinoid receptor 2 (CB2) in a wide range of disorders with an immune component, directing substantial drug development efforts towards CB2 receptor ligands. However, despite the high therapeutic potential, little is known about CB2-mediated signalling in human immunocompetent cells. Evidence of functional selectivity in CB2 signalling [1, 2] opens up even more possibilities for eliciting finely tuned targeted immunological responses.

Given the paucity of information about CB2 signalling in primary human leukocytes, and current interest in functional selectivity of CB2 signalling, we sought to measure CB2 protein expression and determine signalling and functional consequences of CB2 activation in human peripheral blood mononuclear cells (PBMC) and primary human T lymphocytes. We found that human PBMC and T lymphocytes express CB2 receptor protein, as determined by highly sensitive whole cell binding with a highly selective CB2 radioligand 3H-RO6753361-001 (generously gifted by Roche). We have undertaken a detailed comparison of CB2 *in vitro* signalling in human PBMC, primary human T lymphocytes (isolated using Invitrogen Dynabeads Untouched Human T Cells Kit), and a T lymphoblastic Jurkat cell line in response to a range of cannabinoids, in pERK, cAMP, and pAKT (Perkin Elmer LANCE and AlphaLISA), three major signal transduction pathways of immunocompetent cells. Concentrations of cannabinoids were carefully chosen to avoid off-target effects. PBMC and T lymphocytes were cultured for no longer than 5 hours, and assayed in culture medium with 10% foetal bovine serum so as to closer mimic the state of leukocytes *in situ*.

Applying a CB2 selective agonist HU308 (1μM), we found kinetic differences in CB2 signalling between human PBMC, T lymphocytes, and the Jurkat cell line. HU308 significantly inhibited forskolin-triggered cAMP accumulation, with a peak at 5 min in the Jurkat cell line, at 20 min in T cells, and at 30 min in PBMC. Peak activation of pERK was at 2 min for Jurkat cells, and at 3 min both for PBMC and T cells. Moreover, HU308 had cell-dependent shifts in potency and efficacy. We have also initiated studies to determine the functional consequences of CB2 signalling, measuring cytokine production by PBMC and T lymphocytes (BD Cytometric Bead Array Immunoassays).

The vast majority of CB2 signalling and functional studies have been performed on cell lines heterologously expressing CB2, or in primary immune cells derived from rodents, and often with very high concentrations of ligands, which may produce off-target effects. While such studies represent important foundation research into the functional and signalling potential of CB2, these systems exhibit considerable limitations in terms of modelling human physiology. We have identified differential kinetics and efficacy in CB2 signalling between cell types endogenously expressing CB2, indicating that diversity in CB2 signalling exists even between physiologically relevant models. Our continuing studies will address the functional relevance of these differential signalling patterns, and investigate patterns of functional selectivity in primary human immune cells.

Acknowledgements: Funded by a grant from the Health Research Council of New Zealand.

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CAPSAICIN ANALOGUE DERIVED FROM n-3 POLYUNSATURATED EICOSAPENTAENOIC ACID STIMULATES INSULIN SECRETION IN β-CELLS

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Several animal studies report that administration of capsaicin, the major pungent component of red peppers, leads to reduced blood glucose levels, increased insulin secretion and plasma insulin concentrations.[1-3] However, the clinical use of capsaicin is limited by its unfavourable side-effects, including intense gut pain, hyperalgesia, stomach cramps and nausea.[4] Structure activity relationship (SAR) studies have shown that capsaicin analogues characterized by longer acyl chains and higher degree of unsaturation are non-pungent, and display higher bioactivities and oral bioavailability than capsaicin.[4-7]

In this study, two capsaicin analogues, namely *N*-eicosapentaenoyl vanyllylamine (EPVA) and *N*-docosahexaenoyl vanyllylamine (DHVA), were synthesized by lipase-catalyzed *N*-acylation of vanyllylamine with ethyl ester of eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), respectively. These fatty acid amides were investigated for their potential effect on glucose-induced insulin secretion on pancreatic INS-1 832/13 β-cell line harbouring insulin human clone and mimicking biphasic insulin secretion. Levels of insulin in cell media were detected by insulin ELISA method, Ca^{2+} and ATP were quantified in lysed cells by using the appropriate commercially available kits.[8]

EPVA (2.5 μM) was found to significantly increase insulin levels in pancreatic β-cells (by more than 40%), while capsaicin and DHVA were ineffective at the same concentration. Parallel to this, EPVA was found to increase Ca^{2+} and ATP content in our model (by 74% and 170%, respectively), suggesting that insulin release is mediated through an increase in the intracellular ATP/ADP ratio. In conclusion, our data suggest that EPVA, the capsaicin-like conjugate of EPA, might have potential therapeutic value for diabetes integrated treatments. Further *in vivo* investigations are needed to support its role on insulin secretion and therefore on modulation of glucose homeostasis.

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MODULATION OF ENDOCANNABINOID-MEDIATED PLASTICITY WITHIN THE ORBITOFRONTAL CORTEX BY A PALATABLE DIET

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The orbitofrontal cortex (OFC) plays a key role in the cognitive and emotional processing of decision-making. Dysfunction of the OFC is thought to underlie compulsive behaviours, including obsessive-compulsive disorder, drug and behavioural addictions. It is well established that the endogenous cannabinoid (endocannabinoid) system is important for appetite regulation. However, its precise role within the OFC in modulating eating has yet to be elucidated. Using in-vitro patch clamp electrophysiology, we show that CCK-expressing GABAergic synaptic inputs onto pyramidal neurons within layer II/III of the OFC are sensitive to endocannabinoids. Specifically, they exhibit endocannabinoid-mediated short-term depression (depolarization-induced suppression of inhibition, DSI) and long-term depression (iLTD, via theta-burst stimulation). Since obesity is typically associated with an overactive endocannabinoid system, we examined whether consumption of a palatable, high-fat and energy-dense cafeteria diet altered endocannabinoid signalling within the OFC. We found there was a reduction in inhibitory GABAergic synaptic transmission onto OFC pyramidal neurons following extended access (24 hr), but not restricted access (1 hr) to a cafeteria diet. This suppression of inhibition was partly reversed by the neutral CB1 receptor antagonist, NESS-0327 (0.5 μM), indicating the presence of tonic levels of endocannabinoids in obese animals. Associated with this endocannabinoid tone, we observed an enhancement of DSI and an impairment of iLTD. Furthermore, we showed that the upstream mechanism underlying these palatable diet-induced changes was activation of Group 1 metabotropic glutamate receptors (mGluRs). Specifically, mGluR-iLTD induced by the Group 1 mGluR agonist, DHPG (50 uM) was impaired in obese animals, and endocannabinoid tone was blocked in the presence of the mGluR5 antagonist, MTEP. In addition, we further show that iLTD is rescued in obese animals via restoration of glutamate homeostasis by N-acetylcysteine (0.5 μM). This rescue of synaptic transmission depends on activation of the astrocytic glutamate transporters, glutamate transporter 1 (GLT-1) and the cysteine-glutamate exchanger. Together, our findings suggest that long-term exposure to a palatable diet alters glutamatergic synaptic transmission within the OFC, resulting in enhanced endocannabinoid signalling and tone mediated by Group 1 mGluR activation, which leads to a decrease in GABAergic synaptic transmission. This endocannabinoid-mediated disinhibition is thought to result in hyperexcitation of OFC pyramidal neuron output, which may be a cellular mechanism for overeating. The rescue of palatable diet-induced changes in synaptic plasticity by restoring glutamate homeostasis might be a potential strategy for treating obesity.

CANNABIDIOL INHIBITS ENDOCANNABINOID SIGNALING IN AUTAPTIC HIPPOCAMPAL NEURONS

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Δ^9-THC and cannabidiol (CBD) are two main cannabinoid constituents of marijuana and hashish. The pharmacology of Δ^9-THC has been extensively studied, while our understanding of the pharmacology of CBD has remained limited, despite excitement in CBD's potential role in treating certain pediatric epilepsies and its reputation for attenuating some Δ^9-THC-induced effects. It was established early on that CBD binds poorly to the orthosteric site of CB_1 or CB_2 cannabinoid receptors and its actions were commonly attributed to other non-cannabinoid receptor mechanisms. However, recent evidence suggests that CBD does indeed act at cannabinoid CB_1 receptors as a negative allosteric modulator (NAM) of CB_1 signaling. By altering the orthosteric signaling of a GPCR, allosteric modulators greatly increase the richness of GPCR pharmacology. We have recently surveyed candidate CB_1 NAMs in autaptic hippocampal neurons, a well-characterized neuronal model of endogenous cannabinoid signaling, and have now tested CBD in this model.

We find that while CBD has no direct effect on excitatory transmission it does inhibit two forms of endogenous cannabinoid-mediated retrograde synaptic plasticity: depolarization-induced suppression of excitation (DSE) and metabotropic suppression of excitation (MSE), while not affecting signaling via GABA-B receptors. These results are consistent with the recently described NAM activity of CBD and suggest interesting possible mechanisms for CBD's therapeutic actions.

THE EMERGING ROLE OF PEPTIDE ENDOCANNABINOIDS AS ENDOGENOUS ALLOSTERIC MODULATORS OF CANNABINOID RECEPTORS

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Pepcan-12 (RVD-hemopressin; RVDPVNFKLLSH) is the major peptide of a family of endogenous peptide endocannabinoids (pepcans) shown to act as negative allosteric modulators (NAM) of cannabinoid CB1 receptors (Bauer et al. 2012, Straiker et al., 2015). Noradrenergic neurons and adrenals were identified as sites of pepcan production (Hofer et al. 2015). We have more recently shown that Pepcan-12 acts as a potent (K_i value ~50 nM) hCB2 receptor positive allosteric modulator (PAM) (Petrucci et al., 2017). Pepcan-12 significantly potentiated the effects of CB2 receptor agonists, including the endocannabinoid 2-arachidonoyl glycerol (2-AG), for [35S]GTPγS binding and cAMP inhibition (5-10 fold). To assess its selectivity, pepcan-12 was profiled in a panel of 168 GPCRs. Employing an assay measuring cooperativity for orthosteric CB2 receptor binding we performed alanine scanning on the peptide sequence to assess amino acids crucially involved in CB2 receptor binding.

In mice, the putative precursor pepcan-23 (SALSDLHAHKLRVDPVNFKLLSH) was found together with pepcan-12 in brain, liver and kidney whereas in human blood pepcan-23 and pepcan-12 were detected in blood cells. Interestingly, in the brain pepcan-23 seems to be the major peptide . Noteworthy, Pepcan-12 increased upon endotoxemia and ischemia reperfusion damage where CB2 receptors play a protective role. Current data indicate that the adrenals are a major endocrine site of production/secretion of constitutive pepcan-12, as shown by its marked loss after adrenalectomy. However, it remains unclear whether the adrenals mediate the production of pepcans in peripheral tissues or release them. Upon I/R damage pepcan-12 was strongly increased in the liver (from ~100 pmol/g to ~500 pmol/g) independent of adrenals. The wide occurrence of this endogenous CB2 receptor PAM, with unforeseen opposite allosteric effects on cannabinoid receptors, suggests its potential role in peripheral pathophysiological processes.

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ENDOCANNABINOID HYDROLYSIS INHIBITION UNRAVELS THAT ARACHIDONIC ACID STIMULATES A ROBUST SYNTHESIS OF 2-ARACHIDONOYL-GLYCEROL IN HUMAN LEUKOCYTES

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CONTEXT. Endocannabinoids such as 2-arachidonoyl-glycerol (2-AG) modulate immune responses, either by activating cannabinoid receptors (CB) or through their multiple metabolites, notably eicosanoids. In this respect, 2-AG hydrolysis inhibition decreases eicosanoid levels, increase 2-AG levels and diminish inflammation in mice. Thus, 2-AG hydrolysis inhibitors might represent a novel anti-inflammatory strategy in humans. However, 2-AG synthesis by human leukocytes is ill-defined and the documented syntheses of 2-AG are suboptimal, the measured levels being below the concentration range needed to activate CB receptors. Our working hypothesis was that endocannabinoid hydrolysis inhibition would allow the synthesis of relevant 2-AG concentrations by activated leukocytes.

RESULTS. We isolated human leukocytes from the blood or the lungs of healthy volunteers and found that each type of leukocyte expresses at least two 2-AG hydrolases. As such, non-specific 2-AG hydrolysis inhibitors such as MAFP and palmostatin B dramatically increased 2-AG half-life in neutrophils. In MAFP-treated neutrophils, we found that arachidonic acid (AA) induced a robust synthesis of 2-AG, achieving 2-AG concentrations of ~325 nM at 10 μM AA, which is sufficient to activate the CB_2 receptor. The efficacy of AA at inducing 2-AG synthesis in neutrophils is ~1000-fold greater than those of GPCR or TLR agonists. AA also induced the synthesis of 2-AG in human eosinophils, PBMCs, and alveolar macrophages, but not in platelets or erythrocytes. Triascin C, an inhibitor of fatty acyl-CoA synthetases and thimerosal, an inhibitor of acyl-CoA transferases both inhibited the AA-induced 2-AG synthesis in neutrophils, implying that AA remodeling is essential in this process. Additional experiments are currently underway to delineate the additional biosynthetic steps involved in the AA-induced 2-AG synthesis by human leukocytes. Finally, While GPCR agonists such as PAF, fMLP and LTB_4 modestly induced the synthesis of 2-AG in neutrophils, they inhibited the AA-induced 2-AG synthesis by ~50%, suggesting that endocannabinoid synthesis by leukocytes may be 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 AA to synthesize significant concentrations of 2-AG in presence of MAFP and that hijacking the immune system with endocannabinoid hydrolysis inhibitor might diminish inflammation in humans by promoting some anti-inflammatory effects of AA.

Acknowledgements: This work was funded by a grant from The Natural Sciences and Engineering Research Council of Canada (NF) and by the Canada Research Excellence Chair in the Microbiome-Endocannabinoidome Axis in Metabolic Health (VDM). CT is the recipient of a doctoral award from the Canadian Institutes for Health Research; ASA is the recipient of a doctoral award from the Fonds de Recherche du Québec–Santé.

CB1R- AND CB2R-LINKED ACTIONS SPECIFIC TO VENTRAL MIDBRAIN AND NUCLEUS ACCUMBENS MAY MEDIATE ANTI-ADDICTION EFFECTS OF CB1R ANTAGONISTS AND CB2R AGONISTS

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We and others have previously reported that cannabinoid CB1 receptor (CB1R) *antagonists* produce anti-addiction effects against a wide range of addictive drugs in a remarkably wide range of animal models relating to drug abuse. We have more recently reported that the same effects are seen with cannabinoid CB2 receptor (CB2R) *agonists*. Even more recently, we have reported that the *combined* CB1R *antagonist*/CB2R *agonist* delta-8-tetrahydrocannabivarin ()8-THCV) produces robust anti-nicotine effects in 7 different animal models relating to nicotine addiction. These effects of)8-THCV appear to be synergistic as compared to individual CB1R antagonists or CB2R agonists alone. It is well-accepted that addictive drugs exert their reinforcing and incentive motivational properties by acting – either directly or indirectly – on the mesolimbic dopamine (DA) tract originating in the ventral tegmental area (VTA) and projecting via the medial forebrain bundle (MFB) to the nucleus accumbens (NAc). We have reported that CB2Rs are located on VTA DA neurons, and have more recently shown that CB1Rs are found in the VTA on both glutamatergic excitatory and GABAergic inhibitory neurons. Using RNAscope technology, we have found a specific subtype of glutamate neuron in the VTA – subcortical glutamatergic neurons that express VgluT2. CB1 mRNA is absent in the midbrain of VgluT2-*CB1*$^{-/-}$ mice – suggesting that these CB1Rs are located on local VTA glutamatergic neurons. Double-staining RNAscope imaging shows CB1 mRNA in VTA glutamatergic neurons, VTA GABAergic neurons, and GABAergic neurons outside the VTA. Activation of CB1Rs on VTA glutamatergic neuronal fibers would decrease VTA DA neuronal activity by inhibiting VTA glutamate release. Conversely, activation of CB1Rs on VTA GABAergic neurons or afferents reduces local GABA release – thus disinhibiting VTA DA neurons. We propose that local interactions between VTA neurons containing CB1Rs or CB2Rs may mediate the anti-addiction effects of CB1R antagonists and CB2R agonists. CB2R agonists acting directly on VTA DA neurons would be expected to inhibit DA neuronal activity in the VTA-NAc pathway, possibly subserving an anti-addiction effect. CB1R antagonists would be expected to have VTA-mediated anti-addiction properties if the final net effect of opposing actions on VTA CB1Rs on glutamatergic and GABAergic neurons favors the latter. However, more distal actions (e.g., within the NAc) may also need to be considered. NAc CB1R activation enhances NAc DA release. Thus, CB1R antagonism may be expected to have anti-addiction effects. Congruent with this hypothesis, intra-NAc injections of CB1R antagonists have clear anti-addiction effects in animal models. Also, activation of CB2Rs on NAc DA terminals produces inhibition of DA tone, which could contribute to anti-addiction efficacy. Further research will be necessary to fully understand the brain substrates/mechanisms underlying the anti-addiction effects of CB1R antagonists and CB2R agonists – leading hopefully to improved anti-addiction pharmacotherapy.

Acknowledgements: Funded by the Intramural Research Program, NIDA, NIH.

CHANGED ENDOCANNABINOID SIGNALING IN THE PREFRONTAL CORTEX IS RELATED TO CHRONIC PAIN INDUCED DEPRESSION

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Patients with chronic pain often suffer from depression, but the underlying mechanisms are still unclear. The medial prefrontal cortex (mPFC) is a key brain area regulating depression. There are critical links of mPFC synaptic function to depression, with signaling through the endocannabinoid (eCB) system, whereby eCBs 2-Arachidonoylglycerol (2-AG) and N-arachidonylethanolamide (AEA) pre-synaptically suppress GABAergic inhibitory input to mPFC pyramidal neurons via their receptors (CB1Rs), thereby keeping the mPFC active. Anatomical and electrophysiological studies also show that afferent nociceptive pathways connect to the mPFC. eCB signaling is activity dependent. So, it is possible that noxious stimuli acutely activate and chronically depress eCB signaling in the mPFC, which results in depression. The present study was designed to address this hypothesis. We found that spared nerve injury (SNI) reliably induces neuropathic pain and significantly decreases performance of rats on behavioral tests relevant to depression, including the sucrose preference test, novelty-suppressed feeding test, and forced swimming test. Thus, SNI was followed by development of multiple indicators of a depression phenotype. Levels of 2-AG but not AEA in mPFC were elevated 3 days after SNI surgery, but normalized by 34 days. Neuropathic pain acutely (3 days) increases, but chronically (34 days) decreases, spontaneous activity of pyramidal neurons in the mPFC in single unit in vivo recordings. Brain slice electrophysiological recordings showed that CB1Rs in GABAergic presynaptic terminals are desensitized 34 days after SNI surgery, which results in increased inhibitory innervation of mPFC pyramidal neurons. Together, these data indicate that depressed eCB signaling in the mPFC may contribute to chronic pain induced depression, and that eCB signaling is a potential target for treating depression induced by chronic pain.

Acknowledgement: This work is supported by the NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation (#2510).

CANNABINOIDS AS INVERSE AGONISTS FOR GPR3 AND GPR6

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GPR3 and GPR6 are constitutively active, G protein-coupled receptors (GPCRs) without confirmed endogenous ligands. These orphan GPCRs have been shown to be involved in neurological disorders such as Alzheimer's disease and Parkinson's disease. GPR3 and GPR6 are phylogenetically related to the cannabinoid receptors. In a recent study using a β-arrestin2 recruitment assay, both GPR3 and GPR6 have been identified as novel molecular targets for phytocannabinoid cannabidiol (CBD).

In the current study, the activities of a variety of endocannabinoids, phytocannabinoids, and synthetic cannabinoids were examined on both GPR3 and GPR6, using a β-arrestin2 recruitment assay and a cAMP accumulation assay.

Our results demonstrate that none of the endocannabinoids tested had any significant effects on either β-arrestin2 recruitment or cAMP accumulation for either GPR3 or GPR6. Among the phytocannabinoids tested, CBD was the most effective in inhibiting GPR3- and GPR6-mediated β-arrestin2 recruitment, but had no effects on cAMP accumulation. Synthetic cannabinoid WIN55212-2 and its enantiomer WIN55212-3 inhibited both GPR3- and GPR6-mediated β-arrestin2 recruitment and cAMP accumulation. In contrast, CB1 antagonist SR141716A and CB2 antagonist SR144528 inhibited GPR3- and GPR6-mediated β-arrestin2 recruitment, but had no effects on cAMP accumulation.

In conclusion, our data demonstrate that CBD, as well as SR141716A and SR144528, are biased inverse agonists for β-arrestin2 recruitment for both GPR3 and GPR6. This suggests it might be possible to block some of the deleterious effects of GPR3 and GPR6 (e.g. β-amyloid production in Alzheimer's disease for GPR3) while keeping the beneficial actions of GPR3 and GPR6 (e.g. neuroprotection) intact. Although WIN55212-2 and WIN55212-3 had low potency for GPR3 and GPR6, these aminoalkylindoles provide molecular scaffolds upon which ligands with higher potencies may be developed. Furthermore, the fact that SR141716A and SR144528 acted on GPR3 and GPR6 suggests that some of the previously reported effects of these synthetic antagonists for CB1 and CB2 might be partially attributed to their actions on GPR3 and GPR6.

Acknowledgments: This study was supported in part by University of Louisville Research Infrastructure Funds and National Institutes of Health Grants DA011551 (Z.H.S.), DA003934 and DA021358 (P.H.R).

SEX-SPECIFIC EFFECTS OF ENDOCANNABINOID ACTIONS ON FEAR CONDITIONING AND EXTINCTION

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Experiencing a traumatic event is twice as likely to cause post-traumatic stress disorder in women as it is in men. Despite this imbalance, most of what we know about the neural mechanisms that underlie PTSD comes from research in male animals. One especially under-studied area is the endocannabinoid (eCB) system, whose role in modulating the stress response is just beginning to be uncovered. A better understanding of sex differences in these processes is critical to progress in developing more personalized therapies for PTSD patients of both sexes. To explore the influence of eCB signaling on aversive learning and memory processes, we tested male and female rats in a standard cued fear conditioning and extinction paradigm after systemic administration of FAAH inhibitor URB597, CB1 receptor antagonist AM251, MAGL inhibitor MJN110, or vehicle.

We found that females were more responsive to eCB manipulations than males, exhibiting an increase in freezing after URB597 administration, and an increase in active "darting" behavior after administration of MJN110. Together, these results suggest that AEA and 2AG pathways have divergent effects on fear behavior in females, implicating a potential novel, sex-specific mechanism by which the eCB system mediates aversive associative learning.

Acknowledgments: Funded by NIH R56MH 114193

CONTINUOUS MORPHINE INFUSION DETERIORATES LOCOMOTOR RECOVERY AND ENHANCES CHRONIC NEUROPATHIC PAIN IN SPINAL CORD INJURY MICE: EFFECTS OF CANNABIDIOL TREATMENT

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The gold standard opioid analgesic morphine is widely used in the clinic to treat acute and chronic pain. However, morphine not only has high potential for addiction but may also lead to neuroinflammation and exacerbate secondary injury, including that produced following spinal cord injury (SCI). For example anecdotal clinical evidence suggests that treatment with opioids following SCI may worsen recovery. We recently found that cannabidiol (CBD), a non-psychoactive ingredient in Cannabis, ameliorates thermal sensitivity following SCI in a mouse model by modulating the immune response. Therefore we hypothesized that morphine would exacerbate recovery following SCI and that this would be attenuated by co-administration with CBD.

Female C57Bl/6 mice were exposed to sham surgery or SCI and implanted with pumps containing saline, 2.52mg/100ul, or 3.78mg/100ul morphine which is infusion 0.5ul per hour, 24 hours continuously for 7 days. A subset of spinal cord injured mice with 3.78mg/100ul pumps were given vehicle or CBD treatment (2.5 mg/kg) 1 hour post injury and every 24 hours for 7 days by intraperitoneal injection. Changes in locomotor and bladder function, hindpaw thermal and tactile sensitivity, and spinal immune cell population in vehicle versus treated mice were evaluated.

T cell and macrophage/microglia population increased in sham mice infused with 3.78mg/100ul morphine but decreased in SCI mice. Morphine infusion significantly decreased locomotor function and bladder recovery in SCI mice, as well as increased thermal and mechanical sensitivity. CBD treatment significantly ameliorated morphine's effect on thermal sensitivity and produced a trend at protecting against mechanical sensitivity. However, CBD treatment did not protect against morphine's effects on motor and bladder recovery.

Morphine can attenuate locomotor and bladder recovery and contribute to chronic neuropathic pain in spinal cord injured mice. CBD treatment can ameliorate morphine's effect on neuropathic pain but not motor and bladder dysfunction.

Acknowledgments: Department of Defense award W81XWH-14-1-0389 "Novel strategies to mitigate spinal cord injury and secondary inflammation following spinal cord injury (PI: SJW)

ORAL EHP-101 ALLEVIATES SKIN AND LUNG FIBROSIS IN A BLEOMYCIN MODEL OF SCLERODERMA

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Systemic sclerosis (SSc) or scleroderma is a chronic multiorgan autoimmune disease of unknown etiology characterized by vascular, immunological and fibrotic abnormalities. The disease is complex and dynamic, and the interrelationship among the main hallmarks of SSc results in a wide spectrum of clinical presentations ranging from limited skin involvement (limited cutaneous SSc) to widespread internal organ fibrosis (diffuse cutaneous SSc). The pathogenesis of SSc not fully understood, and it is believed that inflammation, as well as vascular injury in the initial stages, drive the autoimmune response and precede fibrosis in the course of the disease. Several lines of evidence have shown that the endocannabinoid system (ECS) may play a role in the pathophysiology of SSc. Thereby, structurally different CB_2 agonists such as ajulemic acid, JHW-133, and a novel cannabidiol (CBD) derivative (VCE-004.8) have been shown to alleviate skin fibrosis and inflammation in experimental models of SSc.

We have shown previously that VCE-004.8, a CBD aminoquinone derivative, prevents skin fibrosis through PPARγ and CB_2 pathways (Del Río, et al., Sci Rep. 2016; 6:21703). Herein we report that EHP-101, an oral lipidic formulation of VCE-004.8, prevents skin and lung fibrosis in a bleomycin-model of SSc with an efficacy comparable to ajulemic acid, which is also dual PPARγ/CB_2 agonist. EHP-101, which has been granted Orphan Drug designation by the FDA and EMA, reduced dermal thickness and prevented the infiltration of inflammatory cells in a dose-dependent manner. In addition, transcriptomic and qPCR analyses, as well as determination of plasmatic biomarkers measured by Proteome Profiler Antibody arrays, demonstrated that EHP-101 downregulated the expression of several key genes and plasmatic biomarkers associated with fibrosis and inflammation. A pharmacokinetic study has demonstrated good oral bioavailability for EHP-101 with a T*max* of 2.5-3 hours. Altogether the results indicate that this synthetic cannabinoid qualifies as a novel compound for the management and possible treatment of scleroderma and, potentially, other fibrotic diseases.

This work was supported by the MINECO grants RTC-2014-1877-1 and SAF2017-87701-R to EM.

THE ANTI-DEPRESSIVE EFFECTS OF ELECTROCONVULSIVE THERAPY (ECT) IN DEPRESSED–LIKE MICE: THE ROLE OF MICROGLIA, NEUROGENESIS AND ENDOCANNABINOIDS

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Electroconvulsive therapy (ECT) is one of the most effective treatments for major depression. Although it has been used for decades, particularly for treatment of patients with antidepressant drug resistance, the mechanisms underlying ECT's action are still elusive. Our recent study on "depressed-like" mice revealed that the therapeutic effects of ECT are dependent on the presence of intact microglia and the levels of microglial activity. Here we assessed the effects of ECT on the hippocampal transcriptomic signature of "depressed-like" mice, and on the physical interactions between microglia and hippocampal adult neurogenic cells (doublecortin (DCX)-positive cells).

ECT (3 sessions/week for 2.5 weeks) was administered to "depressed-like" mice (which were exposed to 5 weeks of chronic unpredictable stress (CUS), and was found to reverse the CUS-induced reductions in sucrose preference (anhedonia) and social exploration. These effects were microglia-dependent, evidenced by blockade of these therapeutic effects by the microglial inhibiting drug minocycline, or by near-complete depletion of brain microglia. We show that ECT induced significant changes in hippocampal endocannabinoid gene transcription, but this effect was not maintained in microglia-depleted "depressed-like" mice. Additionally, we show that ECT induced increases in mRNA levels of the adult neurogenesis-related genes, and of DCX-positive neurogenic hippocampal cells. These effects did not occur when microglial activation was inhibited by minocycline. Furthermore, ECT induced a significant increase in the rate of physical interaction between microglia and DCX-positive cells, as assessed by confocal microscopy. In conclusion, we show that the anti-depressive effects of ECT are dependent on the presence and activity level of microglia in the hippocampus. Furthermore, the therapeutic effects of ECT seem to be mediated by the interaction of microglia with adult neurogenic cells. The involvement of ECT-induced endocannabinoid gene transcription will be discussed.

AN INVESTIGATION INTO THE ROLE OF THE PUTATIVE CANNABINOID RECEPTOR GPR55 IN CORTICAL NEURON SIGNALLING AND APOPTOSIS

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The orphan G-protein coupled receptor GPR55 is widely expressed throughout the body and is responsive to cannabinoids. To date research on GPR55 has been largely carried out using overexpressing cell line models, which do not accurately reflect the function of the receptor physiologically. GPR55 is expressed in the neurons and glia of the brain and has been suggested to have a regulatory role in synaptic plasticity[1] and neuronal growth[2, 3]. Its function in neuropathological conditions is little understood, although the suggested endogenous ligand for GPR55, L-α-lysophosphatidylinositol (LPI), exerts microglia-dependent neuroprotection after excitotoxic lesion[4], suggesting that GPR55 may have a regulatory role in neuroinflammation and neurodegeneration. This makes GPR55 an attractive therapeutic target for the treatment of neuropathological conditions where inflammation is a characteristic feature. Alzheimer's disease (AD) is a neurodegenerative disease associated with neuroinflammation, neuronal loss and cognitive decline. The present study aims to utilise a rat cortical neuron model to examine the effects of GPR55 signalling and to elucidate the role of GPR55 in AD.

Cultured primary cortical neurons obtained from neonatal rats were treated with the novel GPR55 agonist *N*-((4-(*N*-Phenylsulfamoyl)phenyl)carbamothioyl)-[1,1′-biphenyl]-4-carboxamide[5] (referred to as 17g in this study) and the suggested endogenous agonist for GPR55, LPI. 17g- and LPI-induced signalling effects were assessed using phospho-cAMP element binding protein (pCREB) immunocytochemical staining and confocal microscopy; and ratiometric Fura-2 imaging of intracellular calcium ($[Ca^{2+}]i$) responses. Neuronal apoptosis was assessed by active caspase-3 immunocytochemistry.

It was found that 17g (1 μM) significantly increased CREB phosphorylation levels 1.5-fold from control following 15 min of stimulation ($p<0.001$, ANOVA & Student Newman-Keuls, 45 cortical neurons from n=3 independent cultures). This response was inhibited by the selective GPR55 antagonist CID16020046 (CID; 10 μM). 17g (1 μM) and LPI (10 μM) modified $[Ca^{2+}]i$ activity in cortical neurons. Basal Ca^{2+} activity exhibited a synchronous pattern, indicative of network activity, in certain neuronal populations and 17g appeared to increase this activity (24 neurons from n=3 independent cultures). LPI (10 μM), in contrast, diminished the frequency of $[Ca^{2+}]i$ increases (22 neurons from n=3 independent cultures). Treatment of the pathological marker of AD, Aβ, for 72 hours significantly enhanced cellular caspase-3 activation compared to control (2.44±0.50 units; $p<0.01$, ANOVA & Student Newman-Keuls, n=6 independent cultures). 17g (1 μM) significantly attenuated this Aβ-evoked caspase-3 activation (1.64±0.26 units; $p<0.05$, ANOVA & Student Newman-Keuls, n=6 independent cultures), suggesting that 17g may have neuroprotective effects.

This study suggests that GPR55 plays a possible role in the regulation of gene expression, $[Ca^{2+}]i$ activity and neuronal apoptosis.

This research was funded by a Trinity Postgraduate Research Scholarship.

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INVESTIGATING THE ROLE OF THE CANNABINOID 2 RECEPTOR IN *SCN1A* DERIVED EPILEPSY

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Epilepsy, a disease of spontaneous, recurring seizures, affects over 50 million individuals globally, making it one of the most common neurological disorders. Approximately 30% of epilepsy patients do not respond to currently available treatments, thus, the development of novel therapies is critical. The endocannabinoid system has emerged as a potential therapeutic target for treatment-resistant epilepsies. Most research to date has focused on the cannabinoid 1 receptor (CB1R), given its widespread expression in the central nervous system and known role in regulating neuronal excitability. However, activation of CB1Rs can be accompanied by a number of unwanted side effects. Activation of the cannabinoid 2 receptor (CB2R) has been shown to confer protection in models of several neurological disorders, and may represent a viable treatment target with a more favorable side effect profile. However, we still know very little about the specific contributions of CB2Rs to seizure resistance or whether they could serve as targets for therapeutic intervention.

Recent evidence from clinical trials has demonstrated the success of cannabidiol, a compound that can have activity at CB1Rs and CB2Rs, in treating *Scn1a* derived epilepsies. Here, we evaluate the effect on seizure susceptibility of reducing CB2R expression in mice expressing the human *SCN1A* R1648H mutation, which was identified in a family with genetic epilepsy with febrile seizures plus (GEFS+). We crossed heterozygous *Scn1*$^{RH/+}$ mutant mice with heterozygous CB2R knockout mice (*Cnr2*$^{+/}$) and tested susceptibility to acutely induced seizures in the offspring from this cross. Latencies to seizure generation were reduced in *Cnr2* mutants as well as *Scn1a/Cnr2* double mutant mice when compared to wild-type (WT) littermates. Our results indicate that reduced CB2R expression exacerbates seizure phenotypes, thus supporting a potential role for CB2Rs as novel treatment targets for epilepsy. Our future work will continue to investigate the role of CB2Rs in *Scn1a* derived epilepsies.

ENDOCANNABINOID SIGNALLING GATES STRESS-LIKE STEREOTYPIC BEHAVIORS

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Endocannabinoid (eCB) signalling is known to gate many aspects of the stress response, including its ability to 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. This would suggest that eCB signaling regulation of CRH neurons in the PVN could be a critical mechanism by which eCB signaling regulates the stress response.

Recently, Fuzesi and colleagues (2016) demonstrated using optogenetic approaches, that activation of CRH neurons produces a characteristic behavioral 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 behavioral 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 indicate that administration of the CB1 receptor antagonist AM251 (3mg/kg) to non-stressed mice parallels key aspects of the stress response, such as increasing circulating corticosterone and a significant increase in self-directed behaviors. This is consistent with previous studies indicating that tonic eCB signaling gates activation of the stress response and that a disruption of eCB signaling produces a stress-like state. 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, and stress-associated behaviors.
Acknowledgements: Funded by CIHR (Grant. no. #60-28350-0000-10012124).

IDENTIFICATION OF AMINO ACID RESIDUES INVOLVED IN 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. Therefore, there is growing interest in exploring CB1 biased signaling to refine cannabinoid therapeutics. Biased ligands could elicit therapeutic effects while inducing fewer adverse effects. The molecular mechanism implicated in biased signaling is the stabilization of receptor upon ligand binding in different conformational states that favor coupling to G-proteins or β-arrestins. However, little is known about the CB1 receptor conformational state that promotes β-arrestin coupling. Evidence from molecular dynamics simulations of CB1 bound to Org27569, an allosteric modulator that promotes interaction with β-arrestins, suggests that during β-arrestin biased signaling there is 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 specific amino acid mutations that are predicted to bias CB1 signaling toward β-arrestins by stabilizing Y7.53 Chi1 dihedral in trans: I2.43A, I2.43T and S7.57E. Wild-type (WT) and mutated human CB1 receptors were stably transfected into HEK293 cells, and ERK1/2 phosphorylation (pERK1/2) was analyzed.

None of the mutations altered the EC_{50} or the E_{max} for pERK1/2 after 15min of 2-AG treatment. To evaluate $G\alpha_{i/o}$-independent signaling, we analyzed pERK1/2 after 2-AG treatment in the absence or presence of pertussis toxin (PTx). In the I2.43A mutation, pERK1/2 was PTx-insensitive at every time point analyzed, and at 10min PTx-insensitive pERK1/2 was significantly higher in I2.43A compared to WT. For the I2.43T mutation, although PTx partially reduced pERK1/2 at 10 and 15min, PTx-insensitive pERK1/2 was significantly higher compared to WT at 5, 10 and 15min. For the S7.57E mutation, PTx insensitive pERK1/2 was detected at 10min, but this response was significantly lower than obtained for WT in the absence of PTx. At later time points, PTx partially reduced pERK1/2 in S7.57E. These findings suggest that stabilization of Y7.53 Chi1 dihedral in trans can bias CB1 signaling toward $G\alpha_{i/o}$-independent mechanisms, although S7.57E mutation seems to impair both $G\alpha_{i/o}$-dependent and independent signaling. Further β-arrestin knockdown studies will reveal whether the changes in pERK1/2 response induced by these CB1 mutations are mediated by increased β-arrestin signaling.

Support: NIH: R01 DA003934, T32 DA007237 and P30DA013429.

THC-INDUCED HYPERNAUSEA ASSESSED IN THE CONDITIONED GAPING MODEL IN RATS

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The psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC), is known to produce several aversive effects in human users and experimental animals. THC is a partial agonist of the cannabinoid 1 (CB1) receptor of the endocannabinoid system in the brain. Recent research has found that high doses of THC can produce nausea/vomiting in both humans and animals. There is also a condition recently described in humans called "Cannabinoid Hyperemesis Syndrome" (CHS), characterized by cyclical episodes of nausea and vomiting in long-term, high dose cannabis users. These findings are paradoxical, however, because low doses of THC are known to reduce nausea and vomiting in humans undergoing chemotherapy treatment and animal models of toxin induced nausea and vomiting. The mechanism responsible for the nauseating effects of THC remain unclear. It is hypothesized that a dysregulation of the endocannabinoid system, in particular the CB_1 receptor, is involved in the pathophysiology of CHS.

The conditioned gaping model, a rat model of nausea, was used to examine the nauseating effects of high dose THC in the taste reactivity paradigm. In experiment 1, male Sprague Dawley rats underwent 3 daily conditioning trials where they received an intraperitoneal (i.p.) injection of 0.5, 5, or 10 mg/kg of THC, or vehicle (VEH) following an intraoral infusion of a novel saccharin solution for 2 min at a rate of 1 ml/min. The dose of 0.5 mg/kg THC is known to prevent lithium chloride induced conditioned gaping/nausea. The day following the final conditioning trial, rats underwent a drug-free test where they were only exposed to the intraoral saccharin infusion. Experiment 2 evaluated the ability of the CB1 antagonist/inverse agonist, rimonabant (SR141716A; 1 mg/kg) or VEH administered i.p. 30 min prior to each conditioning trial to interfere with the establishment of conditioned gaping produced by 10 mg/kg THC

Doses of 5 and 10 mg/kg of THC produced conditioned gaping reactions, whereas 0.5 mg/kg THC and the VEH did not. Pre-treatment with 1 mg/kg rimonabant prior to conditioning prevented the establishment of conditioned gaping produced by 10 mg/kg THC. These results suggest that high doses of THC can produce nausea through activation of the CB_1 receptor. Subsequent experiments are implementing qPCR to investigate changes in CB1 receptor, fatty acid amide hydrolase (FAAH), monoacylgycerol lipase (MAGL) and diacylglycerol lipase (DAGL) expression in nausea related brain regions (Interoceptive insular cortex and dorsal vagal complex) as well as thermoregulatory (hypothalamus) and control regions.

Acknowledgments: This research was supported by grants from NSERC (03629) and CIHR (137122) to L. Parker.

ENDOCANNABINOIDS CONTROL FLIGHT BEHAVIOR IN A MOUSE MODEL OF GENERALIZED ANXIETY DISORDER

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Our current knowledge about implications of endocannabinoids in fear and anxiety is largely based on fear conditioning paradigms and approach-avoidance conflicts. Recently we established the ethobehavioral beetle mania task (BMT), which is based on confrontation of the mice with an erratic moving robo-beetle. We employed this new active fear task to demonstrate in high-anxiety behavior (HAB) mice decreased tolerance but increased avoidance of the approaching beetle. Treatment with the MAGL inhibitor JZL184 (8 mg/kg) increased flight behavior, but did not affect tolerance. The FAAH inhibitor URB597 (0.3 mg/kg), in contrast, reduced flight behavior and enhanced tolerance to the robo-beetle. This effect could be blocked by co-treatment with the CB1 receptor agonist SR141716 (3 mg/kg). Preliminary data obtained in conditional CB1 knock-out mice suggest that CB1 receptors on GABAergic neurons mediate the panicolytic effects of endocannabinoid signaling.

OVEREXPRESSION OF THE ENDOCANNABINOID ANANDAMIDE DEGRADING ENZYME IN THE BASOLATERAL AMYGDALA DECREASES ANXIETY AND FEAR MEMORY

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Inhibition of anandamide (AEA) hydrolysis by the enzyme fatty acid amide hydrolase (FAAH) within the basolateral complex of the amygdala (BLA) has been shown to reduce anxiety, neuroendocrine responses to stress and promote fear extinction. To determine if impairments in AEA signaling within the BLA would produce the opposite effects, and induce a stress-like state characterized by heightened anxiety and sustained fear, we examined the effects of overexpression of FAAH locally within the BLA on behavioural indices of anxiety and fear memory dynamics.

Male adult Sprague Dawley rats were bilaterally infused in the BLA with an Herpes simplex virus type 1 vector, which infects principal neurons, containing FAAH and green fluorescent protein (HSV-FAAH-GFP) or a control vector containing only GFP (HSV-GFP). Rats were, then, tested for biochemical measurements, anxiety or fear memory behaviour.

Seventy-two hours following HSV administration, a time point in which protein transfection is maximal, we found increased FAAH-mediated AEA hydrolysis together with decreased AEA levels within the BLA, confirming that the virus did successfully increase FAAH expression. At this same time point, a separate cohort of rats was tested for anxiety behaviour in an elevated plus maze, a light/dark box and an open field task. Quite surprisingly, we found that the overexpression of FAAH induced consistent anxiolytic effects in all three behavioural tasks we performed, relative to HSV-GFP rats. An additional cohort of animals was tested for fear memory in an auditory fear conditioning paradigm. Animals were bilaterally cannulated in the BLA, a week later were fear conditioned and 24 h after conditioning, the animals were injected with HSV-FAAH-GFP or its control vector. Seventy-two hours following HSV administration rats were re-exposed to the tone alone for 4 consecutive days to examine fear extinction dynamics. We found that rats infused with the HSV-FAAH-GFP vector exhibited a dramatic reduction in fear expression during the extinction training and first extinction retrieval sessions when exposed to the tone, as compared to their HSV-GFP control rats. Furthermore, the effects of FAAH overexpression on fear memory were blocked by intra-BLA injections of the FAAH inhibitor URB597 and by the GABAA antagonist bicuculline, suggesting that these effects might involve BLA GABAergic transmission. These findings suggest that the exact modes of action of AEA within the amygdala in the regulation of emotional states and memory are still far from being clear, thus, opening the avenue to investigate new potential mechanisms by which these processes may occur.

ADOLESCENT ENDOCANNABINNOID SIGNALLING INFLUENCES THE DEVELOPMENT OF SOCIAL BEHAVIOUR IN FEMALE RATS

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Studies using CB1 receptor agonists have reported impairments in social behaviour that are evident several weeks after the final drug exposure, implicating adolescent CB1 receptor signalling in normative development of social behaviours. In support of a role for adolescent endocannabinoid signaling in the development of social behaviours, we previously reported increased social interaction with an unfamiliar peer in female rats exposed to the CB1 receptor antagonist AM251 (1 mg / kg) in adolescence (postnatal days 30-44) when tested 48 h after cessation of treatment, with no effect observed in males (Simone et al., *Neuropharm* 128 (2018) 433-47).

Experiment 1: To ensure our previous finding of increased social interactions in AM251 females were not the result of residual drug in the system, we repeated the experiment with a longer delay (5 days) between final drug exposure and social interaction testing. AM251 females showed more social interaction 5 days after the cessation of treatment compared with Vehicle females, confirming our earlier results; AM251 did not affect social interaction in males. There was no effect of AM251 in either sex on exploratory behaviour in an open field, nor on latency to approach or time spent investigating a novel object. Thus, the effect of AM251 in females is specific to social interaction rather than a general increase in novelty-seeking.

Experiment 2: We previously reported reduced CB1 receptor expression in the dorsal hippocampus of adolescent AM251-treated females (Simone et al., *Neuropharm* 128 (2018) 433-47). Given the involvement of the dorsal hippocampus in social behaviour (and specifically the CA2 subfield), we investigated whether differences in dorsal hippocampal activation underlie the AM251 effects on social behaviour. We thus repeated Experiment 1 in females only and collected brains 1 h after the end of social interaction testing for immunohistochemical labelling for the protein product (EGR-1) of the immediate early gene *zif268* as a marker of neural activation in the CA1, CA2, and CA3 subfields of the dorsal hippocampus. AM251 females spent significantly more time in social interactions than did vehicle treated females (with no overlap in the distribution of AM251 and Vehicle rats), and with no differences in exploratory or novelty-seeking behaviour. No group differences were observed for EGR-1 expression in any of the hippocampal subfields, however, when controlling for Drug group, there was a significant negative correlation between EGR-1 expression in CA2 and time spent in social interactions. Regression analysis using drug treatment and EGR-1 cell counts in the three subfields resulted in a model with an adjusted R^2 of 0.79, and both drug treatment ($\beta = .815$, $p < 0.001$) and EGR-1 in CA2 ($\beta = -0.440$, $p = 0.002$) emerged as unique predictors of individual differences in social interaction.

Conclusion: Our findings provide support for a role of adolescent endocannabinoid signalling in the development of social behaviour and suggest that changes to dorsal hippocampal CA2 may underlie the effects.

Acknowledgements: Research was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant (C.M.M.) and a NSERC Alexander Graham Bell Canada Graduate Scholarship (J.J.S.).

SEX DIFFERENCES IN THE MODULATION OF ENDOCANNABINOID CONTENT BY PERIPHERAL INFLAMMATION

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Utilizing sex as a biological variable (SABV) is necessary for complete understanding of disease outcomes, as well as potential factors underlying disease risk and resiliency. Women show a greater incidence of Crohn's Disease in large population cohort studies and they show a greater occurrence of extra-intestinal manifestations compared to men. Additionally, women tend to have a greater preponderance of anxiety disorders. However, it is unclear if women have a greater comorbidity of anxiety in Crohn's Disease. We are currently investigating the role that endocannabinoids play in mediating comorbid anxiety-like behaviours using a preclinical model of Crohn's Disease. In adult male rats, we have previously shown that AEA levels were decreased and FAAH levels were increased in the amygdala, hippocampus and medial prefrontal cortex, 7 days after induction of colitis. Colitis also produced an increase in anxiety-like behaviour in male rats, which was reversed by an acute central administration of a FAAH inhibitor. Furthermore, we also showed that 2-AG levels were increased at 7 days post-colitis onset. Given sex differences in the endocannabinoid system, and sex differences in Crohn's disease and anxiety disorders, the purpose of this study was to investigate how endocannabinoid content is altered in the brains of female rats with colitis.

Adult, female, Sprague Dawley rats, were exposed to trinitrobenzene sulfonic acid (TNBS, 0.45 mL, 50 mg/mL, 50 % [vol/vol] in ethanol/water), to induce colitis. Control female rats were exposed to the same volume of saline. Female rats exposed to TNBS had a significant increase in macroscopic tissue damage compared to those exposed to saline (Saline: 1.41±0.48 AU, n=12; TNBS 8.43±1.40 AU, n=12; $p<0.0001$, $t(22)=4.73$). These levels of inflammation are similar to that observed in males (7.867 ± 1.12 AU, n=12). AEA and 2-AG levels were measured in the amygdala, hippocampus, hypothalamus and medial prefrontal cortex 7 days after the onset of colitis. AEA levels were decreased in the amygdala (-14 %), hypothalamus (-9 %) and medial prefrontal cortex (-20 %) in colitic female rats. Although similar to the effects observed in males, the magnitude of these changes is less than what was previously seen in males (-30 % in the amygdala and medial prefrontal cortex). Differently from males, where there was an increase in 2-AG in the hippocampus and medial prefrontal cortex, females had no changes in 2-AG levels as a result of colitis.

These results show that females exhibit alterations in central endocannabinoid levels as a result of colitis, albeit not the same degree as males. Future work will determine if FAAH inhibition in females can similarly attenuate both inflammatory processes in colitis as well as the comorbid anxiety.

INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN THE ELECTROPHYSIOLOGICAL ALTERATIONS ASSOCIATED WITH AMYGDALA KINDLING IN RATS

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Comorbid psychiatric disorders are common in patients with epilepsy. These comorbidities include depression, anxiety, psychoses, and cognitive dysfunctions. It has been extensively documented that temporal lobe epilepsy, the most prevalent form of adult epilepsy in humans, is often associated with interictal memory deficits and negative emotional disturbances. However, the underlying mechanism of seizure-induced emotional impairment is still unclear.

We investigated whether amygdala kindling caused chronic interictal behavioural alterations in memory and emotionality. We then correlated the seizure-induced behavioural changes with alterations in excitatory and inhibitory transmissions and in endocannabinoid-mediated plasticity within the amygdala.

Kindling procedure consisted of 20, once daily, electrical stimulations. One week after the last evoked sham or kindled seizure, rats were tested in the elevated plus maze task to assess anxiety and fear conditioning to test fear memory dynamics. In the same rats, patch clamp recordings of pyramidal neurons of the basolateral amygdala were performed to parallel behavioural alterations with changes in glutamate and GABA transmissions and endocannabinoid-mediated short- and long-term synaptic plasticity.

Preliminary data show alterations of the emotional behaviour and fear memory in rats subjected to amygdala kindling relative to the sham group. Moreover, behavioural emotional alterations were associated with dysregulation of GABA synaptic transmission, alteration of endocannabinoid-mediated plasticity at GABAergic synapses and increased glutamate transmission.
These results suggest that repetitive seizures cause long-lasting changes of the physiology and the endocannabinoid-mediated synaptic plasticity in the amygdala, which are paralleled with emotional disturbances.

THE CANNABIS TERPENE MYRCENE EXHIBITS ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES IN A RAT MODEL OF INFLAMMATORY ARTHRITIS

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Inflammatory arthritis (IA) is an auto-immune condition where persistent inflammation causes chronic pain and degrades the joints. Medical cannabis has been employed to alleviate the chronic pain associated with IA but euphoric and adverse side effects have limited its use (Ware et al. Pain. 102 (2003) 211-216). As a result, research has shifted to focus on the utilization of non-euphoric cannabinoids and cannabis terpenes (McPartland & Russo. J Cannabis Therapeutics. 1 (2001) 103-132). Myrcene is the most abundant monoterpene in cannabis, and has been shown to act as an analgesic when given systemically (Rao et al. J Pharm Pharmacol. 42 (1990) 877-878; Paula-Friere et al. Physiotherapy Res. 27 (2013) 1220-1224). Myrcene can also reduce inflammatory mediators in osteoarthritic human chondrocytes (Rufino et al. Eur J Pharmacol. 750 (2015) 141-150). The purpose of this study was to investigate the analgesic and anti-inflammatory properties of myrcene when administered locally in a well-known rat model of IA.

Inflammatory arthritis was induced by injecting 50µl of Freund's complete adjuvant (FCA) into the right knee joint of male Wistar rats (300-417g). Inflammation was assessed seven days post FCA-induction using intravital microscopy. Individual cohorts received a 50µl bolus of vehicle (soy bean oil), or myrcene (0.1-0.5mg/kg) topically over the exposed knee joint. Additional cohorts were treated with either the CB1-receptor antagonist AM281 or the CB2-receptor antagonist AM630 (75µg/50µl) ten minutes prior to receiving myrcene. Rolling leukocytes, white blood cells migrating slower than blood flow, were examined at 5, 15, 30 and 60 minutes in all treatment groups. Pain behaviour was also assessed on day seven in FCA-injected rats using Von Frey hair algesiometry. Cohorts received 50µl of vehicle or myrcene (0.1mg/kg) subcutaneously over the knee joint. Hind paw withdrawal thresholds were examined throughout a three-hour time course.

Local administration of myrcene caused a dose-dependent reduction in leukocyte rolling compared to vehicle ($n=6\text{-}7$; $P<0.0001$). The cannabinoid receptor antagonists AM281 ($n=8$; $P<0.001$) and AM630 ($n=9$; $P<0.05$) prevented the reduction of rolling leukocytes thirty minutes after myrcene application. Myrcene also significantly increased hind paw withdrawal threshold compared to vehicle treated rats ($n=8$; $P<0.01$).

This study found that myrcene reduced leukocyte trafficking and alleviated referred pain in a rat model of inflammatory joint disease. The anti-inflammatory and analgesic properties of mrycene occurred via activation of the endocannabinoid system. These data suggest that cannabis strains containing high levels of myrcene may be beneficial for the treatment of arthritis.

Acknowledgements: This work was funded by the Arthritis Society. MKM is the recipient of a Nova Scotia Graduate Scholarship.

EFFECT OF CANNABIDIVARIN SYMPTOMATIC AND PREVENTATIVE TREATMENTS IN AN ANIMAL MODEL OF AUTISM SPECTRUM DISORDER

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Recent studies highlight the potential beneficial effects of phytocannabinoids in the context of neurological diseases. Interestingly, some phytocannabinoids modulate symptoms and conditions often present in subsets of patients with autism spectrum disorder (ASD), such as cognitive functions, sociability, anxiety and epilepsy [1-4]. Despite this evidence, no studies have specifically investigated the possible beneficial effect of phytocannabinoids in animal models of ASD.

In this study, we investigated the effect of chronic cannabidivarin (CBDV) administration on autism-like behaviors induced by prenatal valproic acid (VPA) exposure in rats.

To this aim, pregnant Sprague-Dawley rats were injected with VPA at the dose of 500 mg/kg i.p. on the 12.5 day of gestation and CBDV was administered in male offspring of VPA-treated dams using two different treatment protocols. A group of rats was treated with CBDV (0.2, 2, 20 or 100 mg/kg/day i.p.) from PND 34 to 58 to assess the ability of CBDV to reverse autism-like behaviors (symptomatic treatment) whereas another group of animals received CBDV (2 or 20 mg/kg/day i.p.) at very early stages of postnatal development (PND 19-29) to test CBDV's ability to prevent the appearance of autism-like traits (preventative treatment). At the end of both treatments, sociability and preference for social novelty were evaluated using the three-chamber test, short-term memory was assessed in the novel object recognition test, locomotor activity and compulsive self-grooming were measured for 20 minutes in the activity cage to evaluate motor impairments and stereotyped behaviors.

Administration of CBDV at the dose of 20 mg/kg, but not 0.2, 2 or 100 mg/kg, in symptomatic male rats was able to recover social impairments, social novelty preference, short-term memory deficits, repetitive behaviors and hyperlocomotion. Preventative CBDV treatment at the dose of 20 but not 2 mg/kg prevented sociability and social novelty deficits, short-term memory impairments and hyperlocomotion, without affecting stereotypies. At the biochemical level, Western blot analysis revealed that 1) prenatal VPA exposure altered the endocannabinoid system and triggered inflammatory processes in behaviorally relevant brain regions, and 2) symptomatic CBDV treatment at the dose of 20 mg/kg was associated with normalization of these alterations.

Overall, data obtained in this study highlight the ability of symptomatic and preventative CBDV treatments to ameliorate some of the autism-like behaviours induced by prenatal VPA in male rats. The association between CBDV treatment and alterations of the endocannabinoid signaling and neuroinflammatory markers justifies further study of the underlying molecular mechanisms of action.

Acknowledgements: Funded by GW Research Ltd (Cambridge, UK)

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THE ENDOCANNABINOID SYSTEM: A PREDICTOR OF CANNABIS USE BEHAVIOR?

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The endocannabinoid system (ECS) is a complex biological system with fatty acid derivatives that signal through cannabinoid receptors and regulates many biological processes. Its functioning can be altered by exogenous cannabinoids such as the intoxicating cannabis-derived cannabinoid THC. In the context of increasing legality of recreational cannabis in the U.S. as well as greater user exposure to higher THC potent products, it is imperative that researchers observe the interdependent relationship between the ECS and cannabis use behavior. Fatty acid amide hydrolase (FAAH), an enzyme that rapidly degrades endocannabinoid and CB1 agonist anandamide (AEA), is of primary clinical interest. A single nucleotide polymorphism (SNP) in the *FAAH* gene (C385A) results in reduced enzymatic activity of FAAH, and in turn, varying levels of AEA activity. It has also been associated with decreased risk in developing Cannabis Use Disorder (CUD) compared to individuals with the wild-type C/C genotype. To observe the relationship between use behavior and ECS signaling, we recruited current cannabis users in Boulder, Colorado (age mean=30.22, SD=9.4; 22 M and 19 F) and conducted an experimental session in a mobile pharmacology lab, which included three timepoints: pre-, post-, and 1-hour post- *ad libitum* self-administration of a high THC potent product ranging between 16 to 24%. Blood was collected at all three timepoints and used to quantify AEA and THC from plasma, as well as extract DNA to assay *FAAH* (C385A). Two A/A individuals were combined with the heterozygote A/C group for a combined A allele group (n=12) and was compared to the wild-type C allele group (n=26).

As we expected, *FAAH* genotype group significantly predicted AEA levels at each experimental timepoint: pre- (p=.027), post- (p=.01), and 1 hour post self-administration of cannabis (p=.049); with the C allele group having lower AEA on average than the A allele group. This relationship was more significant after controlling for THC levels at each corresponding timepoint: pre- (p=.006), post- (p=.017), and 1 hour post- (p=.001). Additionally, AEA levels before smoking predicted THC levels at each timepoint: pre- (p=.027), post (p=.000), and 1-hour post-use (p=.014) as well as predicted the change in THC levels from pre- to post-use (p=.052) and from post- to 1-hour post-use (p=.05). On average, those in the C allele group had a greater increase in THC levels from pre- to post-use and a greater decrease in THC from post- to 1-hour post-use compared to the A allele group. These data elucidate the ECS role in cannabinoid metabolism, such that individuals in the A allele group show greater AEA levels at each experimental timepoint and in turn, lower levels of THC post-use, and showed less of a decline in THC levels at the 1 hour post-administration timepoint. It also shows that ECS could be a predictor of cannabis use behavior and an indicator THC is metabolism. The rate of cannabinoid metabolism, and thus their pharmacological influence, has implications for cannabis dependence and withdrawal, which is a growing concern in states with legal recreational cannabis markets.

Acknowledgements: Funded by NIDA (Hutchison) and the state of Colorado (Bidwell).

MODELING THE IONOTROPIC CANNABINOID RECEPTOR 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^{o}C, acidic conditions, and cannabinoids. When exposed to an agonist, TRPV1 activation occurs and transmits a painful, burning sensation which is then followed by desensitization. When desensitization occurs, TRPV1 no longer sends the painful signal resulting in a paradoxical analgesic effect. This effect can be exploited for use in pain management.

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 TRPV1 to the effect of capsaicin, though at a lower potency than anandamide.[2]

We have constructed a 3D model of TRPV1 based upon the CryoEM TRPV1 structure reported by Y. Gao, et al.[3] This model has been incorporated in a fully hydrated lipid bilayer and the full system has been equilibrated. The binding interactions of endocannabinoids with this model will be presented. [Support: NIDA DA003934]

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

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COUPLING OF THE HUMAN RECOMBINANT CB_2 CANNABINOID RECEPTOR TO AKT/PROTEIN KINASE B ACTIVATION/PHOSPHORYLATION IN CHINESE HAMSTER OVARY CELLS

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Background: Cannabinoids affect the proliferation and differentiation of neural precursor cells in rats and mice. In oligodendrocytes, cannabinoid receptors influence stem cell survival and differentiation via the phosphatidylinositol 3-kinase:protein kinase B (PI3K-Akt, http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=285) signalling pathway(Gomez, Sanchez-Rodriguez et al. 2011). Recruitment to the plasma membrane and binding to phosphatidylinositol 3,4,5-trisphosphate allows phosphorylation and activation of Akt. The main objective of this investigation was to quantify the coupling of CB_2 receptors to activation of the Akt signalling pathway.

Methods: Chinese hamster ovary cells were stably transfected with human recombinant CB_2 cannabinoid receptors. Activation of the Akt signalling pathway was assessed using a phospho-pan-Akt antibody and in cell immunocytochemistry in 96-well microtitre plates quantified with a Licor Odyssey using fetal bovine serum as a positive control.

Results: Multiple CB_2 cannabinoid receptor agonists were investigated including the endogenous ligands; 2-AG and anandamide, as well as the synthetic ligands; CP55940, fenofibrate, HU210 ,WIN55,212-2, JWH015 and JWH133. A rank order of potency was CP55490 (pEC_{50} 7.9 ± 0.04) ≥ WIN55,212-2 (7.6 ± 0.1), HU210 (7.4 ± 0.1) > fenofibrate (7.1 ± 0.1), JWH015 (7.0 ± 0.1) > 2-AG (6.7 ± 0.2), JWH133 (6.3 ± 0.1) > anandamide (5.8 ± 0.1). The maximal effect induced by the different agonists was indicated as the E_{max} relative to the FBS response with 2-AG producing a maximum response at 149 ± 4 %, HU210 (142 ± 10), CP55940 (139 ± 12), fenofibrate (139 ± 11), WIN55,212-2 (116 ± 10), JWH015 (100 ± 10), JWH133 (98 ± 7), anandamide (71 ± 13). The phosphorylation/activation of the Akt signalling pathway was significantly attenuated by pre-treatment with the CB_2 selective antagonist AM630 (1 μM) or pertussis toxin (100 ng/mL).

Conclusion

This is the first study to quantify coupling of the CB_2 receptor to Akt phosphorylation using cell immunocytochemistry. In common with other signalling pathways, 2-AG appears to act as a full agonist, while anandamide is a partial agonist, in the phosphorylation of Akt.

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ANTINOCICEPTIVE EFFECTS OF FAAH INHIBITORS IN A RAT MODEL OF POST-OPERATIVE PAIN FOLLOWING INGUINAL HERNIA REPAIR SURGERY

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Approximately 20 million inguinal hernia repairs are performed worldwide annually (Kingsworth et al., Lancet 362 (2003) 1561–1567) and approximately 40% of patients experience moderate to severe acute post-operative pain following this type of surgery (McGrath et al., Canadian J. Anesthesia, 51(9) (2004) 886-891). Endocannabinoids have analgesic effects at peripheral, spinal and supraspinal levels (Starowicz and Finn, Adv Pharmacol 80 (2017) 437-475). The aim of the present study was to investigate the effects of pre-surgical administration of URB937, a peripherally restricted FAAH inhibitor, and URB597, a centrally active FAAH inhibitor, on post-operative pain-related behaviour in an anatomically relevant rat model of the Lichtenstein inguinal hernia repair procedure (Bree et al., J. Pain 16(5) (2015) 421-435). We hypothesised that FAAH inhibition would attenuate the post-operative pain-related phenotype.

Forty-two adult male Lister-Hooded rats were randomly assigned to one of six treatment groups (n=7 per group) and baseline measurements of home cage locomotor activity, open field activity and hindpaw and inguinal area mechanical hypersensitivity (von Frey testing) were made 24hrs pre-surgery. Rats received a subcutaneous injection of either vehicle, URB937 (1 mg/kg) or URB597 (1 mg/kg) immediately prior to undergoing either sham surgery or the inguinal hernia repair procedure under isoflurane anaesthesia. Immediately following surgery, animals were returned to their home cage where locomotor activity was monitored for 4 hours post-surgery. Animals were then placed in the open field arena for 5 mins, after which they underwent von Frey testing. Animals were euthanised and trunk blood, spinal cord, brain and inguinal tissue samples were collected. Data were analysed by repeated measures ANOVA followed by Tukey post-hoc tests. Non-parametric data were analysed by Kruskal Wallis followed by Mann Whitney tests.

Inguinal hernia repair surgery induced a reduction in home cage vertical locomotor activity, and induced mechanical allodynia in the ipsilateral inguinal area and ipsilateral hindpaw, compared with sham controls. There was no effect of either FAAH inhibitor on surgery-induced changes in locomotor activity, compared with vehicle-treated controls. Mechanical allodynia in the inguinal area was significantly attenuated by both URB937 and URB597 while only URB597 attenuated mechanical allodynia of the ipsilateral hindpaw.

These results suggest that pre-surgical administration of either peripherally restricted or centrally acting FAAH inhibitors can attenuate post-surgical pain-related behaviour in a rat model of postoperative pain following inguinal hernia repair. The results also indicate development of mechanical allodynia in this model at a site distal to the surgery (ipsilateral hind paw), suggestive of a 'pain spreading' phenomenon, and that this phenomenon may be regulated by central FAAH substrates while allodynia at the surgery site may be regulated by both central and peripheral FAAH substrates.

Acknowledgements: Funded by a scholarship from the College of Medicine, Nursing and Health Science, NUI Galway and Science Foundation Ireland (SFI) with co-funding under the European Regional Development Fund under Grant Number 13/RC/2073.

ROLE OF BIOTRANSFORMATION IN THE ADVERSE EFFECTS OF SYNTHETIC CANNABINOIDS AB-PINACA AND JWH-018

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Synthetic cannabinoids (SCBs) induce numerous adverse effects, but significant individual variability exists among human users. Hydroxylation of SCBs by cytochrome P450s (CYPs) and subsequent conjugation by UDP-glucuronosyltransferases (UGTs) produces numerous metabolites. We hypothesize that some of these metabolites might act "in concert" with their parent drugs to produce the distinct pharmacologic effects and toxicity of SCBs in humans. In this study, we investigated the actions of Phase I and Phase II metabolites of SCBs both in vitro and in vivo.

Pooled human liver microsomes (HLMs) were used to determine the role of glucuronidation of active Phase I metabolites of the abused SCBs, JWH-018 and AB-PINACA, in the presence and absence of UDPGA. Hepatic clearance of 4-OH-AB-PINACA and JWH-018 M2 were compared and quantified by UPLC analysis. In observational studies, mice received a 2-hr pretreatment of either vehicle or the universal CYP inhibitor, 1-ABT, followed by convulsant doses of either JWH-018, 5F-AB-PINACA, or pentylenetetrazol (the chemical convulsant positive control). Convulsions were scored using an observational rating scale. In a separate study, the effects of 1-ABT on hypothermia induced by JWH-018 or 5F-AB-PINACA were determined in mice using radiotelemetry.

Results show extensive glucoronidation of JWH-018 M2, reduced glucoronidation of 4-OH-AB-PINACA, and almost no glucoronidation of 5-OH-AB-PINACA in HLMs. 1-ABT pretreatment protected against convulsant effects of JWH-018, but not against those of PTZ or 5F-AB-PINACA. Interestingly, the intensity and duration of hypothermia elicited by JWH-018 was increased following inhibition of Phase I metabolism by 1-ABT. In conclusion, active Phase I metabolites of AB-PINACA mostly bypass Phase II glucoronidation, but active JWH-018 metabolites do not. Furthermore, our data support the involvement of active Phase 1 metabolites in the convulsant effects of JWH-018, but not those of 5F-AB-PINACA. This suggests that Phase I metabolites potentially linger in the body and interact with the parent SCB drug to contribute to the reported adverse effects associated with SCB use.

Acknowledgements: These studies were funded in part by DA03914 and DA03914-02S1.

IN SILICO DISCOVERY OF NEW PROTEIN TARGETS FOR CANNABINOIDS

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Cannabinoids are promising leads for treating central and peripheral nervous system diseases because of their action at multiple protein targets in the brain and body. Numerous chemicals from cannabis, and also their synthetic derivatives, are active in the treatment of epilepsy, anxiety, pain, cancer, inflammation and more. While they do have therapeutic potential on their own, an additional benefit of these actions is that by treating disease, cannabinoids help to identify the protein targets responsible for causing it. This leads to the potential that we can map protein targets, and perhaps even individual binding sites, to their associated pathologies. Knowledge of this map then allows for drug screening and targeted drug design to discover new compounds that more effectively treat disease. How do we exploit the therapeutic potential of cannabinoids to achieve this?

Similarity Ensemble Algorithm (SEA) is a technique to classify protein targets not by their phylogeny or biological function, but by the chemistry of their ligands[1]. The basic premise is that two related ligands, by being chemically similar, are more likely to bind the same target than two unrelated ligands. High similarity between the ligands of two targets indicates the ligands may bind both targets, reducing the search space for drug screening.

We have performed SEA on cannabinoid drugs. By making hundreds of millions of pair-wise comparisons, we have related the chemical similarity of cannabinoids to groups of ligands that bind hundreds of protein targets. The similarity scores resulting from SEA indicate which protein targets the cannabinoids are likely to bind. These results allow us to rationalise the existing therapeutic activity of cannabinoid drugs by suggesting putative interactions with protein targets. This information can be used to characterise new binding sites and guides drug screening and design.

1. Keiser, M. J. *et al.* Relating protein pharmacology by ligand chemistry. *Nature biotechnology* **25**, 197-206 (2007).

MODULATION OF ANXIETY BY THE ENDOCANNABINOID ANANDAMIDE SIGNALING IN THE PREFRONTAL CORTEX IS DEPENDENT ON THE EMOTIONAL AROUSAL STATE

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Many people who suffer from anxiety disorders self-medicate with cannabis, which can reduce anxiety. However, cannabis usage has also been shown to have the opposite effect and some people have reported feeling increased anxiety and depressive mood when using cannabis. Preclinical studies have indicated that cannabinoid drugs can cause these distinct opposite effects on anxiety behaviour depending on both the animal stress levels and the aversiveness of the environmental conditions. Furthermore, animal studies have shown the critical involvement of the prefrontal cortex (PFC) endocannabinoid system in the regulation of stress and anxiety behaviours. The goal of this study was to evaluate whether the endocannabinoid anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in the PFC, differentially regulate anxiety behaviour depending on the level of environment-associated emotional arousal.

Male sprague-dawley rats were divided in two groups and tested for anxiety in the Elevated Plus Maze (EPM). To increase the level of environment-associated emotional arousal, one group was not handled nor habituated to the experimental room and tested under high light condition (High-Arousal group; HA); the second group was extensively handled and habituated to the experimental room prior to the EPM and tested under red light condition (Low-Arousal group; LA). We measured AEA and 2-AG levels immediately after the EPM in the PFC and evaluated the effects of intra-PFC administration of the AEA hydrolysis inhibitor URB597 (10 ng/side) or the 2-AG hydrolysis inhibitor KML29 (0.2 ug/side) on anxiety behavior in HA and LA rats. As expected, the HA group exhibited significant higher anxiety as compared to the LA group. In addition, HA rats showed decreased anandamide levels in the PFC as compared to their home cage control group. Moreover, URB597 increased the anxiety response shown by LA rats without affecting emotional behavior in the HA group. KML29 injections did not alter anxiety response in the LA or the HA group.

Taken together, these findings show how the endocannabinoid system is differentially activated to regulate the anxiety response, depending on the level of the environment-associated emotional arousal and help to shed light on the neurobiological mechanisms involved in the differential impact of stress on emotionality.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL ROLE-PLAYERS IN 2-AG BIOSYNTHESIS

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The endocannabinoid 2-AG is an important signaling lipid that interacts with the cannabinoid receptors CB1 and CB2. Diacylglycerol lipase α and β (DAGLα, DAGLβ), are thus far the only well-described 2-AG synthesizing enzymes in an endogenous system. DAGLα and DAGLβ knockout populations were created using CRISPR/Cas9 technology in Neuro2A cells (murine neuroblastoma), to investigate their respective contribution to 2-AG synthesis in this cell line. Knockout of either or both of the diacylglycerol lipases yielded no significant changes in 2-AG levels as measured by lipidomics. However, 2-AG levels were completely abolished upon treatment with DAGL inhibitor DH376. These data suggest the presence of alternative 2-AG synthesis pathways in Neuro2A, which can be targeted by DH376. We are currently working on the target identification and characterization using an activity-based chemical proteomics approach.

THE EFFECT OF KYNURENIC ACID ON CANNABINOID RECEPTOR TYPE 1 FUNCTION IN THE BRAIN

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There are increasing number of reports demonstrating the interactions between the endocannabinoid and kynurenine system, mostly in the CNS. Kynurenic acid (KYNA) is one of the most significant metabolite of the kynurenine pathway both in terms of functional and therapeutic value. It is an *N*-methyl-D-aspartate (NMDA) receptor antagonist, but it can also behave as an agonist on the G-protein coupled receptor 35 (GPR35), which shares several structural and functional properties with cannabinoid receptors. Additionally, previously our group demonstrated that systemic chronic KYNA treatment altered opioid receptor G-protein activity depending on the opioid receptor type and brain region (Zádor et al., CNS Neurol. Disord. Drug Targets 13 (2014) 1520-1529). Taking into account these findings, our aim was to examine the direct *in vitro* and systemic, chronic *in vivo* effect of KYNA on cannabinoid receptor type 1 (CB_1) binding and G-protein activity. In our study competition and saturation radioligand binding and [^{35}S]GTPγS G-protein binding assays were applied using rat brain membrane homogenates.

According to our results, KYNA alone *in vitro* did not show significant binding towards the CB_1 receptor and did not induce G-protein stimulation, nor did it alter CB_1 ligand binding and agonist activity. However, when rats were chronically treated with KYNA (single daily, i.p., 128 mg/kg for 9 days), the level of constitutively active G-proteins significantly increased compared to control (47.7 ± 6.3 to 88.9 ± 10.8 fmol/mg, $P < 0.005$). Furthermore, when the CB_1 receptors were stimulated by specific ligands the level of active G-proteins was also significantly higher in KYNA treated brain samples independently from the applied ligand concentrations. Interestingly, the overall maximum efficacy of the G-protein and the potency of the CB_1 ligands to stimulate the CB_1 receptor coupled G-protein did not differ significantly between control and KYNA treated brain samples. Finally, the maximum binding capacity of the CB_1 receptors in rat brain also significantly increased (1365 ± 49.51 to 1996 ± 65 fmol/mg, $P < 0.005$) after long-term KYNA administration. Thus, KYNA indirectly affects the availability of active CB_1 receptors in rat brain, without modifying the overall activity of the receptor. Supposedly this can be a compensatory mechanism on the part of the endocannabinoid system triggered by the long-term KYNA exposure. In our further studies we will map the brain region specificity and explore the possible role of the NMDA receptor regarding the CB_1 receptor related effects of KYNA.

Acknowledgements: Funded by the Development and Innovation Operational Programme (Grant no. GINOP-2.3.2-15-2016-00034).

CANNABINOID CB2 RECEPTORS MODULATE GLUTAMATE RELEASE IN THE NUCLEUS ACCUMBENS IN MICE

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It is well known that cannabinoids modulate glutamate release by activation of presynaptic CB1 receptors. However, little is known as to whether CB2 receptors (CB2Rs) also modulate glutamate release in the brain. Recent studies indicate that CB2Rs are expressed in midbrain dopamine (DA) neurons and hippocampal glutamate neurons, and functionally modulate neuronal activities in both the midbrain and hippocampus. In the present study, we investigated whether systemic or intracranial local administration of CB2R ligands modulates glutamate release in the nucleus accumbens (NAc) using *in vivo* brain microdialysis with HPLC assays.

We found that systemic administration of JWH133, a selective CB2R agonist, dose-dependently increased, while intra-NAc local administration of JWH133 decreased extracellular glutamate in wild-type (WT) and CB1R knockout ($CB_1^{-/-}$) mice, but not in $CB_2^{-/-}$ mice. Pretreatment with AM630, a selective CB2R antagonist, blocked JWH133-induced increase or decrease in NAc glutamate, which provides additional evidence supporting CB2R-mediated effects. In contrast to JWH133, systemic or intra-NAc local administration of AM630 alone failed to alter extracellular NAc glutamate level, suggesting lack of tonic endocannabinoid modulation of basal glutamate release. Furthermore, JWH133-induced increase in glutamate release was action potential-dependent (i.e., tetrodotoxin-sensitive) and was blocked by systemic administration of sulpiride, a D2-like DA receptor antagonist, suggesting that JWH133-enhanced glutamate release in the NAc is derived from glutamatergic projections from brain regions outside the NAc. *In situ* hybridization assays detected CB2 mRNA expression in midbrain DA neurons and cortical/hippocampal glutamate neurons. We propose that activation of CB2Rs in midbrain DA neurons may inhibit DA release in DA projection areas such as NAc and PFC. The decrease in DA release in the PFC may subsequently disinhibit cortical glutamate neurons – causing an increase in glutamatergic input into the NAc. Similarly, activation of CB2Rs in glutamate neurons in the PFC or glutamatergic terminals in the NAc may inhibit glutamate release. Thus, JWH133-enhanced glutamate release after systemic administration could be a final net outcome of two opposite actions – direct inhibition and indirect disinhibition of glutamate release in the NAc. (Supported by NIDA IRP)

EFFECTS OF MARIJUANA USE HISTORY AND URINE THC ON BRAIN FUNCTIONAL MRI CONNECTIVITY

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Although there is an extensive and growing literature on the molecular physiology of cannabinoids on brain receptors, there remains sparse information about the aggregate effects of cannabinoids on functional brain networks. Predicting high-level network effects on attention, language, sensory perception, and executive function is not intuitive given cannabinoid and opioid receptor pharmacology. Such high-level network physiology can be measured using functional MRI connectivity, or synchrony between brain regions of fMRI signal. We studied 1206 subjects from the Human Connectome Project dataset for whom urine THC assays and Semi Structured Assessment for the Genetics of Alcoholism (SSAGA) were obtained, including 1003 subjects (534 female, 469 male) with 1 hour of high-resolution functional MRI FIX ICA processed data.

Pairwise functional connectivity measurements were obtained for each subject between 361 ROIs including 333 cortical regions, 14 subcortical subject-specific Freesurfer-segmented regions, and 14 cerebellar regions. Functional connectivity for each region pair included subject-level regressors for age, sex, mean head motion, age at first marijuana use, number of times marijuana has been used, marijuana abuse or dependency, and detectable urine THC.

11% of 1206 participants had detectable urine THC, and 54% reported ever trying marijuana. Detectable THC was significantly correlated with lower age of first use and higher number of times used, but uncorrelated with marijuana abuse or dependency in the dataset. Functional connectivity values showed significant, widespread increases in functional connectivity for participants with detectable THC predominantly in connections between the default network and sensory networks (visual, somatomotor, auditory). Decreased connectivity between the striatum (putamen and globus pallidus) and sensory networks was observed in association with abuse or dependency score for marijuana.

These findings suggest that detectable urine THC was associated with overconnectivity of the default network with sensory cortex, possibly indicating greater internal attention to sensory stimuli. These effects were independent of frequency of use (number of times used), suggesting that this is more likely to represent a direct action of THC rather than a population selection bias. In contrast, sensory stimuli were underconnected with the striatum in subjects reporting abuse or dependency of marijuana, possibly indicating decreased responsiveness of the striatum to sensory stimuli in these individuals.

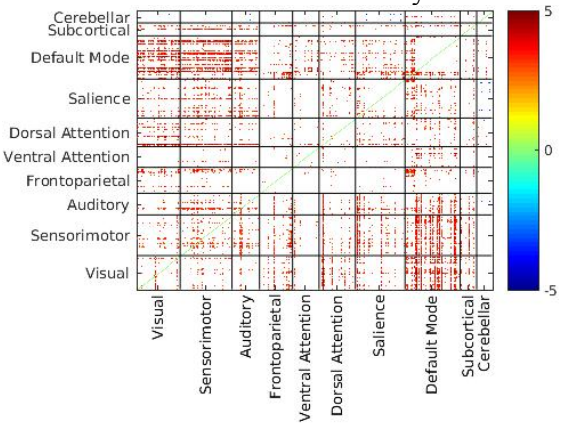

Figure 1: Functional MRI connectivity associated with detectable urine THC in 1003 subjects. Color scale represents t-statistic. All colored squares represent significant correlations between 361 gray matter region pairs grouped by network (Results are FDR corrected)

COMBATTING RAT OSTEOARTHRITIS PAIN AND INFLAMMATION WITH DUAL INHIBITION OF MONOACYLGLYCEROL LIPASE AND CYCLOOXYGENASE-2

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Chronic pain is the major reason osteoarthritis (OA) patients visit their physician. OA pain is linked to intermittent inflammatory flares which cause sensitisation of joint sensory nerves. First line therapies such as cyclooxygenase (COX) inhibitors provide limited symptom relief and their side effect profile preclude long-term use. Thus, there is a need for novel, safe, and improved therapeutic options for OA patients. The endocannabinoid system can be harnessed by inhibiting the rapid breakdown of ligands *in vivo*. Monoacylglycerol lipase (MAGL) is responsible for the rapid metabolism of 2-arachidonoylglycerol (2-AG) which limits the therapeutic potential of this endocannabinoid. Moreover, 2-AG can also act as a substrate for COX-2 leading to the formation of pro-inflammatory prostaglandins. The present study aimed to investigate if combining MAGL inhibition with a selective COX-2 inhibitor could be more efficacious at reducing pain and inflammation in a model of OA compared to either treatment alone.

Experimental OA was induced in male Wistar rats (298-441g) by intra-articular injection of sodium monoiodoacetate (MIA; 3mg) into the right knee joint. Pain behaviour was assessed using von Frey hair algesiometry and dynamic incapacitance. Inflammation was assessed by measuring leukocyte trafficking in the synovial microvasculature. One day following MIA injection, animals were treated with either vehicle (50µl; DMSO:cremophor:saline;1:1:18), KML29 (700µg/50µl s.c.), celecoxib (CXB) (3mg/kg i.p.), or a combination of both inhibitors (KML29/CXB). Pain assessments were carried out over 240-minutes and inflammation was measured at 360-minutes post-drug administration.

On day 1 post-MIA induction, combined inhibition of MAGL and COX-2 significantly improved hindpaw withdrawal threshold and decreased adherent leukocytes when compared to either treatment alone ($p<0.0001$; $n=8$). The combination therapy significantly improved hindlimb weight bearing when compared to KML29 alone ($p<0.0001$; $n=8$) but was not significantly different from the CXB treatment alone ($p>0.05$; $n=8$). Additionally, the combined inhibition significantly decreased rolling leukocytes when compared to CXB alone ($p<0.0001$; $n=8$) but was not different from KML29 alone ($p>0.05$; $n=8$).

This study demonstrates that the combination of KML29 and CXB is more effective at reducing pain and inflammation than the individual drugs. Thus, endocannabinoids offer a means of reducing COX-2 inhibitor dosage, therefore providing a safer side effect profile for long-term treatment of OA patients.

Acknowledgements: Funding provided by The Arthritis Society of Canada (Grant. no. 35901).

TRAUMATIC BRAIN INJURY AND STRESS ALTER BRAIN ENDOCANNABINOID SYSTEM PROTEIN EXPRESSION

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Traumatic brain injury (TBI) and post-traumatic stress disorder (PTSD) are major health concerns. Nearly 2.5 million Americans sustain a TBI each year, most of which are mild TBIs (mTBI). Individuals who experience life-threatening trauma are also at risk for developing post-traumatic stress disorder (PTSD). Both TBI and traumatic stress negatively impact amygdala function and alter brain endocannabinoid (eCB) signaling, and amygdala dysfunction and brain eCB signaling are each attributed a role in addictive behavior. Our lab is specifically interested in exploring a potential role for trauma-induced changes in amygdala eCB function in post-trauma addictive behaviors.

In Experiment 1, we tested the effect of mTBI on operant alcohol self-administration and endocannabinoid system protein expression in the central (CeA) and basolateral amygdala (BLA) of male and female rats. Adult Wistar rats were trained to self-administer alcohol using an operant task, then underwent craniotomy. Three days later, mild-to-moderate TBI (25ms, 30-35 PSI) or sham injury was delivered via lateral fluid percussion over the sensorimotor cortex. Operant alcohol self-administration was measured every two days post-injury, then animals were sacrificed 11 days post-TBI. Following sacrifice, brains were excised, CeA and BLA were bilaterally dissected, and micropunches were used for Western blot analysis of eCB protein expression (eCB synthetic and degradative enzymes and CB1 receptors). Post-TBI physiologic measures (i.e., apnea, respiratory rate, righting reflex) suggest female drinkers may be more severely impacted than male drinkers exposed to equal intensity mTBI. Preliminary data also suggest that mTBI alters eCB protein expression in basolateral amygdala (BLA) and central amygdala (CeA) of alcohol drinking rats, which may represent a mechanism by which mTBI increases alcohol drinking.

In Experiment 2, we used a predator odor (bobcat urine) model of traumatic stress in male Wistar rats to test the effects of stress on BLA eCB system protein expression over time. Some rats were trained to self-administer alcohol (10% w/v) and water in an operant setting. Rats were then stressed (or not stressed) and divided into groups based on their avoidance of the odor-paired context: Avoider, Non-Avoider, and unstressed Control groups. Rats were sacrificed 2, 16 or 21 days post-stress, brains were excised, BLA was bilaterally dissected, and micropunches were used for Western blot analysis of eCB protein expression, as described in Experiment 1. Alcohol-naïve rats exposed to predator odor exhibited a trend toward higher DAGLα expression in BLA 16 days after stress, and a trend toward lower MAGL expression 2 and 16 days after stress. Rats with a history of alcohol drinking exposed to predator odor exhibited a trend toward higher DAGLα expression 21 days after stress. These results suggest that traumatic stress may induce long-term alterations in BLA eCB signaling.

Acknowledgments: Funding was provided by NIH grants AA023305 (NWG), AA026531 (NWG), MH104373 (JGT), AA007577 (PEM), and VA grant BX003451 (NWG).

EFFECTS OF EARLY CANNABIDIOL TREATMENT IN THE Δ^9-THC ANIMAL MODEL OF SCHIZOPHRENIA

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Epidemiological and clinical studies suggest that a neurodevelopmental dysfunction could be one of the main exploratory hypotheses of schizophrenia (SCZ), which symptoms lead to severe personal and social dysfunctions. A variety of animal and human studies found a dysregulation of the endocannabinoid system (both in term of cannabinoid receptors CB1 or CB2 and endocannabinoid ligands anandamide or 2-arachidonoylglycerol) in psychosis; thus, the pharmacological exploitation of the endocannabinoid system could be a novel approach for treating SCZ. In the present study, we aimed to investigate 1) the potential effects of prenatal/perinatal administration of the delta-9-tetrahydrocannabinol (Δ^9-THC), the main psychotropic compound of *Cannabis sativa* on neurophenotypic presentations using a set of behavioral test battery, and 2) if the pharmacological modulation of the endocannabinoid signaling could reverse the schizophrenia-like phenotype. At adulthood, Δ^9-THC -exposed rats engaged in less social interaction as well as they shown cognitive impairment, which were reversed by the chronic treatment both with the non-psychotropic phytocannabinoid cannabidiol. These results suggest that pharmacological modulation of the endocannabinoid tone could be a novel potential therapeutic target for the treatment of schizo-affective disorders.

Acknowledgments
This work was supported by the project financed from the SoMoPro II programme. The research leading to these results has acquired a financial grant from the People Programme (Marie Curie action) of the Seventh Framework Programme of EU according to the REA Grant Agreement No. 291782 (to V.M.).

FAAH (C385A) MUTANT MICE HAVE A REDUCED MORPHINE REWARDING EFFECT

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Opioid dependence crisis represents a main concern in the United States, and although there are pharmacological treatments available, these treatments are confounded by abuse potential. Additionally, treatments are not effective in all individuals. Individual genetic variation/polymorphism may account for differential response to drugs of abuse, resulting in either vulnerability or resilience. The interactions between the opioid and the endocannabinoid system, at the level of neurochemical, neuroanatomical and molecular pathways, place the endocannabinoid system as a possible target for the development of new pharmacological treatments. Endocannabinoid signaling is regulated in part by fatty acid amid hydrolase (FAAH), the primary catabolic enzyme for the endocannabinoid anandamide (AEA). A common human mutation in the gene for FAAH (C385A; rs324420) results in decreased expression and activity of FAAH and thus increased levels of AEA. FAAH inhibitors have shown to reduce certain aspects of morphine withdrawal effects, however there is no data showing the role of FAAH inhibitors, or FAAH mutants on the effects of morphine reward in pre-clinical models. Thus, we first studied the effects of morphine in our FAAH (C385A) mutant mice. Using the morphine conditioned place preference (CPP) model, we found that adult male knock-in mice expressing the FAAH C385A SNP exhibit significantly lower preference for morphine compared to wildtype mice. Similarly FAAH mutant mice exhibit an attenuated locomotor sensitized response to morphine compared to wildtype mice. Interestingly, examination of morphine's effects on analgesia, and tolerance was no different in FAAH mutant mice compared to wildtype, suggesting an interaction of the cannabinoid system and the opioid system impacting rewarding properties of morphine as a consequence of FAAH enzyme activity, without affecting aspects of morphine observed during pain management. In order to mimic the FAAH enzyme activity obtained in our FAAH mutant mice, we tested the acute effects of PF-3845, a FAAH inhibitor on morphine preference. Preliminary data showed that the rewarding effects of morphine is not affected in presence of this inhibitor. In summary, our findings suggest that altered endocannabinoid signaling as a consequence of the FAAH C385A SNP that results in lower levels of FAAH; results in a decrease in the rewarding effects of morphine. However this may be a result of altered connectivity as acutely inhibiting FAAH and raising AEA levels does not recapitulate this observed decrease in morphine reward in the FAAH SNP mice.

Acknowledgements: Funded by the Pritzker Foundation and the NIH National Heart, Lung, and Blood Institute (Martínez-Rivera, A; T32HL135465).

Δ^9-TETRAHYDROCANNABINOL REVERSAL OF PAIN-INDUCED PLACE AVERSION IN RATS

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Human pain includes sensory, affective, and cognitive components. Opioid analgesics such as morphine are known to decrease both sensory and affective/cognitive aspects of human pain, but the latter is attenuated by much lower doses of morphine than those required to dampen sensory pain. It has been suggested that in rodents, pain-induced conditioned place aversion may model the affective/cognitive aspect of pain, in part because doses of analgesics required to reverse pain-induced place aversion are much lower than those required to attenuate withdrawal responses to noxious stimuli. The aim of this study was to determine whether THC would block pain-induced conditioned place aversion at low doses that do not decrease classical pain-related rodent behavior (e.g., hindpaw licking). On Day 1, adult Sprague-Dawley rats were placed into a place conditioning apparatus, and time spent in each side of the apparatus (which differed by wall color and floor type) in 15 min was recorded. On Day 2, rats were given vehicle or THC (0.01-1.0 mg/kg) i.p., and 30 min later, a 2% formalin injection into the right plantar hindpaw (i.pl.), and then restricted to one side of the place conditioning apparatus for 45 min. On Day 3, rats received injections opposite to what they received on Day 2 (e.g., if THC i.p. + formalin i.pl. was given on Day 2, then vehicle i.p. + saline i.pl. to left hindpaw was given on Day 3), and rats were restricted to the opposite side of the chamber for 45 min. Pain-related behavior (number of hindpaw licks) and rearing were recorded during the Day 2 and 3 conditioning trials. On Day 4, rats were placed into the apparatus for 15 min, and time spent on each side was recorded.

When data were considered without regard to the side of the apparatus that had been paired with formalin, vehicle-treated rats showed a slight aversion to the formalin-paired side, but THC did not significantly reduce this aversion. However, when data were examined by the side of the apparatus which had been paired with formalin, place aversion was only significant in rats that had received formalin on the "white wall/bar floor" side, and THC dose-dependently attenuated this place aversion, whereas THC had no consistent effect on place conditioning in rats that had experienced formalin paired with the "black wall/grate floor" side. Thus, THC's attenuation of pain-induced place aversion was context-dependent, suggesting that THC altered the affective/cognitive experience of pain. There were no differences in the number of hindpaw licks (classical pain-related behavior) or rears (exploratory behavior) between rats that had experienced formalin in each side of the apparatus; thus, the conditioning-side effect is probably not due to rats experiencing greater pain intensity on the "white wall/bar floor" side, or exploring that side more than the other. These results suggest that THC may reduce the affective/cognitive aspect of pain at doses that are lower than those that attenuate classical pain-related rodent behaviors (typically, $\geq$ 1.0 mg/kg). We will examine factors that may contribute to the apparatus side-dependent effect of formalin-induced place aversion, and whether the THC doses tested in this study produce a place preference or aversion in rats that are not in pain.

Acknowledgements: Funded by the National Institute on Drug Abuse (DA016644).

CHARACTERIZATION OF ENDOCANNABINOID METABOLIZING ENZYMES IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS UNDER INFLAMMATORY CONDITIONS

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Our laboratory previously examined mechanisms by which inflammation is resolved in response to the toll-like receptor ligand, lipopolysaccharide (LPS), in mice. LPS induced pro-inflammatory mediators such as IL-6 and simultaneously inhibited endocannabinoid metabolizing enzymes. This pathway has the potential to increase endocannabinoid levels and serve as a possible negative feedback system to reduce inflammation. Huntington's Disease (HD) is a human genetic disease causing neurodegeneration and chronic systemic and central nervous system inflammation. Previous studies have reported increased IL-6 levels in plasma of individuals with HD, and decreased activity of an endocannabinoid metabolizing enzyme, fatty acid amide hydrolase (FAAH), in lymphocytes. We therefore investigated the effects of systemic inflammation on peripheral blood mononuclear cells (PBMCs) from healthy controls and individuals with HD. We hypothesized that high IL-6 levels observed in HD patients would inhibit monoacylglycerol lipase (MAGL) and carboxylesterase (CES) activity, increasing 2-AG levels, as a mechanism to limit inflammation.

Human PBMCs were isolated from whole blood of HD and healthy donors for ex vivo LPS stimulation in the absence and presence of an IL-6 neutralizing antibody. IL-6 levels were measured by ELISA in plasma and cell culture supernatants. 2-AG hydrolase activity in PBMC lysates was measured using liquid chromatography mass spectrometry. Furthermore, 2-AG hydrolase activity was characterized utilizing inhibitors of specific endocannabinoid metabolizing enzymes, specifically JZL184, an inhibitor of both MAGL and CES1, and WWL113, a potent and selective inhibitor of CES1. We found that LPS induced IL-6 production from PBMCs with >70-fold increase, which could be partially blocked by an IL-6 neutralizing antibody (>20-fold decrease), but there was no difference between diseased and healthy PBMCs. There was an increased trend in IL-6 levels in the plasma of HD subjects compared to healthy controls. The 2-AG hydrolytic activity of PBMC lysates did not differ between HD and healthy subjects and was not affected by either LPS treatment or treatment with LPS and the IL-6 neutralizing antibody. However, evaluation using JZL184 and WWL113 using healthy naïve PBMC lysates showed that there is some CES1-dependent 2-AG hydrolase activity in human PBMCs, but the dominant 2-AG hydrolytic enzyme is MAGL.

Acknowledgments: Funded by a grant from the MSU-CVM Office of Research and Graduate Studies.

CROSS-SECTIONAL STUDY OF CHRONIC PAIN PATIENTS USING MEDICAL CANNABIS: THE EFFECT OF CENTRALIZED PAIN

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Chronic pain affects over 100 million Americans, and costs an estimated $635 billion dollars per year in the US alone.(Institute of medicine (US). 2011) However, treating chronic pain is difficult, with centralized pain (i.e., pain of central nervous system origin) being especially challenging to manage. Cannabis is a promising analgesic for many chronic pain conditions, with studies reporting that individuals with chronic pain are substituting cannabis for opioids and other pain medication.(Boehnke et al. J. Pain 17 (2016) 739-44) However, few studies performed to date have examined whether cannabis is more or less effective in certain underlying mechanisms of pain. Thus, we examined whether pain centralization (as measured by the 2011 American College of Rheumatology Survey Criteria for Fibromyalgia) (Wolfe et al. J. Rheum 38 (2011) 1113-22) affected treatment outcomes in medical cannabis users throughout the US. Adults (≥18 years old) who used cannabis for chronic pain were invited to participate through an anonymous link sent from participating dispensaries. The 2011 FM survey criteria can range from 0-31, with 0 being no pain centralization, 31 being the highest degree of centralization. This survey also included questions on substitution of cannabis for other medications and rationale for doing so, and changes in quality of life and pain. Differences between FM score tertiles were gauged using Pearson Chi-Square (pain and health change) as well as Analysis of Variance and paired t-tests (quality of life).

The sample (N=503, 56% female) had greatest representation from California (32.9%), Arizona (21.4%), New Hampshire (13.6%) and Nevada (13.2%). Roughly 50% had an Associate's degree or above, and 65% made <$70,000 per year. The population was split into tertiles using FM score, reflecting low, moderate, and high degrees of pain centralization. Those in the highest tertile reported greater pain, pain interference, sleep impairment, anxiety, and fatigue than their counterparts (all $p<0.001$). Participants reported substituting cannabis for multiple pain medications (including n=300 for opioids), citing fewer adverse effects, better symptom management, and fewer withdrawal effects as their top three reasons for doing so. 89% reported that their pain had decreased since starting cannabis. 48% of individuals in the highest tertile reported their pain decreased 'a lot', compared to 55% and 68% in the moderate and low tertiles, respectively ($p=0.02$). Similarly, 96% reported that their health had improved a lot or a little after starting cannabis, and fewer participants in the low group reported health improvement compared to their counterparts ($p=0.042$). Consistent with previous studies, we found that medical cannabis users reported substituting cannabis for opioids and other pain medications as well as improved pain and quality of life. Those with greater centralized pain appeared to respond less well to cannabis than those with less or no centralized pain, suggesting that currently available cannabis products may be less effective for those with centralized pain, or that such individuals have other co-morbid health issues that prevent cannabis from being as effective of a treatment.

Acknowledgements: We are very thankful to the patients who participated in this study.

FISH OIL DERIVED AMIDES AND INFLAMMATION IN CANCER

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Inflammation plays a crucial role in cancer development, tumour growth, metastasis development and the development of cancer-induced muscle wasting (cachexia). Attenuating the inflammatory microenvironment of tumour tissues might be a promising approach to increase efficacy of cancer treatment. PGE2 and IL6 are pro-inflammatory regulators secreted by tumour tissues and often found to be elevated in cancer patients.

Previously we have shown that conjugates of the polyunsaturated *n*-3 fatty acids docosahexaenoic acid (DHA), docosahexaenoyl ethanolamide (DHEA) and docosahexaenoyl serotonin (DHA-5-HT), can be endogenously formed [3] and inhibit the expression of pro-inflammatory cytokines and PGE2 in macrophages. In these cells, DHEA predominantly reduced levels of several COX-2-derived eicosanoids [2] and DHA-5-HT potently inhibited the IL23-IL17 axis in LPS stimulated macrophages [1]. We have also shown that a fish oil diet, in combination with high protein and leucine, improved muscle function and decreased PGE2 plasma levels in the C26-colon adenocarcinoma cachexia mouse model [4,5].

Our hypothesis is that the fish oil derived amides, DHEA and DHA-5-HT, can decrease the release of inflammatory regulators IL6 and PGE2 also in the murine cachexia-inducing C26-colon adenocarcinoma cells and therefore contribute to the attenuation of the inflammatory microenvironment of the tumour. C26-colon adenocarcinoma cells were incubated for 24h with different concentrations of DHEA or DHA-5-HT with or without 0.5 μg/ml LPS or 0.5 μg/ml Pam_3CSK_4. The secretion of PGE2 and IL6 in the culture medium was measured by performing enzyme-linked immunosorbent assays. Here we show that 1 μM DHEA and 100 nM DHA-5-HT reduce the levels of PGE2 in C26 tumour cells with approximately 40% ($p<0.05$). At a concentration of 1 μM, DHA-5-HT also decreases the release of IL6 in TLR4 and TLR1/2 stimulated C26-colon adenocarcinoma cells with respectively approximately 60% and 70% ($p<0.05$) whereas DHEA does not have a significant effect on the secretion of IL6 in these cells. DHEA and DHA-5-HT were more effective to decrease levels of PGE2 and IL6 compared to DHA.

From these results we may conclude that fish oil derived endocannabinoid congeners DHEA and DHA-5-HT can be promising compounds in attenuating the inflammatory microenvironment of the tumour.

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GENETIC REDUCTION OF 2-ARACHIDONOYLGLYCEROL SIGNALING IN THE FOREBRAIN SELECTIVELY IMPAIRS COGNITIVE MEMORY IN MICE

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The endocannabinoid system may play a critical role in the control of cognition and memory. A number of studies have shown that acute activation of type 1 cannabinoid (CB_1) receptors by exogenously administered agonists impairs memory formation and consolidation, whereas CB_1 receptors inverse agonists such as rimonabant exert beneficial effects on cognition in young rodents. Moreover, young CB_1 knockout mice perform better than do their wild-type controls in various learning and memory paradigms, an effect that is highly dependent on the age of the animals. On the other hand, our recent study revealed that endocannabinoid 2-arachidonoyl-*sn*-glycerol (2-AG) signaling is required for long-term potentiation at the lateral perforant path (LPP-LTP) that is associated with episodic memory. Furthermore, dysfunctional 2-AG signaling is accompanied with impaired LPP-LTP in the *Fmr1*-KO mice, an animal model for Fragile X syndrome, a leading monogenic cause for autism spectrum disorder. Therefore, the identity, localization, and the plastic changes of the endocannabinoid signal involved in the control of cognition require further study.

Here, we studied cognitive phenotype of monoacylglycerol lipase-transgenic (MGL-TG) mice that overexpress in forebrain neurons the presynaptic hydrolase, MGL, that deactivates 2-AG. MGL-TG mice showed a significant decrease in forebrain 2-AG levels compared to wild-type control mice, which was accompanied with profound deficits in location memory in all test ages ranging from 2 to 13 months old. Of interest, MGL-TG mice displayed normal behaviors in object recognition memory, indicating that the genetic reduction of forebrain 2-AG signaling mainly affected cognitive memory that is mediated by the hippocampus. In addition, wild-type mice that had received daily administration of Δ^9-tetrahydrocannabinol (THC) during adolescence displayed significant reduction in 2-AG signaling, which was associated with persistent impairment in location memory. The results suggest that endogenous CB receptor signaling is required for encoding cognitive memory in the hippocampus, and that genetic or environmental impairments in the 2-AG signaling may underlie deficits in cognitive memory caused by Fragile X syndrome or adolescent THC exposure.

Acknowledgement: Funded by the National Institute on Aging grant to D.P. (Grant. No. 5R21AG052336)

IDENTIFICATION AND OPTIMIZATION OF INHIBITORS FOR THE CALCIUM-DEPENDENT N-ACYLTRANSFERASE PLA2G4E

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In 2016, the enzyme PLA2G4E was discovered to be a calcium-dependent *N*-acyltransferase capable of producing *N*-acyl phosphatidylethanolamines (NAPEs) from phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE). Together with the calcium-independent members of the phospholipase A/acyltransferase (PLA/AT) family PLA2G4E is suggested to be the enzyme responsible for the production of NAPE in the endocannabinoid system (ECS). In this study, two activity-based protein profiling (ABPP) assays were developed for PLA2G4E. Both a conventional gel-based assay and a fluorescence-polarization (FluoPol) assay were developed, making use of the broad-spectrum serine hydrolase probe fluorophosphonate-TAMRA. Gel-based ABPP allows for the visualization of multiple enzymes at the same time, whereas the FluoPol assay is performed in 96-wells format allowing for higher-throughput screening of inhibitor compounds.

Using the two assays, an in-house compound library was screened against hPLA2G4E derived from HEK293T cell overexpression and inhibitors were identified. New molecules were designed, synthesized and tested for activity on PLA2G4E. Selectivity over PLA2G4 family members and other enzymes within the ECS was studied by gel-based ABPP. Via this approach, WEN091 was identified as a potent inhibitor of PLA2G4E with an IC_{50} of 10 nM. The activity and selectivity of this compound is currently being investigated in biochemical and cellular assays.

DELTA-9-TETRAHYDROCANNABINOL IN NEUROPATHIC PAIN AND COMORBID INSOMNIA

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Neuropathic pain (NP) is a major health problem characterized by high suffering, low productivity and substantial health care costs. An estimated 50% to 88% of patients with NP develop sleep disturbances (Finan et al., 2013) and only a restricted number of effective drugs are clinically available for NP associated to insomnia with several side effects. The delta-9-tetrahydrocannabinol (THC) may offer a novel approach to the chronic pain management and insomnia, but its mechanism of action in these two conditions is not yet fully understood. The effects of THC in NP and in the sleep-wake cycle (EEG/EMG) was thus here examined. We induced NP (sciatic nerve ligation) in Wistar rats who 14 days later were treated with vehicle (VEH; 5%Tween 80, 5% PEG and saline) or THC (1, 1.5, 2, 2.5 and 5 mg.kg, i.p.) to assess mechanical allodynia. Then, rats with NP (sciatic nerve injury) were implanted with six stainless-steel wire electrodes in the skull and EEG/EMG was recorded for a period of 6 h (from 6 AM to 12 PM).

THC (2.5 and 5 mg/kg) decreased mechanical allodynia in rats with NP ($p<0.001$) vs VEH, 1h. after administration in a dose dependent-manner, reaching an antiallodynic plateau of 6 gr. This effect lasted about 3.5 hours. EEG/EMG analysis showed that rats with NP had a reduced time in non-rapid eyes movement (NREM) sleep (-35%, $p<0.05$) and increased wakefulness (+39.6%, $p<0.05$) compared to naive rats. THC (5 mg/kg) was able to restore the normal sleep-wake cycle in NP rats. The findings suggest that THC has a moderate analgesic effect and good hypnotic effect in a NP paradigm, similar to clinical outcomes reported in humans.

Acknowledgments: Funded by the Ministère de l'Économie, Science et Innovation (MESI) du Québec, (Grant. no. PSR-SIIRI-988)

AN EXAMINATION OF CBD:THC RATIO BASED PRODUCTS PREFERRED BY MEDICAL CANNABIS USERS

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Cornerstone Research is a medical cannabis collective that generates detailed profiles of its members cannabis use to enhance their overall personalized care and improve the consultant's ability to understand each member's individual needs. This research intends to examine member profiles in order to study the various self-reported medical reasons for using cannabis-based treatments and the type of cannabis-care preferred by members including method of intake, frequency of use and products consumed. This study will examine the overall preferred ratio of CBD to THC, which types of cannabinoid-based products are most commonly used and which varietals are self-reported to alleviate specific ailments. In addition, the study will compare differences in preferred types of cannabis-care and the self-reported effects of products and varietals by medical group.

This study considers a wide variety of medical reasons for using cannabis. The largest group reporting cannabinoid-based treatments include chronic pain, psychological disorders, and stress management. Other groups represented include autoimmune disorders, cancer, neurological disorders, sleep disorders, and gastrointestinal disorders. Overall, the preferred method of cannabis intake is smoke inhalation, with vapor inhalation as the second preferred method, and edibles as the third. The majority of users report using cannabis on a daily basis to cope with their symptomatology. Cannabis products are preferred as THC only, second as a ratio of 1:1 CBD:THC, and third as a ratio of 2:1 CBD:THC. The most frequently used flower varietals overall include Golden Pineapple for mood elevation, Little Dragon for pain relief, C3 of mental calm, and Blueberry OG 2:1 for anxiety reduction. Lastly, medical group members exhibit similar preferences for specific ratios, flower varietals, and cannabis-based products. The demographic outline elucidates variability in the types of cannabis-care preferred for self-treatment and provides insight into the products selected by these users to alleviate a variety of medical conditions. The preference for CBD:THC ratios may support the existence of a synergistic relationship between CBD:THC. Furthermore, the preference to inhale cannabis flowers to help with symptomology may support the preference for whole plant products and give insight into understanding the entourage effects of cannabis. This research helps bridge the gap between the medical cannabis community and clinical research to create opportunities for further examination of cannabinoid-based treatments within specific medical populations.

EXPRESSION OF CANNABINOID-RELATED G PROTEIN-COUPLED RECEPTORS AND RELATED PROTEINS IN HEK293, ATT20, BV2, AND N18 CELL LINES AS REVEALED BY MICROARRAY ANALYSIS

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Thousands of research studies have utilized heterologous expression systems such as human embryonic kidney cells (HEK293). Though often treated as 'blank slates', these cell lines nevertheless endogenously express GPCRs and related signaling proteins. The outcome of a given study can be profoundly influenced by this largely unknown complement of receptors and/or signaling proteins. Little easily accessible information exists that describes the expression profiles of the genes in cell lines. What is accessible is often limited in scope. For example, of the hundreds of GPCRs and related proteins, it is rare to find information on expression of more than a dozen genes in a given cell line. Microarray technology allowed rapid analysis of mRNA levels of thousands of candidate genes, but though often publicly available, the results can be difficult to efficiently access or even to interpret.

To bridge this gap, we used microarrays to measure the mRNA levels of gene products in four cell lines frequently used for research: HEK293, AtT20, BV2, and N18. We have published a survey of GPCRs based on this data set (Atwood et al., 2011) but have not published information relating to expression of cannabinoid-related genes. This includes enzymes involved in cannabinoid signaling as well as assorted TRP-family receptors.

This study provides researchers an easily accessible mRNA profile of the endogenous cannabinoid-related signaling repertoire that these four cell lines possess. This will assist in choosing the most appropriate cell line for studying cannabinoid signaling. It also provides a better understanding of the potential interactions between cannabinoid receptors and various components of the cannabinoid signaling system.

THE DERIVATION OF SERUM FAAH ACTIVITY

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Background: We recently described endocannabinoid hydrolysing enzyme activities (FAAH and MAGL) in different blood fractions to show a differential expression in these fractions. FAAH activity was highest in isolated erythrocytes (39 ± 3 pmol/min/mL blood), while MAGL activity was markedly higher in platelets (160 ± 13 nmol/min/mL blood) than erythrocytes (17 ± 1 nmol/min/mL). Interestingly, FAAH activity was low but detectable in serum, while MAGL was variable or undetectable in serum (Anajirih et al., ICRS 2017). We have extended these study to identify the mechanisms for how clotting causes these changes and precisely which enzymes are involved.

Methods: Following approval from the School of Life Sciences Ethics Committee, University of Nottingham, overnight fasting blood samples were taken in the morning from 8 healthy males. Blood fractions were isolated, including platelet-rich plasma (PRP), packed erythrocytes, washed platelets, plasma and serum. Thrombin was employed to trigger clot formation, particularly with packed erythrocytes and platelet-rich plasma. Following the generation of a clot, the supernatant layer from a low speed centrifugation, equivalent to serum, was harvested and assayed for endocannabinoid hydrolase activity. Using selective inhibitors, the activities of FAAH, MAGL and ABHD6 were quantified using both radiometric and ABPP assays. Immunoblotting was also applied to attempt to detect the expression of FAAH and MAGL proteins.

Results: Thrombin caused rapid platelet activation and clot formation within 5 minutes of its addition. Our results indicate that the majority of FAAH activity liberated from these fractions derived from erythrocytes, while very low levels of FAAH activity was released from platelets.
In contrast, the activity of MAGL was significantly reduced in erythrocytes and PRP releasate induced by thrombin compared to nonactivated erythrocytes and platelets. In addition, low activity of ABHD6 was detected in erythrocytes and washed platelets, however, its activity was degraded during clot formation. The reduction of MAGL and ABDH6 activities observed during clot formation was not due to the direct proteolytic effects of thrombin.
Using human washed platelet samples, human MAGL-like immunoreactivity was found to have a slightly higher molecular weight than immunoreactivity in rat liver samples. The expression level of FAAH protein in blood samples was too low to be detected in immunoblots. The level of FAAH and MAGL activities was stable in washed platelet and erythrocyte samples after being stored in liquid nitrogen, while more than 50 % of their activates was lost after 3 days of storage at -80 °C.

Conclusion: although we have identified that formation of blood clot results in a marked loss of MAGL activity, these studies identified that thrombin-induced clotting in vitro changes the distribution of endocannabinoid hydrolysing enzymes activities. It is found that blood clot formation releases FAAH activity into serum from either erythrocytes or platelets.

Acknowledgement: Funded by the Saudi Cultural Bureau.

PREGNENOLONE ANALOGS AS SIGNAL SPECIFIC ALLOSTERIC MODULATORS OF THE CB1 RECEPTOR

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Pregnenolone, an endogenous negative allosteric modulator of the CB1 receptor, modulates BArr-mediated signaling, but does not affect the G-protein mediated pathway. The proposed binding site (TMH1/Hx8 exosite), previously identified through a fragment based MMC approach and receptor mutation experiments, is in an elbow region reported to move during BArr signaling. Pregnenolone has also been shown to protect the brain from cannabis intoxication. Further investigations regarding this endogenous ligand and its CB1 binding site are of particular interest.

We have designed a series of non-steroidal pregnenolone analogs with improved Lipinski parameters that can fit the pregnenolone binding site. These analogs contain a tetralone moiety and while stability issues occur with 2-tetralones, the 1-tetralone isomers are considerably more stable.

Some of the analogs decrease ERK signaling produced by CP55940 in the absence and in the presence of pertussis toxin, suggesting that these negative allosteric modulators are biased toward reducing BArr signaling of CP-55,940.

Acknowledgement: Supported by R01 Grant 2R01DA003934-27 to P. H. Reggio, T32DA007237 (L. Leo), and P30DA013429 (L. Leo, M.E. Abood).

THE INFLUENCE OF FAAH GENETIC VARIATION ON THE DEVELOPMENT OF DIET-INDUCED OBESITY IN MICE

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The endocannabinoid system is an important regulator of energy balance. This includes the combined actions of anandamide and 2-arachidonoyl glycerol (2-AG) in the brain and periphery to modulate energy intake (i.e., feeding), energy expenditure (EE), and metabolism. Impaired anandamide metabolism via genetic knockout has been shown to promote energy storage and fat mass accumulation in mice. Similarly, in humans, a single nucleotide polymorphism (SNP) of the FAAH gene (C385A SNP), which results in reduced FAAH expression and increased anandamide levels in A-allele carriers, has been associated with a vulnerability to become overweight and develop obesity. The current study assessed the effects of this SNP on diet-induced obesity using a genetic knock-in mouse expressing the human FAAH C385A gene mutation.

Male FAAH C385A mice (6-7 weeks old) of all 3 genotypes (wildtype C/C, heterozygous C/A and homozygous for the A/A SNP) weighing approximately 25g at the start of experiments were exposed to a 14-day baseline dietary period (Research Diets #D12450H; 10% fat; 70% carbohydrate). This was followed by 9-weeks of high fat diet (#D12451; 45% fat; 35% carbohydrate). Body composition was assessed weekly by nuclear magnetic resonance (NMR) scans, and energy metabolism data were collected using CLAMS metabolic chambers.

Homozygous FAAH A/A mice displayed greater fat mass (16%) relative to wildtype (C/C; 6%) and heterozygous (C/A; 7%) animals at the onset of low fat diet exposure in CLAMS. A/A mice also gained more fat mass during LFD feeding (8% increase) compared to C/C (+4%) and C/A (+3%) mice. Interestingly, A/A mice lost fat mass (4% decrease) during the first week of subsequent HFD exposure and this corresponded with increased EE relative to C/C (2% fat increase) and C/A (1% fat increase) mice. Fasting/re-feeding manipulations also revealed genotype differences in respiratory quotient with A/A mice exhibiting an earlier but slower shift in substrate use towards lipids during a 16-hr food-restriction period; whether this a protective adaptation of A/A to negative energy balance remains to be determined. Taken together, these findings suggest that genetic variation in anandamide signaling plays a role in response to changing metabolic demands on high carbohydrate/fat diets. Future experiments will assess how energy balance is affected by this polymorphism under differing dietary conditions.

Acknowledgements: Funded by the Canadian Institutes of Health Research (CIHR) and Natural Science Engineering Research Council of Canada (NSERC)

PHARMACOLOGICAL CHARACTERIZATION OF ABUSED SYNTHETIC CANNABINOIDS AND THEIR HYDROXYLATED METABOLITES

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A variety of wide spread adverse health events[1] and a growing number of deaths have been attributed to synthetic cannabinoid use.[2] In addition to the parent compounds, consideration of the pharmacology and toxicology of their metabolic products is warranted as these may contribute to the in vivo effects of these drugs. For example, hydroxylated metabolites of JWH-015[3] and JWH-073[4] retain in vitro and in vivo activity in laboratory assays and rodent models, respectively, suggesting that active metabolites of abused synthetic cannabinoids may contribute to the variety of adverse effects observed in humans. To further examine the effect of hydroxylation of the alkyl side chain on the pharmacology of abused synthetic cannabinoids, AB-PINACA, 5F-AB-PINACA, AMB, 5F-AMB, ADB, 5F-ADB, APINACA, 5F-APINACA, and 5F-CUMYL-PINACA and a variety of their side chain hydroxylated metabolites were synthesized and are being tested in a series of in vitro pharmacological assays of affinity and efficacy.

Compounds were characterized in receptor equilibrium binding as well as the agonist-stimulated [^{35}S]GTPγS functional assay to determine their affinity, potency and efficacy at human cannabinoid type-1 (hCB_1) and type-2 (hCB_1) receptors. Membrane (P2) preparations of HEK293 cell expressing either the hCB_1 or hCB_2 receptors were used for all studies. For binding, Cheng-Prusoff correction was applied to displacement curves of 1 nM [^{3}H]CP55,940 to determine pK_i values whereas [^{35}S]GTPγS functional data were fit to three parameter nonlinear regression for determination of E_{max} and pEC_{50} values.

We report that all hydroxylated metabolites retained affinity and efficacy at both hCB_1 and hCB_2 receptors and describe their pharmacological parameters in relation to the parent compounds. These studies suggest that hydroxypentyl metabolites can contribute to the in vivo effects of these synthetic cannabinoids. Future studies will determine the effects of these compounds and their metabolites to produce cannabis-like effects using the drug discrimination procedure.

Acknowledgements: R01DA040460 and R01DA03672

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USING CONTINUOUS CELL IMPEDANCE MONITORING TO MEASURE CANNABINOID SIGNALLING

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The cannabinoid receptor CB1 exhibits functional selectivity in response to endogenous and phytocannabinoids. Previous work has shown that intestinal permeability is differentially modulated by cannabinoids, as measured by trans-epithelial electrical resistance measurements and fluorescent dextran flux. This suggests that the CB1 receptor exhibits ligand biased signalling properties. Using a real-time cellular analysis platform, iCELLigence (ACEA Biosciences, San Diego), this study sought to investigate whether changes in cellular impedance resulting from stimulation with cannabinoid ligands could be detected and how impedance profiles compared.

CHO cells stably expressing the human CB1R, differentiated Caco-2 intestinal cells and HIEC-6 cells were cultured on E-plates (gold microelectrodes fused to the bottom of each well). Anandamide (AEA, 1 and 10μM), cannabidiol (CBD, 1 and 10μM) and WIN 55,212-2 (10nM-10μM) were applied to cells in serum-free medium, with or without the CB1R antagonists SR141716A (100nM and 1μM) AM251 (100nM and 1μM) for up to 2 hours and cellular impedance measured continuously. The unit-less Cell Index (CI) was collected every 30 seconds or every minute and values were normalised by dividing the CI at the time prior to ligand addition. Baselines (vehicle controls) were corrected by subtracting the CI from vehicle treated cells. Replicates of 2-4 were used for each treatment per experiment. Data analysis was performed using RCTA software (v 1.0.0.1304) provided with the system.

Impedance profiles could be divided into several phases, which differed with both ligand and cell type. In CHO-hCB1R cells, both AEA and WIN induced a rapid rise in CI within 5 minutes. After an initial decay, there was a second ascending peak around 12 minutes that returned to baseline within 30 minutes and dropped below baseline for the remaining timeframe (2 hours). The WIN effect was approx. 2-fold greater than AEA. CBD had no effect on CHO-hCB1R cells within this timeframe. In Caco-2 cells, there was no significant effect from any of the cannabinoids within the 2 hour timeframe of the experiment. In HIEC cells, there was no secondary peak for any of the cannabinoids. CBD peaked around 30 minutes and sustained this level for the 2 hour timeframe, whereas AEA peaked within 15 minutes and then impedance dropped back to baseline around 1 hour and continued to fall below baseline for the remaining time. The CBD maximum was approx. 2-fold greater than AEA. Interestingly, WIN dose-dependently had an inverse effect, with maximum peak CI achieved with 10nM and only the 1μM and 10μM dropping below baseline after 40 minutes. Reduced changes in impedance was achieved using the CB1R blockers SR141716A and AM251, although this was not complete.

Electrical impedance assay is a useful tool to dissect receptor mediated signalling. However, endogenous receptor levels may be a factor in the sensitivity of the assay. A functionally different CB1 receptor (or isoform) may be expressed in intestinal epithelium that differs from the engineered CB1R in CHO cells. Finally, there may be additional factors, such as cell number or the presence of other cannabinoid receptors that could lead to changes in impedance.

AN OBSERVATIONAL STUDY OF MEDICINAL CANNABIS EFFECTS IN ADULTS WITH MEDICALLY REFRACTORY EPILEPSY

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Despite the availability of many different anti-epileptic drugs, some with novel mechanisms, approximately 30% of people with epilepsy continue to have seizures (Brodie et al, 2012; Kwan et al., 2000). This condition, called Medically Refractory Epilepsy (MRE), is an extremely debilitating condition that is associated with severe morbidity and increased mortality (Devinsky et al., 2016). Quality of life in MRE is severely compromised in numerous ways, including inability to drive, decreased employment, decreased marriage, social isolation, and increased rates of comorbid psychiatric disorders (Baker et.al, 1997; Sperling, 2004). This gap in treatment has led patients to seek alternative treatments, such as cannabis.

Research from a number of different types of studies support the potential use medicinal cannabis for the treatment of seizures in epilepsy. Both Δ9 tetrahydrocannabinol (THC) and cannabidiol (CBD) show anti-convulsant properties in animal models, although THC has also shown pro-convulsant effects in some cases (Devinsky et al., 2014). Case studies have found seizure exacerbation after marijuana cessation in two male patients with epilepsy. Survey research found 84% of parents reported a reduction in their child's seizure frequency while taking CBD-enriched cannabis (Porter and Jacobsen, 2013), although another study found similar rates of parent perception of seizure reduction that were not supported by EEG recordings (Press et al., 2015). More recently, results of a randomized control trial in children with Dravet syndrome showed the "median frequency of convulsive seizures per month decreased from 12.4 to 5.9 with cannabidiol, as compared with a decrease from 14.9 to 14.1 with placebo" (Devinsky et al., 2017). This study provides the first conclusive evidence that CBD is an effective, well-tolerated treatment for seizures, at least in children with very severe forms of the condition. Research on adults is lacking.

For this study, researchers plan to enroll thirty-five adults from Colorado with MRE. Recruitment has just begun. Participants are followed for a period of six months, 1 month before and for 5 months after they begin medicinal cannabis use. This study is observational; no medicinal cannabis is provided to the participants by the researchers. Over the course of the study, participants make three lab visits. At each of the three lab visits, participants fill out behavioral questionnaires. These questionnaires include Quality Of Life In Epilepsy (QOLIE-31-P; Cramer et.al, 2003; Borghs et al., 2012), Liverpool Adverse Events Profile (LAEP; Baker et al., 1993), Seizure Severity Questionnaire (SSQ; Cramer et al., 2002), Hospital Anxiety and Depression Scale (HADS; Zigmond et al., 1993), and the Medical Cannabis Survey (MCS; Swift et. al., 2005). At each visit participants are also asked to provide blood and/or urine samples that are processed to identify 11 different cannabinoids and metabolites.

A unique aspect of this study is that participants are outfitted with an E4 wristband (Empatica) for daily wireless physiological recording. Physiological variables collected include heart rate, motion/acceleration, blood volume pulse, temperature, and acceleration. The wristband includes an event marker to be pressed by participants at the start of a seizure (if possible) and when they administer cannabis. This will allow researchers to examine the autonomic consequences of seizures as well as allow them to track the effects of medicinal cannabis on the participants' physiology. Monthly seizure reports will be produced for each participant using an algorithm developed by Empatica. Participants are also asked to keep a seizure diary using seizuretracker.com. Progress on the study to date will be described. Preliminary physiological data will be shown.

CANNABIS BIOACTIVE COMPOUNDS RESPONSIBLE FOR ENTOURAGE EFFECTS: CHANGES DURING PROCESSING

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We have investigated the metabolome of various Cannabis plants and products thereof employing high resolution mass spectrometry (HRMS), a technique that enables screening not only of the 12 major cannabinoids for which analytical standards are available (CBDA, CBD, Δ9-THCA-A, Δ9-THC, Δ8-THC, CBN, CBGA, CBG, CBC, CBDVA, CBDV, THCV), but also for a number of other bioactive compounds that might be responsible for entourage effects. Using the database of 243 phytocannabinoids (reported in scientific literature) together with 152 other compounds such as phenolics, terpenoids etc., the changes in their profile during various production/processing practices (mainly heat treatment) were documented. The knowledge of the specific composition of cocktails of bioactive chemicals and their transformations are important for regulatory bodies overseeing clinical studies concerned with clinical effects of, and adverse events attributable to, cannabinoids and other cannabis compounds being utilized by patients.

NAAA INHIBITOR AM11095 TARGETS THE BREAST CANCER INFLAMMATORY NETWORK AND DISEASE PROGRESSION

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While the link between inflammation and cancer initiation is well established, very little is known on its role in metastatic disease. Recent studies show that lipids play important roles in metastasis and cancer progression. Although fatty acid synthesis (FAS) overexpression confers a survival advantage to cancer cells, new compounds that target de novo FAS, haven't reached the clinic. Therefore, there is an unmet need for safer and more effective therapies for triple negative breast cancer and breast cancer metastasis in the brain.

Palmitoylethanolamide (PEA) is an endogenous bioactive lipid synthesized "on demand" by most mammalian cells, and is involved in the regulation of inflammation and pain processes. N-acylethanolamine acid amidase (NAAA) is a lysosomal enzyme, which plays a central role in the deactivation of PEA. PEA reduces peripheral inflammation and exerts neuroprotective and antinociceptive effects. This compound may putatively act as a modulator of inflammation and lipid deregulator in cancer. Thus, enhancement of endogenous PEA levels, by inhibition of NAAA enzyme, using specific NAAA inhibitors, represents a novel approach to treat tumor inflammation, tumor growth and metastasis. To this end, we have discovered a new selective NAAA inhibitor AM11095, at the Center for Drug Discovery, Northeastern University, with high specificity and potency toward NAAA and with no overt signs of toxicity. AM11095 selectively inhibits human NAAA (IC_{50} = 20 nM).

Based on our preliminary studies using this compound, we hypothesize that the bioactive lipid PEA plays important roles in breast cancer metastasis via modulation of lipid signaling and inflammation. Our preliminary studies showed: **1)** Significant increase in the expression of full length and splice variants of NAAA in human breast cancer cells, as compared to normal breast epithelial cells. **2)** Exposure of tumor cells to PEA or AM11095 inhibitor resulted in significant decrease of secretion of inflammatory cytokines/chemokines from tumor cells as well as inhibited secretion of VEGF, endothelin-1, and endoglin, all angiogenic important factors in breaching the BBB integrity and facilitating tumor migration and tumor growth. **3)** Both PEA and AM11095 inhibited tumor cell invasion, proliferation and migration. **4)** Breast tumor cells treated with either AM11095 or PEA showed inhibition of NF-kB, which indicates that one of the pathway mechanism of breast tumor progression and metastasis inhibition with PEA and AM11095 is through the NF-kB pathway. These in-vitro preliminary results provided an insight into the biological roles of the bioactive lipid PEA on breast tumor and its metastasis to the brain. Modulation of the endogenous levels of PEA by inhibiting its hydrolysis using AM11095 NAAA inhibitor, is a novel approach to treat breast tumor growth and metastasis to the brain through the inhibition of the breast tumor inflammatory network microenvironment.

PERINATAL EXPOSURE TO THC DISRUPTS CEREBELLAR DEVELOPMENT

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The recent increase in legalization and positive public perception of cannabis has resulted in a higher prevalence of perinatal exposure, raising the urgency for better understanding of the role of endogenous cannabinoid (eCB) signaling in brain development, and the neurodevelopmental consequences of perinatal exposure to phytocannabinoids. Our previous results demonstrate that expression of eCB system in the cerebellum is prominent and dynamic throughout development.

In this study, using gross anatomical criteria (size and extent of foliation), we show that eCB signaling plays a prominent role in cerebellar development. Furthermore, we show that perinatal exposure to phytocannabinoid tetrahydrocannabinol (THC) alters cerebellar developmental trajectory, leading to a decrease in size and an increase in foliation, including robust differences in the anterior region of the midvermal cerebellum. In addition, we found this region to be variable between males and females – a majority of males have 3 folia, while a majority of females have two. Interestingly, psychotropic effects of phytocannabinoids are reported to differ between sexes, and developmental exposure to cannabis is associated with higher risk for the development of psychiatric disorders that exhibit sexually dimorphic prevalence, such as schizophrenia. Our results indicate that perinatal exposure to THC erases sex-dependent differences in the development of anterior cerebellar vermis, leading to bigger loss of area in males and bigger increase in foliation in females, so that the phenotype of exposed animals – smaller size and increased foliation – is indistinguishable between sexes.

Acknowledgements: Funded by NIDA R21 (Grant No. 00504055).

ENDOCANNABINOIDS MEDIATE STRUCTURAL PLASTICITY IN CEREBELLAR BASKET CELL SYNAPSES

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Cerebellum plays an important role in automation and sequencing of motor, cognitive and emotional behaviors including language, attention, anxiety, and reward. Cerebellar Purkinje cells (PCs) are the primary cerebellar co-incidence detectors, integrating all excitatory inputs and generating the pattern of cerebellar output. Interestingly, impaired PC function has been implicated in Autism spectrum disorder in human patients and Autism-like behaviors in rodents, including impaired social behavior, repetitive actions, and vocalizations. Inhibitory cerebellar interneurons, the basket cells (BCs), 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.

Endocannabinoids (eCB's) have been implicated as predominantly retrograde signals in synaptic plasticity in both inhibitory and excitatory synapses, and are involved in modulation of behaviors and physiological functions ranging from mood, anxiety, and pain to temperature homeostasis. In the cerebellum, eCB signaling has been shown to regulate both activity-dependent and homeostatic synaptic plasticity in excitatory synapses on both short and long timescales.

In this study, we set out to explore the role of endogenous cannabinoid signaling in structural plasticity of BC/PC synapses, and the effects of exposure to phytocannabinoids on BC/PC synaptic structure and function. We show that in the mature cerebellum cannabinoid receptor 1 (CB1) is prominently expressed in BC axons and in the pericellular and pinceau presynaptic compartments, while the enzymes involved in the synthesis of eCBs (2-AG and anandamide) are 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. Next, we investigated the changes in the size and shape of the pinceau synapses at two month in CB1 knockout mice and following brief exposure to THC during the second postnatal week. Our results demonstrate that BC/PC synapses undergo structural plasticity, which is regulated by eCB signaling. Strikingly, the effect of CB1 ablation has binary effect on BC/PC synapse structure between males and females, matched by sexually dimorphic alterations in cerebellar-controlled behaviors such as rotarod learning and forepaw coordination in sunflower seed opening.

Acknowledgements: Funded by Indiana University in Bloomington College of Arts and Sciences start-up fund for AK

PILOT DATA FROM A PROSPECTIVE OBSERVATIONAL NATURALISTIC STUDY OF CANNABIDIOL-RICH DRIED FLOWER IN THE TREATMENT/REDUCTION OF ANXIETY

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Background: We report on a current prospective naturalistic observational pilot study following out-patients (n=27, ♂21 ♀5) reporting clinically significant symptoms of anxiety/stress. In this study we focus on CBD rich chemovars (6.44%THC: 9%CBD, 9.22%THC:14.15%CBD, 3.31%THC:11.24%CBD) recommended to research patients who were instructed to self-titrate and report weekly, answering the Beck Anxiety Inventory (BAI>10 entry criterion) and the Global Quality of Life Scale, with repeated measure designed analysis.

Methods: Using a previously reported standardized electronic questionnaire and visualization instrumentation program, (Moller et al., 2015) we captured severity self-rating (5-point Likert Scale) on 5 core symptoms: pain, sleep quality, mood, relaxation and stress perception, a Composite Wellness Score (CWS) was computed over a sample of 27 enrolled patients over an 8-week duration. Data was collected at weekly intervals using a standardized telephone interview.

Results: We report on data collected from the first 17 subjects that completed 8 weeks. Most notable results were seen in significant decreases in BAI scores, GQLS scores increased congruently. We also noticed increases in CWS, from this we saw subject perceived stress reduced.

	CWS mean	SD	BAI mean	SD	GQLS mean	SD
t=0(initial)	2.1	0.7	24.8	5.6	61.3	14.7
t=1(4 weeks)	2.8	0.7	17.4	11.6	71.5	11.6
t=2(8 weeks)	3.0	0.6	8.7	5.4	74.7	9.5

Conclusions: These subjects' self-reports indicated a general improvement across several facets of mental health which warrant further scientific inquiry. Early data show promise of CBD as a possible anxiolytic, as a pilot study, data showed reductions in anxiety with use of chemovars containing CBD. This study is intended to gauge efficiency of the compound in active treatment, we continue to collect data for further investigation as the study is not complete.

Reference:
Moller HJ, Saynor L, Sudan K, Tabak D. (2015) Wellpad: A New Standardized Assessment and Data Visualization Tool for Clinicians and Researchers. IACM 2015 8th Conference on Cannabinoids in Medicine 17-19 September, Sestri Levante, IT

2-AG SIGNALING THROUGH CB1 RECEPTORS REGULATES OPERANT RESPONDING TO PREDICTIVE INCENTIVE CUES FOR A SUCROSE REWARD

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The endocannabinoid system has been implicated in reward-seeking behavior, for example, inhibiting CB1 receptors disrupts operant responding for food and drug reinforcers as well as cue-induced reinstatement. Conversely, upregulation of 2-arachidonyl glycerol (2-AG) signaling via inhibition of its degradative enzyme, monoacyl glycerol lipase (MAGL), increases operant reward seeking. However, it is difficult to separate the effects of these drugs on motivation for the primary reinforcer or the incentive motivational properties of the reward-related cues. The goal of this study was to characterize endocannabinoid modulation of responding in an operant model dependent upon incentive cues (ICs). In this task, rats nosepoke during a distinct 8-second audiovisual cue to obtain 60 □l of a 10% sucrose solution. Rimonabant dose-dependently (1, 3, and 6 mg/kg, i.p.) decreased the response ratio (number of reinforced IC/total IC presented) while increasing the latency to nosepoke. However, the latency to enter the reward cup to obtain the reinforcer was only slightly increased at the highest dose, indicating a greater disruption in the motivational properties of the IC than consummatory behaviors. To test the contribution of 2-AG, we pretreated rats with the MAGL inhibitor MJN110 (1, 5, 10 mg/kg, i.p.) in a modified version of the IC task, in which the volume of sucrose delivered decreased incrementally across the 1-hr session, producing an overall lower baseline of responding. Under these conditions, MJN110 increased the response ratio, had no overall effect on the latency to nosepoke in response to the IC, but decreased the latency to enter the reward cup at the highest dose. Furthermore, pretreatment with rimonabant blocked MJN110's effects, indicating that MJN110 is acting through a CB1 receptor mechanism. Together these data support that CB1 receptor blockade preferentially decreases motivation for ICs, while enhancing 2-AG signaling increases motivation for the primary reinforcer.

A NOVEL BRAZILIAN SPIDER TOXIN ANALOGUE IS ANTINOCICEPTIVE ACTING VIA THE CANNABINOID SYSTEM

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Peptide toxins produced by a variety of organisms have evolved with different targets, including GPCR, enzymes and ion channels, where there are many examples of ligands with high potency and selectivity. The Brazilian scientific team developed peptide analogues of an active component of the venom of the Brazilian wandering spider (*Phoneutria nigriventer*). The synthetic nontoxic peptide PnPP-19 (BR 10 2012 020800-8 A2) induced antinociception involving the inhibition of neutral endopeptidase and activation of CB_1 cannabinoid receptors as well as μ and δ opioid receptors (Freitas, Br J Pharmacol PMID: 26947933). In this work, a novel synthetic nontoxic peptide, Pn1 (11 amino acid residues, MW= 1.35 kDa, in patent application) modeled from δ-Ctenitoxin-Pn1a toxin was examined.

Nociceptive thresholds were measured by the *in vivo* paw pressure test on male Wistar rats (180-200 g). First, to investigate the role of Pn1 in nociception, the peptide was injected (1.5, 2.5, 5 and 10 μg per paw) or vehicle (50 μl) into rat paws-that were hyperalgesic following the administration of carrageenan (250 μg). Pn1 induced a dose-dependent antinociceptive response, with the maximal effects at 10 μg per paw, which peaked at 30 min post-administration. The role of the CB_1 cannabinoid receptor in these effects was investigated with intraplantar co-administration of AM251 (40, 80 and 160 μg per paw). The highest dose totally inhibited the antinociceptive effect of Pn1 (10 μg per paw). Moreover, we investigated whether enzymatic degradation by FAAH, MAGL and anandamide endocannabinoid transporter pathways could be contributing to the antinociceptive activity using intraplantar administration of JZL184 (14 μg per paw); MAFP, (4 μg per paw); or VDM11 (40 μg per paw). The results showed that inhibition of MAGL or the transporter potentiated the antinociceptive activity of the Pn-1. In *in vitro* studies, we evaluated the selectivity of Pn1 using CHO-CB_1 and CHO-CB_2 human receptors and [^{35}S]-GTPγS binding assays. The preliminary results indicated that Pn1 (10 μM) appears to exhibit selectivity and affinity for the CB_1 receptor. Further studies of the downstream signalling pathways for the CB_1 receptor (calcium elevation and ERK1/2 phosphorylation), however, failed to show significant effects of Pn1 (10 μM).

Antinociception induced by Pn1 appears to involve the inhibition of MAGL and anandamide endocannabinoid transporter and the selective activation of CB_1 receptors. This novel peptide could be useful as a new antinociceptive drug candidate.

Acknowledgments: CAPES / University of Nottingham. Drug Discovery nº 041/2014.

L-DOPA-INDUCED DYSKINESIA ATTENUATION CAUSED BY CANNABIDIOL+CAPSAZEPINE IS ASSOCIATED TO THE DECREASE OF PRO-INFLAMMATORY FACTORS IN HEMIPARKINSONIAN MICE

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The endocannabinoid system is emerging as an important modulator of basal ganglia functions. Pharmacologic manipulation represents a promising therapy to alleviate L-DOPA-induced dyskinesia (LID). Recently, our group has demonstrated the association of the major non-psychoactive cannabis constituent, cannabidiol (CBD) and the TRPV-1 antagonist capsazepine (CPZ) modulates LID in hemiparkinsonian mice. This effect was accompanied by the reduction of inflammatory factors involved in LID, such as the enzyme cyclooxygenase 2 and the immediate early gene NF-ƙB. Our aim in this work was to further characterize the anti-inflammatory properties of CPZ+CBD in the LID mouse model.

C57BL/6 mice with a 6-Hydroxydopamine lesion were treated chronically with L-DOPA (25 mg/kg + Benserazide 10 mg/kg i.p.) for 21 days and developed severe axial, limb, locomotor and orofacial abnormal involuntary movements (AIMs). Following this period, the animals were exposed to CPZ (5 mg/kg i.p.) + CBD (30 mg/kg i.p.) 30 minutes prior to L-DOPA during 3 days. After the confirmation of LID attenuation, we performed immunohistochemistry for the glial markers GFAP (for astrocytes) and Iba-1 (for microglia) and ELISA for cytokines TNF-α, IL-1β and IL-6 and for COX-2 metabolite PGE2 in the lesioned dorsal striatum of hemiparkinsonian mice.

CBD+CPZ significantly reduced axial, limbic and orofacial AIMs without altering locomotor AIMs, as previously demonstrated. Molecular analysis revealed this decrease was accompanied specifically by the reduction of the cytokine TNF-α and COX-2 metabolite PGE2. However, they failed in decreasing IL-1β and IL-6 or in reversing the activated glial state found in the dorsal striatum of dyskinetic mice. Although CPZ+CBD might be a potential tool to alleviate LID, their mechanism of action seems to involve specific anti-inflammatory properties, yet to be understood.

Acknowledgement: FAPESP [2017/14207-7], NIDA grant (R13DA016280), CNPq, CAPES.

THE ABHD6 INHIBITOR WWL70 ATTENUATES ACUTE LUNG INJURY IN MICE

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Respiratory diseases are one of the leading causes of death worldwide and lung inflammation plays a crucial role in most of these diseases. Several strategies have been explored over the years to tackle airway inflammation.

It has been shown that monoacylglycerol lipase (MAGL) inhibition and consequently the increase of 2-arachidonoylglycerol (2-AG) has beneficial effects on a model of acute lung injury.[1] We thus wanted to assess the effects of the inhibition of α/β hydrolase domain 6 (ABHD6) in acute lung injury and to compare them with the effects of MAGL inhibition.

Acute lung inflammation was induced by intra tracheal instillation of lipopolysaccharides (LPS, E. coli; O55:B5, 6µg/mouse) and inflammation was assessed 24 hours later. The ABHD6 inhibitor WWL70 (20 mg/kg) and the MAGL inhibitor JZL184 (16mg/kg) were administered (i.p.) to mice 1 h before LPS.

We show that WWL70 and JZL184 decreased leukocyte migration into the lungs, myeloperoxidase activity and pro-inflammatory cytokines and chemokines expression in the lung tissue. Both inhibitors also reduced protein concentration and production of the pro-inflammatory cytokines TNF-α and IL-6 in the bronchoalveolar lavage. Interestingly WWL70 reduced more parameters than JZL184. WWL70 also retained its effects when administered 1 h after LPS thus when inflammation was already ongoing.
Moreover, ABHD6 inhibition not only increased 2-AG levels but also the levels of some lysophospholipids, which were proposed as ABHD6 substrates[2], in the lung. Therefore, we conducted mechanistic studies on primary alveolar macrophages to assess the role of 2-AG and lysophospholipids on LPS-induced macrophage activation. Indeed, macrophages are key players in lung inflammation.

While additional studies are needed to decipher the molecular players involved in effects of the ABHD6 inhibitor on lung inflammation, these data further support the interest of interfering with 2-AG metabolism in the context of lung inflammation.

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Acknowledgements: Pauline Bottemanne is a FRIA Doctoral Researcher and Mireille Alhouayek is a Post-doctoral Researcher at the FRS-FNRS (Fonds de la Recherche Scientifique)

DOES CANNABIS USE MITIGATE THE EFFECT OF POST-TRAUMATIC STRESS DISORDER ON DEPRESSION AND SUICIDAL IDEATION? PRELIMINARY EVIDENCE FROM A REPRESENTATIVE SAMPLE OF CANADIANS

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Post-traumatic stress disorder (PTSD) sharply increases the risk of depression and suicide. Although individuals living with PTSD frequently use cannabis to manage symptoms of the disorder, little research exists evaluating the use of cannabis for therapeutic purposes among people with PTSD. This study was conducted to test whether cannabis use modifies the association between PTSD and experiencing suicidal ideation or a major depressive episode (MDE).

Data was obtained from the 2012 Canadian Community Health Survey-Mental Health (CCHS-MH), a representative cross-sectional survey of non-institutionalized Canadians aged 15 and older. Logistic regression was used to model the relationship between PTSD and: suicidal ideation; and MDE, controlling for demographic characteristics and current mental health and substance use comorbidities, and stratified by cannabis use status.

Among 24,089 eligible respondents, 420 (1.7%) reported a clinical diagnosis of PTSD. In total, 106 (28.2%) people with PTSD reported cannabis use (>1 time) in the previous year compared to 11.2% of those without PTSD ($p<0.001$). In multivariable models adjusted for demographic characteristics and comorbid mental health and substance use disorders, PTSD remained significantly positively associated with recent suicidal ideation and MDE among cannabis non-users (Adjusted odds ratio [AOR] = 4.82, 95% confidence interval [CI]: 2.41–9.63; AOR = 3.29, 95% CI: 1.83 – 5.94, respectively). PTSD was not associated with suicidal ideation or MDE among cannabis-using respondents (AOR = 1.67, 95% CI: 0.78 – 3.56; AOR = 0.97, 95% CI: 0.44 – 2.15, respectively). In sub-analyses of 420 respondents with PTSD, respondents who used cannabis without meeting criteria for a cannabis use disorder were less likely than cannabis non-users to experience suicidal ideation (AOR = 0.40, 95% CI: 0.15 – 1.05) or MDE (AOR: 0.35, 95% CI: 0.14 – 0.92).

This preliminary study provides the first epidemiological evidence to support the hypothesis that cannabis mitigates the effect of PTSD on severe depressive and suicidal states. Furthermore, lower-risk cannabis use may be associated with improved mental health outcomes among individuals suffering from PTSD. These findings highlight a need for high-quality experimental investigation of the effectiveness of cannabis/cannabinoids for the treatment of PTSD.

Acknowledgments: Stephanie Lake is funded by doctoral scholarships from the Canadian Institutes of Health Research and the Pierre Elliott Trudeau Foundation. Thomas Kerr is supported by a Foundation grant from CIHR (FDN-148476). Zach Walsh is the PI of an ongoing clinical trial of cannabis for PTSD sponsored by Tilray, a licensed producer of medical cannabis in Canada. Evan Wood receives support through a Tier 1 Canada Research Chair in Inner City Medicine. M-J Milloy is supported by the United States National Institutes of Health (U01-DA0251525), a New Investigator award from the Canadian Institutes of Health Research and a Scholar award from the Michael Smith Foundation for Health Research. His institution has received unstructured funding from NG Biomed Ltd., a private firm seeking a license to produce medical cannabis from the Canadian government, to support his research.

ALTERED EXPRESSION LEVEL OF MGL AND FAAH IN THE PHENCYCLIDINE MOUSE MODEL OF SCHIZOPHRENIA

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Schizophrenia is a chronic psychotic disease that affects 1% of the world population. It has been documented that increased use of cannabis is a risk factor associated with the onset of psychosis. Accordingly, a number of studies have found significant changes in the endocannabinoid system in patients with schizophrenia and in animal models of schizophrenia. Changes in the endocannabinoid system that were found in patients include genetic polymorphisms, changes in the level of cannabinoid CB_1 receptor and changes in the level of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG). Since these changes were found in different studies it remains unclear whether all these changes occur in the same time in the same brain area. The aim of the current study was to test the mRNA expression level of monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH), both are endocannabinoid inactivating enzymes, in a mouse model of schizophrenia. Schizophrenia-like symptoms were induced by sub-chronic injections of phencyclidine to mice. Phencyclidine is a glutamate NMDA receptor antagonist that induces in human psychosis and other impairments and in mice schizophrenia-like behaviour. The mRNA expression level of selected genes was examined by RT-PCR at age 26 days. We have focused the study to the prefrontal cortex, a brain area that is involved in the pathophysiology of schizophrenia.
The results show that the expression level of MGL and FAAH was significantly altered in the left prefrontal cortex of females, while no significant changes were found in males at the same age.
These results suggest that NMDA receptor inhibition alters the level of anandamide and 2-AG in the same brain area and point to gender differences. This study enhances to our understanding of how alterations in the endocannabinoid system may contribute the pathophysiology of schizophrenia.

Acknowledgments: This study was supported by The Institute for Psychobiology in Israel-founded by The Charles E. Smith Family, Young Investigator Award 244-09-2010, PI SAG, co-PI E. The authors are grateful to The Daniel Turnberg UK/Middle East Travel Fellowship Scheme (to SAG) for supporting of our research.
Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

CANNABINOIDS COMPOUNDS AS A TARGET FOR NOVEL ANTIPSYCHOTIC DRUGS

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The information processing appears to be deficient in schizophrenia which is a highly disabling disease. Prepulse inhibition (PPI), measures the inhibition of a motor response by a weak sensory event is considered particularly useful to understand the biology of information processing in schizophrenia patients. Drugs that facilitate dopaminergic neurotransmission such as amphetamine (AMPH) induce PPI disruption in human and rodents. Clinical effective antipsychotics reverse the AMPH disruptive effect. Cannabidiol (CBD), a non-psychotomimetic constituent of the Cannabis sativa plant, has also been reported to have potential as an antipsychotic. Studies demonstrated that CBD is able to attenuate the disruptive effect of AMPH in the PPI. CBD has been reported to act as an agonist of the vanilloid 1 channel in the transient receptor potential family (TRPV1R) and also to inhibit the hydrolysis and cellular uptake of the endogenous cannabinoid anandamide (AEA). TRPV1R are expressed in limbic areas, that are also part of the circuitry regulating sensorimotor gating. Moreover, in a clinical study with schizophrenic pacients, CBD treatment was accompanied by a significant increase in serum AEA levels, which was significantly associated with clinical improvement. Our aim was to investigate the mechanisms enrolled in the CBD effects. To investigate the involvement of TRPV1R in the CBD effects, male Swiss mice were systemically treated with either CBD or CBD preceded by the TRPV1R antagonist capsazepine (CPZ) prior to AMPH, and were exposed to PPI test. Finally, we investigated the effects of N-arachidonoy-serotonin (AA-5-HT) a dual inhibitor of FAAH and TRPV1R. The PPI test consist of 64 trials irregularly divided into pulse (P, white noise, 105dB), prepulse (PP; pure tone; 7kHz; 80, 85 or 90dB), prepulse + pulse (PP+P) and no-stimuli with white background noise level of 64dB – %PPI=[100-(PP+P/P)*100]. The percentage of PPI was analyzed with repeated measures with the treatment as the independent factor and the prepulse intensity as repeated measure. Duncan´s post hoc test ($p<0.05$) was used to specify differences.

Systemic CBD (30 or 60 mg/kg) attenuated the AMPH disruptive effects on PPI test at prepulse intensities of 80 and 85dB). CPZ blocked the ability of CBD to reverse the AMPH-induced disruption. The pretreatment with AA-5-HT (5 mg/kg) failed to block AMPH-induced disruption in PPI. CBD attenuates the AMPH disruptive effects in the PPI test, and this effect may be mediated by TRPV1R as evidenced by the reversal of CBD effect by CPZ. Corroborating the hypothesis that AEA has a role in the CBD antipsychotic-like effects, Previous study of our group showed that URB597, an AEA hydrolysis inhibitor had similar effects to CBD in the PPI test. Thus, our results demonstrate that the antipsychotic profile of CBD may involve concomitant increase in the availability of AEA and activation of TRPV1R.

Support: FAPESP; CNPq; CAPES; NAPNA; USP.

THE CB_1 POSITIVE ALLOSTERIC MODULATOR, ZCZ011, ATTENUATES SOMATIC SIGNS OF Δ^9-THC WITHDRAWAL

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ZCZ011 is a CB_1 positive allosteric modulator that increases the potency of CB_1 agonists in catalepsy, hypothermia, antinociception and allodynia assays. We hypothesized that ZCZ011 attenuates precipitated Δ^9-THC withdrawal in mice. Equal numbers of male and female 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, Δ^9-THC withdrawal induced paw tremors and head twitches, and also increased struggling in the tail suspension test and suppressed marble burying. Acute ZCZ011 (10 or 40 mg/kg, i.p.) significantly attenuated paw tremors and head twitches. ZCZ011 (10 or 40 mg/kg, i.p.) had no effect on struggling or marble burying. A separate group of male and female mice was administered a single dose of ZCZ011 (2.5, 5, 10, 20, or 40 mg/kg, i.p.) to probe for locomotor effects. Mice administered 40 mg/kg ZCZ011 displayed increased immobility that was not blocked by rimonabant (3 mg/kg, i.p.) pretreatment. These data support the use of CB_1 positive allosteric modulation as a strategy to reduce cannabinoid dependence.

MEDICAL CANNABIS USERS' HEALTH-RELATED QUALITY OF LIFE (HRQOL) AND DISCONTINUATION OF PRESCRIPTION MEDICATIONS

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Background: As legalization and utilization of medical cannabis (MC) continues to expand in the U.S., a small but growing body of evidence supports the use of cannabis and cannabinoids for their analgesic, anticonvulsant and anti-inflammatory properties in the treatment of a range of chronic conditions. While recent findings suggest that some persons using MC may discontinue prescription opioid-based medications in the treatment of chronic pain, the degree to which other HRQoL concerns (e.g., anxiety, insomnia, depression) influence decisions to discontinue prescription medications is relatively unknown.

Methods: We conducted a cross-sectional online survey of persons with a state medical marijuana card in Illinois (N=367) recruited from licensed MC dispensaries across the state. We summarized participants' qualifying conditions, HRQoL domains addressed by MC, MC efficacy in improving HRQoL symptomology, and discontinuation of prescription medications. We then used multivariate logistic regression models to analyze associations of specific conditions and MC efficacy in improving HRQoL symptomology with discontinuation of prescription medications.

Results: Most commonly reported HRQoL domains improved by MC were pain (74.9%), anxiety (65.7%), insomnia (56.4%), and depression (49.4%), and a large majority reported multiple symptoms treated (92.4%). Perceived efficacy of MC in relieving pain ($F=2.41$, $p<.05$), anxiety ($F=4.46$, $p<.01$), and depression ($F=5.31$, $p<.001$) increased with number of total symptoms experienced. Multivariate analyses indicated MC efficacy in relieving pain (OR= 1.61, 95%CI: 1.08, 2.39) and fibromyalgia diagnosis (OR=2.03, 95%CI: 1.16, 3.55) were significantly associated with discontinuation of opioid-based medications; MC efficacy in relieving anxiety (OR=1.88, 95%CI: 1.17, 3.02) and post-traumatic stress disorder diagnosis (OR=3.48, 95%CI: 1,40, 8.64) were significantly associated with discontinuation of benzodiazepines.

Discussion:
The use of MC in this population extends across a range of HRQoL domains. Greater symptom relief appears to be afforded by MC among persons reporting multiple HRQoL-related symptoms. Persons with fibromyalgia and post-traumatic stress disorder diagnoses, in particular, appear to report improved HRQoL via MC and increased likelihood of effectively treating these conditions while discontinuing prescription medications. Future research with human participants may benefit from more granular examination of symptomology, utilization, dosing, and outcomes associated with MC.

PROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF CANNABINOID TYPE 2 RECEPTOR (CB2) ACTIVATION ON BLOOD BRAIN BARRIER

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Previous studies have shown that the receptor-mediated cannabinoid system during neuroinflammation can produce potent neuroprotective and anti-inflammatory effects. Little is known about how selective activation of CB_2 affects the activated state of the brain endothelium and blood–brain barrier (BBB) function during neuroinflammation. Using human brain tissues and primary human brain microvascular endothelial cells (BMVEC), we demonstrate that the CB_2 is highly upregulated during HIV infection and inflammatory insults. In vitro CB_2 agonists increased barrier tightness and increased the amount of tight junction proteins in BMVEC, decreased adhesion/migration of monocytes across BBB models and expression of adhesion molecules in BMVEC treated with proinflammatory mediators. These results were further confirmed in vivo where CB_2 agonists attenuated adhesion to and migration of leukocytes across the BBB (assessed by intravital microscopy), diminished expression of adhesion molecule and attenuated BBB 'leakiness' in mouse model of LPS or TNF-induced neuroinflammation. We recently identified novel CB_2 agonists which tightened BBB, diminished monocyte adhesion/migration across BBB models *in vitro* and protected BBB in animal models after oral administration. We also demonstrated that selective CB_2 activation in human leukocytes diminished their ability to engage the brain endothelium and migrate across BBB in vitro and in vivo preventing its injury. Therefore, CB_2 ligands offer a new strategy for BBB protection during neuroinflammation.

EARLY TREATMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS WITH CANNABIDIOL SUPPRESSES SPLENIC TC1 CELLS AND REDUCES NEUROINFLAMMATION

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Multiple sclerosis (MS) is a neurodegenerative, T cell-mediated, autoimmune disease. In this study, we examined use of the phytocannabinoid, cannabidiol (CBD), in the experimental autoimmune encephalomyelitis (EAE) model to determine CBD's potential as an oral therapy for MS. We hypothesized that 75mg/kg of oral CBD for 5 days after initiation of disease would reduce EAE severity through suppression of either the early peripheral immune or late neuroimmune response. EAE was induced in C57BL/6 mice at two different magnitudes, and peripheral inflammatory and neuroinflammatory responses were measured at days 3, 10, and 18. Th1, Th17, Tc1, Tc17, Tregs, and myeloid derived suppressor cells (MDSC) were identified in lymph node and spleen by flow cytometry to determine if CBD altered these cell populations in the secondary lymphoid tissues. Additionally, neuroinflammation was identified in brain and spinal cord using hematoxylin and eosin (H&E) and CD3 staining to detect cellular infiltrates and T cells, respectively. Our results show a clear correlation between reduction in the percentage of MOG_{35-55} specific Tc1 cells in the spleen at day 10, and reduced neuroinflammation and clinical scores at day 18 in EAE mice treated with CBD; however, this correlation was not seen in the milder form of disease. These results suggest CBD's ability to reduce neuroinflammation in EAE might be due to early suppression of the Tc1 phenotype in the peripheral inflammatory response, and that CBD's ability to suppress disease is dependent on the magnitude of the inflammatory response. Furthermore, this study highlights the effectiveness of oral CBD as a treatment for EAE and suggests that CBD might be effective for treatment of other T cell-mediated diseases.

Acknowledgements: Funded by the MSU-CVM Centers of Biomedical Research Excellence (COBRE) (Grant: P20GM103646), and Mississippi State University Summer Research Experience Program (Grant: 2T35OD010432)

VOLUNTARY EXERCISE ENHANCES THE EXTINCTION OF FEAR AND REDUCES ANXIETY-LIKE BEHAVIORS IN WILD TYPE BUT NOT CB1 RECEPTOR KNOCKOUT MICE

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Introduction: The endocannabinoid (eCB) system has emerged as a promising target for enhancing fear extinction learning, which has therapeutic implications for the treatment of stress and anxiety disorders, such as Posttraumatic stress disorder. Although previous investigations have used exogenous pharmacological approaches, there is a strong rationale to investigate non-pharmacological approaches, such as exercise, that have been shown to activate the eCB system. We previously demonstrated that voluntary exercise significantly enhanced fear extinction, reduced anxiety-like behaviors, and increased amygdala 2-AG content in male ICR/CD-1 wild-type mice. Therefore, the purpose of the current study was to expand the aforementioned findings by examining male CB1 receptor knockout mice in order to more fully understand the role of the eCB system in exercise-induced enhancement of fear extinction.

Methods: Experiments were conducted on male CB1 receptor knockout mice (N = 12). All mice were housed under standard laboratory conditions for a 10-day acclimation period following transfer to the behavioral core. Next, mice completed a series of baseline behavioral tests (one test every 24 hrs) in order to assess anxiety- and depressive-like behaviors (i.e., open-field test, OFT; elevated plus maze, EPM; forced swim test, FST). Forty-eight hours later, all mice were administered a fear conditioning protocol involving the presentation and pairing of an auditory conditioned stimulus with an aversive, inescapable, electric foot shock. Immediately following completion of the fear conditioning protocol, mice were randomly assigned to caging containing either an unlocked (EX) or locked (CON) running wheel, with unrestricted access until 24 hrs following the last fear-extinction session. For the next four days, all mice were placed in the same chambers and taken through a fear extinction protocol involving the presentation of 20 tones in absence of electric shock. Following completion of the fear extinction protocol, mice completed the behavioral tests a second time. Data were analyzed using a series of one-way and mixed model ANOVAs, Pearson correlations, and Cohen's *d* effect sizes.

Results: No significant ($p > .05$) group, time, or group x time interactions were found for the anxiety-like behavioral outcomes, as both groups experienced moderate to large increases in anxiety-like behaviors during the OFT (distance traveled; CON $d = 0.54$; EX $d = 0.59$) and EPM (% open arm entries; CON $d = 1.63$; EX $d = 1.52$) from pre- to post-experiment. There were no significant ($p > .05$) correlations between running wheel revolutions and behavioral outcomes. Regarding fear outcomes, there were no significant ($p > .05$) group differences in the acquisition of fear during the fear conditioning session (day 1). Additionally, no significant ($p > .05$) group, time, or group x time interactions were found regarding freezing behavior during fear extinction sessions (days 2-5), suggesting that exercise did not significantly enhance the extinction of learned fear. Moreover, there were no significant correlations between running wheel revolutions (past 24 hrs) and freezing behavior during subsequent fear extinction sessions on days 2-5.

Conclusion: These preliminary results suggest that voluntary exercise does not significantly enhance the extinction of fear or reduce anxiety-like behaviors in male mice lacking the CB1 receptor. These results are in direct contrast to our previous work that found enhanced fear extinction, reduced anxiety-like behaviors, and increased amygdala 2-AG content in exercised wild-type male mice possessing the CB1 receptor. Taken together, these preliminary results suggest a role for the eCB system in exercise-induced enhancement of fear extinction in male mice.

Acknowledgements: Funded by the UW Virginia Horne Henry Fund and the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin.

DEVELOPMENT OF A PERIPHERALLY RESTRICTED CB1 RECEPTOR ANTAGONIST FOR ALCOHOLIC STEATOSIS

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Type 1 cannabinoid receptor (hCB1) antagonists are useful for treating obesity, liver diseases, metabolic syndrome and dyslipidemias. However, inhibition of hCB1 receptors in the central nervous system (CNS) can produce adverse effects including depression, anxiety and suicidal ideation in patients. Currently, CB1 receptor antagonists that lack CNS penetration are under development. We have synthesized and characterized a series of potent and selective aryl and heteroaryl substituted analogues of otenabant (CP-945,598) with limited brain penetration. Several of these compounds were predicted to have limited brain permeability based on an in vitro model (penetration of MDCK mdr1 cell monolayers) of CNS penetration. A 2-chlorobenzyl piperazine inverse agonist from this series of compounds has favorable properties for continued development. This compound is potent (Ki = 8 nM) and >1000-fold selective for hCB1 over hCB2, has excellent solubility in acidic media (>100 μM), good stability in human liver microsomes, low P450 induction potential and no hERG liability. Additionally, this compound failed to reverse cannabinoid induced hypothermia confirming little to no CNS penetration in mice. Additional behavioral phenotyping studies of this compound are underway. Molecular modeling studies indicated that the ligand environment contains hydrophobic interactions within the diaryl region but the substituted piperazine moiety extends into a more polar region at the top of the transmembrane helices of hCB1 that precedes an access channel to the extracellular space. Finally, the CB1 inverse agonist was tested in a mouse model of alcoholic liver steatosis induced by feeding a Lieber DeCarli diet containing alcohol for 4 weeks. At an oral twice daily dose of 1.25 mg/kg, this peripherally selective inverse agonist blocked liver steatosis. In conclusion, an advanced candidate targeting hCB1 receptors in the liver and other peripheral tissues has been identified for further development to treat alcoholic liver disease and other important indications.

Acknowledgement: This work was made possible by R01AA023256 from NIAAA to RM

RAPID DETECTION OF UNKNOWN COX-2 DERIVED METABOLITES OF N-3 POLYUNSATURATED ENDOCANNABINOIDS IN LPS STIMULATED MACROPHAGES

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Cyclooxygenase-2 (COX-2) is a non-constitutional enzyme that is specifically expressed during inflammation. The enzyme oxygenates poly-unsaturated fatty acids (PUFAs) such as arachidonic acid (AA) as well as neutral lipids like the endocannabinoid arachidonoyl ethanolamide (AEA), leading to various metabolites that have regulatory roles in inflammation. Previously published data on the interaction between the ω-3 derived endocannabinoid docosahexaenoylethanolamide (DHEA) and COX-2 suggested that DHEA may also be a substrate for COX-2. To better understand the interactions between endocannabinoids and COX-2, we designed a new cell free screening assay consisting of hCOX-2 incubations followed by LC-HRMS analysis to qualitatively identify metabolites. To show the validity of this rapid screening assay, we first successfully demonstrated the enzymatic synthesis of known prostaglandins and monohydroxylated PUFA's from AA, and ω-3 PUFAs, respectively. Next, the formation of several novel oxygenated metabolites of DHEA and related congeners was demonstrated, of which 13-HDHEA and 16-HDHEA were shown to be the hCOX-2 products of DHEA. Finally, we showed that 13-HDHEA and 16-HDHEA were also produced in LPS-stimulated murine RAW264.7 macrophages. These results underline the potential of our new LC-HRMS screening assay to rapidly detect *in vitro* formed hCOX-2 products. In addition, this assay demonstrates that hCOX-2 can metabolize DHEA into two previously unknown metabolites, 13-HDHEA and 16-HDHEA.
Acknowledgements: This research project is funded by the VLAG Graduate School of Wageningen University & Research

ENDOCANNABINOIDS IN THE GUT CONTROL NUTRIENT SENSING AND GUT-BRAIN SATIATION SIGNALING

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Gut-brain signaling is important for food intake and energy balance; however, the impact of consuming high-energy nutrients on these pathways is not well established. Our studies suggest that overeating associated with diet-induced obesity (DIO) is driven by heightened gut-brain endocannabinoid (eCB) signaling. When compared to mice maintained on low-fat/low-sugar chow, those fed a western-style diet (i.e., high fat and sucrose) for 60 days displayed large increases in daily caloric intake, meal size, and rate of feeding, which was met with a more than two-fold increase in levels of the eCBs, 2-AG and anandamide, in upper small intestinal epithelium and plasma. Pharmacological inhibition of eCB signaling at cannabinoid CB_1 receptors in the periphery with the peripherally-restricted neutral CB_1 receptor antagonist, AM6545 (10 mg/kg), completely normalized meal patterns in western diet-fed mice to levels found in lean controls. AM6545 had no effect on food intake in control mice. We next tested the hypothesis that eCB signaling at CB_1 receptors in the gut modulates feeding behavior by controlling nutrient-induced gut-brain satiation signaling. Indeed, CB_1Rs were found to be co-localized with cells in the mouse upper small intestinal epithelium that express the gut-derived satiation peptide, cholecystokinin (CCK). Oral gavage of corn oil potently increased CCK-8 levels in plasma of lean mice, an effect blocked by pretreatment with the cannabinoid receptor agonist, WIN 55,212-2 (5mg/kg). The actions of WIN were reversed by co-administration of AM6545, highlighting the role for CB_1Rs in this response. In contrast to lean mice, oral gavage of corn oil failed to affect CCK-8 levels in DIO mice, which have elevated levels of eCBs in small-intestinal epithelium; however, pretreatment with AM6545 normalized the ability for corn oil to induce CCK-8 release. Furthermore, co-administration of AM6545 with a low dose of the CCK-A receptor antagonist, devazepide (0.1 mg/kg), completely blocked the hypophagic actions of AM6545 on meal patterns in DIO mice. Collectively, our data suggest that heightened eCB signaling at small intestinal CB_1Rs drives overeating in DIO by a mechanism that includes inhibiting nutrient-induced release of CCK-8, which in turn, delays satiation.

Acknowledgements: Funding from the National Institutes of Health grant DA034009 to NVD.

THERAPEUTIC EFFECTS OF PROLONGED CANNABIDIOL TREATMENT ON PSYCHOLOGICAL SYMPTOMS, COGNITIVE FUNCTION AND HIPPOCAMPAL SUBFIELD VOLUMES IN REGULAR CANNABIS USERS: A PRAGMATIC OPEN-LABEL CLINICAL TRIAL

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Chronic cannabis use has been associated with hippocampal structural alterations, impaired cognition and elevated psychological symptoms, particularly psychotic-like experiences. Whilst Δ^9-tetrahydrocannabinol (THC) is thought to be primarily responsible for these deleterious effects, cannabidiol (CBD) is purported to have antipsychotic properties and to ameliorate cognitive, symptomatic and brain harms in cannabis users. However, this has never been tested in a prolonged administration trial in otherwise healthy cannabis users. Here we report the first study of prolonged CBD administration to a community sample of regular cannabis users in a pragmatic trial investigating potential restorative effects of CBD on psychological symptoms, cognition and hippocampal subfield volumes. Twenty frequent cannabis users (16 male, median age 25 years) underwent a 10-week open label trial of 200mg of daily oral CBD treatment, whilst continuing to use cannabis as per usual. The majority of participants were daily cannabis users who had used cannabis for several years (median 5 years regular use). Participants underwent psychological, cognitive and MRI assessments at baseline and post-treatment and were monitored weekly throughout the trial.

CBD was well tolerated with no reported side- or adverse effects. Participants retrospectively reported reduced euphoria when smoking cannabis but did not change their cannabis use patterns. Importantly, participants reported significantly fewer depressive and psychotic-like symptoms at post-treatment relative to baseline and exhibited improvements in attentional switching, verbal learning and memory and greatest benefits in dependent users. There was also significant increase in hippocampal subicular complex volume post-treatment, with marked growth in heavy users in the presubiculum and CA1. These changes in brain structure and function were associated with increased plasma CBD concentrations. Prolonged CBD treatment appears to have promising therapeutic effects for improving psychological symptoms and cognition and restoring hippocampal volumetric alterations in regular cannabis users. Our findings require replication given the lack of a placebo control in this pragmatic trial, but suggest that CBD may be a useful adjunct treatment for cannabis dependence as well as a range of other clinical indications (e.g. schizophrenia, Alzheimer's disease).

Acknowledgements: Funded by the Australian Research Council (FT110100752).

A SELECTIVE ACTIVITY-BASED PROBE REVEALS MITOCHONDRIAL LOCALISATION OF MAGL

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Inhibition of monoacylglycerol lipase (MAGL) is thought to have therapeutic value for neuroinflammatory diseases and breast cancer [1,2]. Here, we report the development of LEI-463 as a highly selective activity-based probe for MAGL that enables the study of MAGL expression and activity in tissues and cells. LEI-463 inhibited recombinant human MAGL with a pIC_{50} of 8.0 ± 0.2 in a natural substrate assay and could visualize MAGL activity in a gel-based activity-based protein profiling assay using mouse or rat brain membrane proteome without labeling ABHD6. LEI-463 visualized MAGL activity in an array of mice tissue proteomes, such as brain, liver, kidney, lung, heart, testis, pancreas and spleen, which was not observed in proteomes derived from MAGL knock-out mice. LEI-463 also labeled MAGL activity in a panel of breast cancer cells, including both non-aggressive luminal cells and highly aggressive basal cells. Confocal microscopy revealed subcellular expression of MAGL activity in mitochondria of human MCF7 cells, which was confirmed using high resolution correlative light electron microscopy. Chemical proteomics performed on purified mouse brain mitochondria confirmed the presence of MAGL. In a functional assay, MAGL inhibition led to reduced complex I activity, but not in brain lysates of cannabinoid CB_1 receptor knock out mice. In conclusion, development and application of the novel activity-based probe LEI463 revealed the activity of MAGL in mitochondria.

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POTENT AND SELECTIVE ENDOCANNABINOID REUPTAKE INHIBITORS: PHARMACOLOGICAL POTENTIAL FOR THE MODULATION OF THE ENDOCANNABINOID SYSTEM

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Selective endocannabinoid reuptake inhibitors (SERIs) represent a new class of modulators of the endocannabinoid system (ECS) which are potentially more effective and safer than other cannabinoid drugs. Unlike the covalent inhibitors of endocannabinoid (EC) degrading enzymes (e.g., FAAH, MAGL), SERIs can modulate EC actions in a more time- and space-restricted manner, thus potentially avoiding adverse effects. Recently, we reported the first prototype SERI (WOBE437) and its biological and pharmacological characterization *in vitro* and *in vivo* (Chicca et al., 2017). WOBE437 inhibited anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) uptake in different cell types with nanomolar potency and high selectivity towards all the other components of the ECS. Using a radiolabeled ^{14}C-WOBE437 analog, we showed that the molecule hits a saturable membrane target and only marginally penetrates into the cytosol. *In vivo*, WOBE437 behaved as an "indirect CB1-agonist" showing pronounced anti-inflammatory, analgesic and anxiolytic effects in mice, but lacking the high-dose anxiogenic effects seen with direct agonists of CB1 receptors. WOBE437 is orally bioavailable showing brain penetration in mice and specific modulation of brain EC levels, primarily increasing 2-AG. Over 7 days of treatment, WOBE437 induced a moderate but significant 1.5 fold rise of both AEA and 2-AG compared to vehicle, without triggering a loss of functional CB1 receptors in the brain (desensitization). In pharmacological experiments, the time-dependent distribution of WOBE437 was quantified and correlated to its effects on different metabolites using LC-MS/MS and targeted lipidomics. The observed EC modulation were in agreement with the potent anti-endotoxemia, anti-inflammatory, analgesic and anxiolytic effects of WOBE437 in mice. WOBE437 also showed analgesic and anti-inflammatory effects in a mouse model of monoarthritis, which could be blocked by CB1, CB2 and PPAR-gamma receptor antagonists, thus reflecting the polypharmacology of ECs. Moreover, WOBE437 significantly improved the clinical score in a mouse model of multiple sclerosis. In conclusion, WOBE437 and other SERIs may represent a promising and innovative therapeutic strategy for neuropsychiatric and inflammatory disorders.

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TOWARDS UNDERSTANDING THE ANTICONVULSANT EFFECT OF CANNABIDIOL: STRUCTURAL COMPARISON OF PHENYTOIN AND CANNABIDIOL METABOLITES BY MOLECULAR MODELING

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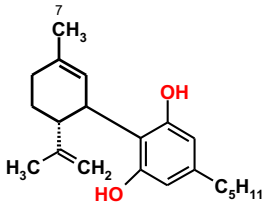
Cannabidiol (CBD; Epidiolex®, GW Pharmaceuticals, Cambridge, UK) is in clinical trials in children with intractable pediatric epilepsies but the mode of anticonvulsant action of CBD remains to be elucidated. However, similarities between the activity of CBD and phenytoin (PHT) in several models of epilepsy have been observed [1], and spatial conformational similarity of the two anticonvulsant drugs has also been noted [2]. Intriguingly, the human pharmacokinetics and pharmacology of the metabolites of CBD have scarcely been studied [3].

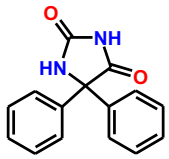
The key pharmacophore of PHT is the weakly acidic and hydrogen bond forming hydantoin moiety. Since hydantoins have been considered to be bioisosteric with carboxylic acids [4] and since most human metabolites of CBD are carboxylic acid species, we speculated that 7-COOH-CBD or similar metabolites of other phytocannabinoids displaying anticonvulsant effects could be responsible for the observed phenytoin-like activity of the parent phytocannabinoid *in vivo*.

To provide chemical rationale for this hypothesis we used molecular modeling to compare PHT with representative oxidized metabolites of CBD and cannabidivarin. For PHT and 7-COOH-CBD similarities were noted between the electrostatic potential maps projected onto the electron density surface of the molecules and obtained using ωB97X-D/6-31G* density functional method (Spartan'16, Wavefunction, Inc., Irvine, CA, USA).

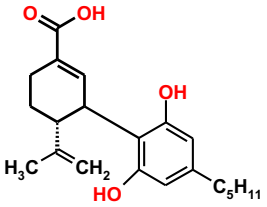
Based on the results we suggest further studies *in vitro* and *in vivo* of COOH-type CBD metabolites to reveal their involvement in the pharmacology and therapeutic effects of CBD-containing preparations.



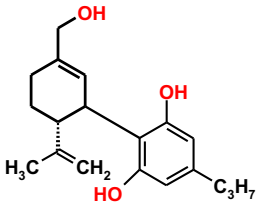
CBD



PHT



7-COOH-CBD



7-OH-cannabidivarin

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EFFECT OF HEAVY CANNABIS EXPOSURE ON NEUROCOGNITIVE FUNCTION IN PERSONS WITH OPIOID USE DISORDER

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Acute cannabis exposure is associated with neurocognitive (NC) impairment, and chronic exposure is associated with impairment in the NC domains of learning and memory. Though long-term cannabis users frequently use other substances associated with NC impairment, few studies have examined the independent NC effects of cannabis. As the U.S. opioid epidemic grows, combined exposure to cannabis and opioids may elevate risk for NC impairment. To test this, we evaluated NC function among persons with opioid use disorder (OUD) who were heavy cannabis users, compared to persons with OUD who were non/occasional cannabis users.

The sample included 91 adults with OUD initiating treatment with methadone or buprenorphine/naloxone in an urban treatment setting. Other than OUD, participants did not have severe psychiatric, neurologic, or major medical conditions. Participants completed measures of cannabis and other substance use (Addiction Severity Index, ASI), depressive symptomatology (Beck Depression Inventory, BDI-II), and NC functioning, including learning, memory, attention/working memory, executive functioning, processing speed, verbal fluency, and motor. Cannabis use was assessed by urine toxicology tests administered by the treatment program.

To assess NC function, we used a comprehensive, well-validated test battery, computed demographically-corrected T-scores for each test, and averaged individual test T-scores to create global and domain-specific T-scores, with NC impairment defined as T-score<40. To determine cannabis exposure, % THC-positive results for each treatment month was calculated, and Growth Mixture Modeling identified distinct cannabis use trajectories from month 1 through month 4. The minimum Bayesian Information Criterion identified two cannabis exposure groups: heavy users (30%) and non/occasional users (70%). We then used *t*-tests and ANCOVA analyses to assess NC differences between heavy and non/occasional users, adjusting for relevant covariates.

Among 91 participants, mean age was 42 years, 76% were male, 23% were Non-Hispanic white, 22% were non-Hispanic Black, and 52% were Hispanic. Depression was prevalent, with 31% scoring in the moderate-severe range on the BDI-II. Current non-opioid, non-cannabis substance use was common, with 41% reporting alcohol use and 40% reporting cocaine use. The sample's mean global NC T-score was in the low average range (M=41.7$\pm$6.4) and 34% were globally impaired, with impairment most prevalent in the NC domains of learning (79%) and memory (67%). Compared to non/occasional users, heavy cannabis users demonstrated significantly worse mean global (M=38.4$\pm$1.2 *vs*. M=42.4$\pm$0.8), learning (M=31.0$\pm$8.6 *vs.* M=35.9$\pm$7.8), and memory (M=31.4$\pm$1.8 vs. M=36.7$\pm$1.1) NC T-scores; all p's<0.01). No other significant group differences were observed in the remaining NC domains (all p's>.05).

In sum, we identified high rates of neurocognitive impairment among cannabis users with opioid use disorder, with heavy cannabis use associated with significantly worse global neurocognitive function and worse functioning in the specific domains of learning and memory. These findings suggest that cannabis use among persons with OUD may have significant neurologic implications.

CHANGES OF THE ENDOCANNABINOID SYSTEM IN HIV-1 TAT TRANSGENIC MICE

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The endocannabinoid system plays an important role in several neurodegenerative and neuroinflammatory diseases including Parkinson's disease, Alzheimer's disease, and neuropathic pain. Human immunodeficiency virus type 1 (HIV-1) infects the brain and, despite combined antiretroviral therapy (cART), many infected individuals suffer from HIV-1-associated neurocognitive disorders (HANDs). HAND is characterized by deficits in attention, executive function and inhibitory control of HIV-infected individuals, which is largely dependent on the fronto-striato-thalamo-cortical circuits, in particular the prefrontal cortex (PFC). In our current studies, HIV-1 Tat transgenic mice were used to investigate an animal model of inhibitory control deficits associated with HAND, where male and female mice were trained to perform the operant conditioning Go/No-Go task. Results demonstrate that transgenic Tat [Tat(+)] mice show less behavioral inhibition and increased impulsivity compared to their wild-type counterparts [Tat(-)]. This impairment was dose-dependently increased following administration of the cannabinoid 1 receptor (CB1R) antagonist rimonabant, specifically for male Tat(+) mice. No effect was noted for the CB2R antagonist SR144528. Further, PET imaging studies demonstrate an upregulation of CB1R in Tat(+) compared to Tat(-) mice using the radiotracer ^{11}C-JHU75528. The deficits in inhibitory control induced by the Tat gene appear to be related to an alteration in synaptic function. *Ex vivo* recordings from medial PFC slices of Tat(+) mice indicate significant increases in glutamatergic neurotransmission (EPSCs). Importantly, bath application of the FAAH inhibitor PF3845 significantly downregulated sEPSCs frequency for Tat(+) compared to Tat(-) slices, suggesting a decrease in excitatory neurotransmission. Understanding the effects of HIV-1 Tat on cognitive function, such as inhibitory control, may help to identify novel therapeutic interventions to benefit individuals suffering from HAND and other cognitive impairments. Our results indicate that the endocannabinoid system plays an important role in neurodegenerative effects of HIV1-Tat protein, and therefore further studies are necessary to reveal whether the manipulation of the endocannabinoid signaling may offer therapeutic solutions for treatment of HAND as well as other neurodegenerative conditions.

Acknowledgements: Funded by NIDA R21 DA041903, R00 DA033878, T32 DA007244, UNC CFAR P30 AI50410, R01 DA032933, and K05 DA021696.

PRO-ADIPOGENIC EFFECTS OF CBG AND CBGA IN CONGENITAL GENERALIZED LIPODYSTROPHIES

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Lipodystrophies (LD) are a heterogeneous group of genetic and metabolic disorders characterized by a generalized or partial fat loss, which may occur in combination with a redistribution of fat or a localized accumulation of subcutaneous adipose tissue. LD is generally associated with metabolic abnormalities, including diabetes mellitus, insulin resistance, hypertriglyceridemia, hepatic steatosis. LD can be divided into inherited or acquired forms and depending on the degree and locality of fat loss could be either generalized or partial (Fiorenza, Chou, & Mantzoros, 2011). So far, several mutated genes have been identified in human LD, pointing out their importance in adipose tissue physiology. In particular, PPARγ plays a key role in the control of lipid and glucose metabolism directly regulating a number of target genes (Jeninga, Gurnell, & Kalkhoven, 2009). Several lines of evidence show that phytocannabinoids (pCBs), such as THC and cannabidiol (CBD) bind to and activate the transcriptional activity of PPARγ (O'Sullivan and Kendall, 2010). However, pCBs strongly influence the adipose tissue (e.g. by induction of browning of white adipocytes, augmentation of lipolysis, thermogenesis, and reduction of lipogenesis) via mechanisms of action not uniquely relying on the presence of PPARγ (Morales et al., 2017; Parray and Yun, 2016). Any effect of cannabinoid therapy upon adipose tissue in these disorders has never been investigated. The aim of this study was to test the effect of these two pCBs in an animal model of congenital generalized lipodystrophy (CGL, Garg. 2011). Cav-1 knockout (KO) mice and genetically matched wild-type (WT) female mice were treated with two doses of CBG and CBGA (10 and 50 mg/kg, via ip) once a day for 5 weeks (vehicle and powder of pCBs were dissolved first in ethanol and injected in 1:1:18 ethanol:cremophor:0.9% saline.). We measured body weight (BW), food intake (FI), body mass index (BMI) and performed several behavioural tests to assess motor coordination, balance, neuromuscular strength before, 14 days and 28 days post treatments. Whereas we measured intraperitoneal glucose tolerance and performed tests for pain and depression at the end of treatments. Moreover, we measured plasma adipokine levels, gene expression and CBG and CBGA levels in adipose tissue depots. We also used the forced swim test and tail suspension test as tests and found that female Cav-1 KO mice were less motivated than WT mice. This phenotype was not sensitive to pCB treatment and was not due to locomotor impairment, as observed in rotarod and wire hanging tests, in which Cav-1 deficient mice showed a slightly better performance in the rotarod test in comparison to WT mice at 12 weeks of age ($p<0.05$) and no difference in the other test. Importantly, we found that Cav-1 KO mice display impaired glucose tolerance, which was reversed by CBG. Furthermore, we found that Cav-1 KO mice exhibit lower circulating levels of leptin and adiponectin, and downregulated PPARγ, FABP4 and cEBP mRNA levels in the perirenal adipose tissue. These data suggest that particularly CBG has the potential to modulate some of the biochemical markers present in lipodystrophy possibly through modulation of genes important in adipogenesis. Although this study have not identified a clear behavioural phenotype in the mouse, data did not show treatment effects upon behavior and further analysis is needed to investigate the effect of CBG on these biochemical parameters also under a high-fat diet regimen, the biochemical results support continued non-clinical investigation of the effects of CBG in LD.

Acknowledgements: Funded by GW Pharmaceuticals.

CANNABIS AND PAIN: EXAMINING THE RELATIONSHIP BETWEEN FREQUENT CANNABIS USE AND PAIN SENSITIVITY

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The extended use of opioid analgesics has the potential to result in a syndrome of pain intolerance and accentuated pain sensitivity described as opioid-induced hyperalgesia. Cannabis-based medicines are increasingly being considered as potential substitutes for opioid analgesics, making the extent to which the use of cannabis analgesics might also result in a hyperalgesic syndrome a clinical concern. However, although recent experimental studies have reported increased sensitivity to experimentally induced pain following cannabis administration, the extent to which frequent cannabis use (CU) influences pain sensitivity beyond the acute effects of cannabis ingestion has not been systematically examined. The present study sought to examine if frequent CU was associated with a differential sensitivity and tolerance to pain, and differences in past-month pain sensitivity and use of analgesics among frequent cannabis users. We also examined whether the effects were moderated or mediated by gender or individual differences in negative affect and sleep quality.

A sample of 40 cannabis users and 40 non-using controls completed the Cold Pressor Task (CPT) and a past-month pain query. The cannabis using group endorsed using cannabis at least twice weekly and used an average of 4.07 grams per week (SD = 5.35). Both groups were asked to abstain from alcohol use and the use of analgesics for at least 12 hours prior to the experimental session. Cannabis users were asked to abstain from CU for at least 12 hours prior to their appointment. Pain sensitivity was defined as the first report of pain (in seconds), tolerance as the duration that the hand was kept in the cold water (in seconds), and pain intensity was measured with a rating on visual analogue scale.

The groups did not differ in terms of pain sensitivity (U = 874.5, p = .47), tolerance (U = 891.5, p = .37), or intensity (U = 804, p = .97). Interestingly, the non-using control group showed a trend of more frequent use of pain medication in the past-month relative to those using cannabis (X^2 = 3.41, p = .07).

These findings suggest that frequent CU is not associated with differences in sensitivity to acute pain. Our results revealed group differences in pain medication use patterns that may be clinically relevant. Given previous experimental research that has shown an association between cannabis intoxication and hyperalgesia to the CPT, questions remain as to the mechanism underlying this association. In contrast to opioids, frequent CU does not appear to lead to hyperalgesia in healthy individuals and therefore might be a more suitable candidate for the treatment of chronic pain.

HIGH PREVALENCE OF MEDICAL AND NON-MEDICAL INTENTIONS FOR CANNABIS USE AMONG PEOPLE AT HIGH RISK OF DRUG-RELATED HARMS IN VANCOUVER, CANADA: A LATENT CLASS ANALYSIS

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BACKGROUND: In North America, uncontrolled outbreaks of HIV and unprecedented numbers of opioid overdose fatalities among people who use illicit drugs are ongoing public health emergencies. Although cannabis use is highly prevalent among people at risk of these harms, the reasons for cannabis use in this population have rarely been described. In light of emerging evidence showing cannabis use might play a beneficial role in addressing the ongoing overdose crisis, we sought to characterize cannabis use intentions among participants of three ongoing community-recruited cohorts of marginalized people who use drugs in Vancouver, Canada, a setting with high levels of drug-related harm and decriminalized access to cannabis.

METHODS: Data for these analyses came from participants in three harmonized open prospective cohorts ongoing since 2005: ARYS (street-involved youth who use drugs); ACCESS (HIV-positive people who use illicit drugs) and VIDUS (HIV-negative people who use injection drugs.) Using interview data from an open-ended question about cannabis use intentions, we employed latent class analysis to assign individuals into classes and multivariate logistic regression to estimate associations between class membership and various individual-, behavioural-, clinical-, social- and structural-level characteristics.

RESULTS: Between December, 2016 and June 2017, 1462 individuals completed a study interview. Of these, 802 (55%) reported cannabis use in the last six months and were included in these analyses. The five most common reasons for using cannabis were "to get high" (56% of participants), pain relief (30%), address stress (27%), treat insomnia (26%), and treat nausea (24%). Fit statistics for the latent class models indicated a four class solution was superior, partitioning individuals into:

1. people mainly using for non-medical reasons (318, 40%);
2. people mainly using for pain, insomnia, nausea and stress (308, 38%);
3. people mainly using for pain, stress, and to substitute for other psychoactive substances, including licit and illicit opioids and illicit stimulants (169, 21%);
4. and a small class of 7 individuals.

In multivariate models, participants in class 1 were less significantly likely to be HIV-positive (Adjusted Odds Ratio [AOR] = 0.53), report chronic pain (AOR = 0.56), or use cannabis ≥ daily (AOR = 0.42.) People in class 2 were significantly more likely to be older (AOR = 1.02 per year), use cannabis ≥ daily (AOR = 1.88), be HIV-positive (AOR = 2.57), report chronic pain (AOR = 1.59), and access cannabis from dispensaries (AOR = 1.61). People in class 3 were less likely to be HIV-positive (AOR = 0.89.)

CONCLUSIONS: We observed a substantial prevalence of cannabis use among participants in three community-recruited cohorts of marginalized people who use illicit drugs. Many cannabis users reported both medical and non-medical reasons for cannabis use. Addressing chronic pain and substituting for licit and illicit opioids and stimulants were common intentions in these participants. Our findings are in concordance with other emerging research describing the use of cannabis by people who use drugs to reduce the likelihood of harm from using other substances. These analyses support further investigation of the possible controlled use of cannabis as a novel strategy to mitigate the risk of accidental drug overdose.

Acknowledgments: The authors thank the study participants for their contribution to the research, as well as current and past researchers and staff. The study was supported by the United States National Institutes of Health (U01DA038886, R01DA021525). This research was undertaken, in part, thanks to funding from the Canada Research Chairs program through a Tier 1 Canada Research Chair in Inner City Medicine which supports Dr. Evan Wood. Dr. Kora DeBeck is supported by a Michael Smith Foundation for Health Research (MSFHR)/St. Paul's Hospital Foundation–Providence Health Care Career Scholar Award and a Canadian Institutes of Health Research (CIHR) New Investigator Award. Dr. Kanna Hayashi is supported by a CIHR New Investigator and MSFHR Scholar Award. Stephanie Lake is funded through doctoral scholarships from CIHR and the Pierre Elliott Trudeau Foundation. M-J Milloy is supported by the United States National Institutes of Health (U01-DA0251525), a New Investigator award from CIHR and a Scholar award from MSFHR. His institution has received an unstructured gift from NG Biomed Ltd., a private firm seeking a license to produce medical cannabis from the Canadian government, to support him.

MEDICAL AND NON-MEDICAL USE OF CANNABIS AMONG HIV-POSITIVE PEOPLE WHO USE ILLICIT DRUGS IN VANCOUVER, CANADA: IMPLICATIONS FOR THE PLANNED LEGALIZATION OF CANNABIS

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The legalization of cannabis for medical and non-medical purposes is imminent in Canada. Cannabis is frequently reported among people living with HIV to manage symptoms of the disease and address side effects of antiretroviral therapy (ART). People who use illicit drugs (PWUD) living with HIV report comparatively higher rates of cannabis use, but little is known about reasons for cannabis use, particularly as they relate to HIV disease management, in this highly marginalized population.

Data was drawn from the AIDS Care Cohort to evaluate Exposure to Survival Services (ACCESS) study, a cohort of HIV-positive PWUD in Vancouver, Canada. This cross-sectional study was restricted to 224 participants who reported cannabis use in the previous six months between June 1 and December 1, 2016. Descriptive statistics were used to summarize sources of cannabis and reasons for use. Chi-square and Wilcoxon rank-sum tests were used to compare characteristics of therapeutic and non-therapeutic cannabis users.

In total, three-quarters of participants (n=169) reported using cannabis for therapeutic purposes, most commonly to address nausea or loss of appetite (40.2%), pain (31.7%), sleep (29.9%), and stress (23.2%). About two-thirds of participants (n=148) obtained cannabis from local dispensaries or compassion clubs rather than dealers or other illicit means. None reported accessing medical cannabis through the government-approved system. Compared to participants who reported non-medical use only, those who reported therapeutic use were more likely to use cannabis daily, have an undetectable viral load, and have a higher CD4 cell count in the previous six-month period ($p<0.05$).

The majority of HIV-positive PWUD in this study reported using cannabis therapeutically, including to manage HIV symptoms/ART side effects, substitute higher-risk substances, and treat mental or physical health. Despite this, no participants had government-authorized access to medical cannabis and most obtained cannabis through a "quasi-legal" source. Although longitudinal research is needed, the findings suggest that therapeutic cannabis use may be associated with favourable HIV disease and treatment outcomes. Monitoring the impact of Canada's planned regulatory system for legal cannabis on access to medical cannabis for this key vulnerable population should be prioritized.

Acknowledgments: We would like to thank the study participants for their contribution to the research, as well as current and past researchers and staff. This study was supported by the US National Institutes of Health (NIH; U01-DA021525). Stephanie Lake is supported by doctoral awards from the Canadian Institutes of Health Research (CIHR) and the Pierre Elliott Trudeau Foundation. Thomas Kerr is supported by a Foundation grant from CIHR (FDN-148476). Dr. Nadia Fairbairn is supported by a Scholar award from the Michael Smith Foundation for Health Research and the Research in Addiction Medicine Scholars Program from the NIH (R25-DA033211). Evan Wood receives support through a Tier 1 Canada Research Chair in Inner City Medicine. M-J Milloy is supported by the US National Institutes of Health (NIH; U01-DA021525), a New Investigator Award from CIHR, and a Scholar Award from the Michael Smith Foundation for Health Research (MSFHR). His institution has received unstructured funding from NG Biomed Ltd., a private firm seeking a license to produce medical cannabis from the Canadian government, to support his research.

THE EFFECT OF DOCOSAHEXAENOYL-SEROTONIN ON HYPOTHALAMIC INFLAMMATION

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Cancer-induced muscle wasting (cachexia) is directly associated with higher mortality in cancer patients and it has a severe impact on the quality of life. Hypothalamic inflammation has been reported to play a key role in cancer cachexia. In this study we tested the effect of docosahexanoic acid (DHA), serotonin and the acyl-amine docosahexaenoyl-serotonin (DHA-serotonin) on hypothalamic inflammation in vitro. The effect on a co-culture of hypothalamic HypoE-46 cells and BV-2 microglial cells was compared to the effect on monocultures of these two cell lines. Microglial cells were seeded on top of hypothalamic cells that had grown for 24h. Inflammation was initiated with the addition of 10 to 31,6 ng/mL LPS. These low concentrations of LPS resemble the physiological concentrations in the hypothalamus. At the same time point that LPS was added, DHA-serotonin was added at different concentrations. IL-6 and MCP-1 excretion was measured with ELISA. LDH, BCA and WST-1 assays were performed to exclude toxicity.

LPS stimulated IL-6 and MCP-1 release in the hypothalamic cells. IL-6 and MCP-1 release were higher in the co-culture (180% and 220% respectively). These LPS concentrations had no influence on cytokine excretion of the microglial cell monoculture. Addition of 0.3 uM DHA-serotonin decreased the IL-6 release in the co-culture with 25% ($p<0.05$), but not in the hypothalamic monoculture. The combination of serotonin and DHA (control) at the same concentrations had no effect. MCP-1 release was not affected by the addition of DHA-serotonin. These data indicate that cytokine production is different in the co-culture when compared to the mono-cultures, suggesting a sensing of the inflammation by the hypothalamic cells and a moderating effect by the microglial cells. DHA-serotonin has potential for reducing this hypothalamic inflammation.

LAMINAR DISTRIBUTION AND CELLULAR LOCALIZATION OF CB1R, NAPE-PLD, AND FAAH IN THE PRIMARY VISUAL CORTEX OF VERVET MONKEYS

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The expression and localization of the endocannabinoid system have been well characterized in recent years in the monkey retina and in the dorsal lateral geniculate nucleus (dLGN). However, few data are available on primate cortical visual structures. The goal of this study is to characterize the expression and localization of the cannabinoid receptor type 1 (CB1R), the synthesizing enzyme N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), and the degradation enzyme fatty acid amide hydrolase (FAAH) in the vervet monkey primary visual cortex (V1). Using Western blots and immunohistochemistry, we investigated the laminar and cellular expression patterns of CB1R, NAPE-PLD, and FAAH across the rostrocaudal axis of the vervet monkey (*Chlorocebus sabaeus*) area V1.

CB1R, NAPE-PLD, and FAAH were expressed in V1 throughout the rostrocaudal axis. CB1R showed very low staining in layer (L) 4, with higher expression in all other layers, especially L1, followed by L2 and L3. NAPE-PLD and FAAH expression patterns were similar, but not quite as low in L4. The low level of CB1R in L4 suggests less direct endocannabinoid modulation of V1 afferents from the dLGN, but that modulation may occur via the higher expression of CB1R in L2 and L3 on the way to the dorsal and ventral visual streams. This is further supported by the higher expression of NAPE-PLD and FAAH in these layers. CB1R, NAPE-PLD, and FAAH are localized in some but not all vGlut2-positive cells, representing glutamatergic projection neurons. They are also localized in somatostatin (SST)-positive cells, a class of interneurons which suppress lateral and feedback interactions. These data indicate that CB1R could influence the network of activity patterns in the visual streams after the visual information has reached V1, and thus may influence visual perception.

Acknowledgements: Funded by the Natural Science and Engineering Research Council of Canada (6362-2017, Dr Maurice Ptito; RGPAS 478115-2015 and RGPIN 2015-06582, Dr Jean-François Bouchard) and the Canadian Institutes of Health Research (MOP-130337, Dr Jean-François Bouchard). Dr Joseph Bouskila is supported by a NSERC postdoctoral fellowship. Pr Jean-François Bouchard was supported by a “Chercheur-Boursier Senior” from the Fonds de recherche du Québec Santé (FRQ-S). Pr Maurice Ptito is Harland Sanders Chair professor in Visual Science.

THE EFFECT OF CANNABIDIOL ON 2,5 DIMETHOXY-4-IODOAMPHETAMINE (DOI)-INDUCED HEAD TWITCHES

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Head twitch response in mice is a distinctive behaviour, which does not resemble other types of head movements and can occur spontaneously at a very low frequency. Administration of DOI, a potent agonist of the serotonin 5-HT_{2A}/5-HT_{2C} receptors, to adult mice increases head twitch response. DOI-induced head twitch response has been proposed to model motor tics of Tourette syndrome. Tourette syndrome is a neuropsychiatric disorder which is defined as part of the tic disorder spectrum. It affects about 1% of the general population with a childhood oneset.

It has been previously shown in other studies that Δ^9-THC reduces DOI-induced head twitch response, further supporting reports describing a significant amelioration of symptoms when cannabis was used by patients with Tourette syndrome. However, the effect of cannabidiol (CBD) in the mouse DOI model of Tourette syndrome has not yet been investigated. Our results show that DOI induces dose-dependently head twitch response in juvenile C57BL/6J mice, resembling the onset of motor tics in children with Tourette syndrome. CBD had a small, but significant, reversal effect on head twitch response. Surprisingly, CBD alone significantly increased the head twitch response in healthy juvenile mice.

These results show that CBD cannot effectively reverse motor-like tics which are mediated by 5-HT_{2A}/5-HT_{2C} receptors. These results suggest that CBD may not effectively treat motor tics in children and may even exacerbate tics in a population of patients.

Acknowledgment: The Research Grant Award, Tourette Association of America (SAG). Kamin Award, Officer of Chief Science, Ministry of Science and Technology, Israel (SAG). Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL SYNAPSES ARE MODIFED BY FAAH DELETION IN 5xFAD ALZHEIMER'S DISEASE MODEL

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Alzheimer's disease (AD) is a pathology characterized by neuroinflammation and neuronal death due to the deposition of the amyloid-beta peptide in the form of neuritic plaques in the brain parenchyma together with the formation of neurofibrillary tangles of tau protein. In this context, the endocannabinoid system (ES) has shown a neuroprotective potential in brain injuries through elevation of endocannabinoids with neuroprotective properties. Not only endocannabinoids are involved in neuroprotection against chronic inflammation but also endogenous receptors such as CB_1 and CB_2 and degradative enzymes like FAAH (Fatty Acid Amide Hydrolase) are essential in this process.

Previous studies in AD mouse models have shown that FAAH gene deletion generates an exacerbated inflammatory response associated with astroglial and microglial activation. Paradoxically, spatial memory, which is impaired in AD models, improves upon FAAH deletion. Thus, in this study we analyze the electrophysiological properties of the AD mouse model 5xFAD/FAAH$^{-/-}$.

In this work we uncover the impact of FAAH deletion on basal synaptic transmission and synaptic plasticity at the CA3-CA1 hippocampal synapse. Our electrophysiological recordings demonstrate that basal synaptic transmission, which is impaired in 6-month old 5xFAD mice, is rescued upon FAAH deletion. LTP is rescued as well in 5xFAD/FAAH$^{-/-}$ mice comparing to 5xFAD animals. Therefore, the increment of anandamide as a consequence of FAAH deletion, ameliorates the impairment in synaptic plasticity and basal synaptic transmission in 5xFAD mouse model.

Acknowledgements: This work was supported by Ministerio de Economía y Competitividad (SAF2016/75959-R), and Universidad Francisco de Vitoria.

CANNABINOID REGULATION OF EXCITATORY SYNAPTIC TRANSMISSION AT HIPPOCAMPAL TA-CA1 SYNAPSES

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The endogenous cannabinoid system, composed of neuromodulatory endogenous lipid ligands and their cannabinoid receptors, has crucial physiological and regulatory roles throughout the body. It is known that cannabinoids produce their biological effects via activation of CB1 and CB2 receptor subtypes (Battista et al., 2012), however in the CNS, the predominant cannabinoid receptor is CB1. Numerous studies have examined the modulatory effects of cannabinoids on excitatory synaptic transmission at hippocampal schaffer collateral (SC)-CA1 synapses. Indeed, evidence suggests that hippocampal cannabinoid receptors not only play a role in learning and memory, but they are also linked to neurodegeneration in Alzheimer's disease (AD). However the effects of cannabinoids on excitatory synaptic function at the anatomically-distinct temporoammonic (TA) input to CA1 neurons is not clear.

Here, standard extracellular recordings were used to examine the effects of different selective agonists for CB1 receptors on excitatory synaptic transmission at juvenile TA-CA1 synapses. Transverse hippocampal slices (350μM) were prepared from 12-18 day old rats and perfused with oxygenated aCSF. Application of (R) - (+) - methanandamide (50nM; 15min) resulted in a transient increase in excitatory synaptic transmission (to 137 ± 7% of baseline) that returned to baseline on washout. Similarly, application of ACEA (100nM; 15min), a highly selective CB1 receptor agonist, caused a transient increase in synaptic transmission (to 132 ± 6% of baseline) that returned to baseline on washout. Addition of a higher concentration of (R) - (+) - methanandamide (100nM; 15min) induced a long term increase (to 148± 5% of baseline) in synaptic transmission. These data indicate that CB1 receptor activation modulates excitatory synaptic transmission at hippocampal TA-CA1 synapses in a dose dependent manner. These findings may be important as the TA pathway plays a role in episodic memory (Remondes & Schuman 2004) and impairments in episodic memory is an early event in AD (Hodges 2000).

DISCOVERY OF SULFONYL-1,2,4-TRIAZOLE UREAS AS DIACYLGLYCEROL LIPASE INHIBITORS BY HIGH THROUGHPUT SCREENING

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Diacylglycerol lipases (DAGLα and DAGLβ) are serine hydrolases responsible for the formation of the endocannabinoid 2-arachidonoylglycerol (2-AG). 2-AG is a full agonist of the cannabinoid CB_1 and CB_2 receptors and functions as the main precursor for arachidonic acid and pro-inflammatory eicosanoids in the brain. Several DAGL inhibitors have been reported in the literature, but most of these compounds do not possess the selectivity or pharmacokinetic properties to act as drug candidates. Thus, there is an unmet need to identify novel chemotypes to modulate DAGL activity. We performed a high throughput screening coupled to an orthogonal activity based protein profiling assay to screen >300,000 compounds for DAGL-α. Sulfonyl-1,2,4-triazole ureas were discovered as novel chemotype and subsequently optimised to low nanomolar *in vitro* potency. *In situ* and *in vivo* activity was subsequently shown, with excellent target engagement in mouse brain. This class represents the most hydrophilic class of DAGL inhibitors reported to date.

Acknowledgements:
The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n' 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7 / 2007-2013) and EFPIA companies' in-kind contribution. We thank the Dutch Research Council–Chemical Sciences for the ECHO-grant to APAJ and MvdS.

ENDOCANNABINOIDS INCREASE SLEEP AND PROTECT AGAINST SEIZURES IN *DROSOPHILA MELANOGASTER*

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Endocannabinoids (eCbs) possess anticonvulsant and hypnotic properties. Several studies suggest that these effects may involve non-canonical cannabinoid (Cb) signaling pathways. *Drosophila melanogaster* is a powerful model organism for studying the molecular mechanisms underlying behavior, but to date the effects of Cbs in flies has not been studied. This is likely due to the fact that flies lack homologs for the canonical Cb receptors. However, flies have detectable levels of eCbs as well as homologs for other putative eCb targets. Fly experiments therefore offer a unique opportunity to study the mechanism of action of eCbs through non-canonical receptors that may be obscured by classical Cb receptor interactions in mammals. The aim of my study is to use *Drosophila melanogaster* to determine the effects of eCbs on sleep and seizures, and to identify the mechanisms and pathways responsible. For sleep studies, flies were fed methanandamide (mAEA), a metabolically stable analog of the eCb anandamide, and sleep was monitored using the Drosophila Activity Monitoring System. Sleep was also monitored in mutant flies with decreased expression of CG8839, a putative ortholog of the eCb catabolizing enzyme FAAH2. Results show that mAEA increased total sleep time, and that mutant flies with decreased expression of CG8839 slept more and had a shorter latency to sleep onset relative to controls. For seizure experiments, *eas* flies, which carry a mutation rendering them susceptible to mechanically induced seizures, were fed mAEA. The proportion of flies seizing after mechanical stimulation was compared. mAEA protected against mechanically induced seizures. This effect was blocked by AMG9810, a TRPV1 antagonist, but not by MK806, a PPARα antagonist. In summary, these studies show that eCbs promote sleep through an unknown mechanism and protect against seizures in a TRPV1 dependent manner in *Drosophila*.

DESIGN, SYNTHESIS AND APPLICATIONS OF NOVEL HIGHLY SELECTIVE CANNABINOID RECEPTOR 2 RADIOLIGANDS

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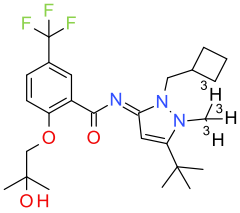
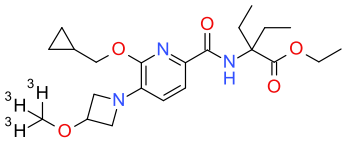
The type 2 cannabinoid receptor (CB_2R) is a promising GPCR drug target for the treatment of tissue injury and inflammatory diseases. Highly selective CB_2R agonists show robust efficacy in various animal models of central and peripheral diseases. The lack of specific anti CB_2R antibodies and suitable biomarkers for target occupancy hampers the clinical development of CB_2R agonists. We have developed various probes and reporter molecules that bind to CB_2R with high selectivity against CB_1R. Our highly selective CB_2 radio ligands will help to address important biological questions. Tritiated CB_2 ligands e.g. allow for localization of the CB_2 protein in tissue sections via binding and autoradiography studies. Furthermore they enable the determination of binding kinetic properties for CB_2 drug candidates[1]. Positron emitting CB_2 ligands also enable the visualisation of CB_2 protein in animal models. In addition, positron emission tomography (PET) is a powerful molecular imaging technique to study dynamic processes such as drug/receptor interactions in humans[2].



[^{3}H]RO6753361
CB_2 Ki 2 nM
Ki Ratio h CB1/h CB2: 7'074
Agonist



[^{3}H]RO6957022
CB_2 Ki 3 nM
Ki Ratio h CB1/h CB2: >1'195
Inverse Agonist

RO6753361 and RO6957022[3] have recently been discovered as highly selective tritiated CB_2 agonists and inverse agonists, respectively. We report here the synthesis and in depth characterization of stably (^{3}H, ^{14}C) and ^{11}C (PET) labelled radio-analogues as well as their application in CB_2 protein detection studies. A detailed discussion of these results as well as the way forward toward to further improved CB_2 radio ligands (^{3}H and PET) will be the subject of this communication.

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THE EFFECT OF MORPHINE AND ETHANOL ON SPINAL CORD INJURY WITH TREATMENT BY CANNABIDIOL AND B-CARYOPHYLLENE

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Rationale: Morphine, an opiate analgesic used for treating acute and chronic pain, and ethanol, a sedative used commonly for its anxiolytic psychological effects, have both been shown to have high abuse liability. Further, both have been shown to activate pro-inflammatory mechanisms potentially leading to exacerbation of spinal injury and worsening of recovery. Cannabinoids, specifically cannabidiol (CBD) and β-caryophyllene (BCP), are both non-psychoactive components of the *Cannabis* plant. Both cannabinoids have been shown to exhibit anti-inflammatory properties in rodent models of alcoholism. **Objectives**: In these studies, our research investigated how morphine with cannabinoid paired treatment, as well as ethanol alone, will affect locomotor recovery from spinal cord injury. To further investigate inflammatory effects of alcohol, we evaluate the hepatic macrophage phenotypes (M1 vs. M2) in control and ethanol fed mice. We hypothesized that morphine combined with cannabinoids will lead to improvement in recovery compared to morphine alone. We additionally hypothesize that ethanol will exacerbate spinal cord injury and lead to robust inflammation at the site of injury. Then, in ethanol animals without SCI, we hypothesize that ethanol will increase hepatic macrophage counts with this increase attenuated by administration of CBD or BCP. **Methods**: SCIs were conducted in female C57/BL6 mice and mini-pumps containing either morphine or vehicle solution were implanted subcutaneously. Morphine infusions were administered 0.5uL per hour continuously for 7 days and a subset of animals were treated with either vehicle, CBD, or BCP I.P. once per day for 7 days. Changes in locomotor and bladder function, hindpaw thermal and tactile sensitivity in injured mice were evaluated. Separately, a Lieber-DeCarli diet (infused with ethanol or control) was provided to injured and non-injured mice 5 days after injury for 10 days. Locomotor and thermal sensitivity were evaluated as well as consumption of diet. To investigate ethanol effects on injury, spinal cords were dissected and stained to measure injury volume and the glial profile of injured and non-injured mice. Livers from ethanol and control mice were evaluated using IBA-1 staining. **Results:** Morphine infusion alone lowered BMS scores in SCI mice and increased thermal and mechanical sensitivity in non-injured mice. Treatment with CBD reversed thermal sensitivity of morphine but also worsened bladder recovery in injured mice. Treatment with BCP did not reverse thermal sensitivity but did not show worsening of bladder recovery. Injured mice given ethanol had smaller lesion sizes compared to non-injured mice and fluorescent staining of glial cells were also lower in ethanol fed mice. Injured mice given control diet consumed more diet post-injury. Mice consuming ethanol had increased 'M2' counts compare to control which was mitigated by CBD treatment. **Conclusions:** Morphine infusion deteriorated locomotor function in injured mice and worsened mechanical sensitivity in sham mice. Treatment with CBD ameliorated thermal sensitivity while BCP did not elicit significant effects to alleviate pain. SCI induces hyperphagia in mice given control diet and ethanol exhibits a neuro-protective effect by decreasing injury volume and preventing the pro-inflammatory effects of neuroglia which is also evidenced by the decrease in M2 counts by CBD in the liver.

Acknowledgements: Funded by the Department of Defense, W81XWH-14-1-0389 (SJW)

COMBINATION CANNABINOID THERAPY IN THE TREATMENT OF ISCHEMIC STROKE IN A MOUSE PHOTO-THROMBOTIC MODEL

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Strokes are the third leading cause of death in United States and the patients who survive suffer major motor and cognitive disabilities. Damage from an ischemic stroke involves the initial infarct due to lack of perfusion and secondary damage caused by activation of the immune response. Many of the current treatments for stroke involve prophylactic care and Tissue Plasminogen Activator to re-perfuse the tissue, but there is currently no treatment targeting the immune response following the onset of a stroke. Our laboratory has previously shown that selective cannabinoid CB2 receptor agonists, such as O-1966, target the secondary immune response and lead to attenuated infarct sizes in a mouse model. Our goal was to determine whether combination cannabinoid therapy, which is a current treatment option for other diseases such as Multiple Sclerosis, would prove even more beneficial in the treatment of ischemic stroke than single cannabinoid therapy.

We selected to determine the effects of the cannabinoid CB2 receptor agonist sesquiterpene beta-caryophyllene (BCP) and the non CB1/CB2 receptor binder cannabidiol (CBD) alone and in combination in a mouse photo-thrombotic stroke model. Combination CBD-BCP therapy showed a statistically significant reduction in infarct size three days following induction. Immunofluorescent staining also demonstrated significant changes in microglial state of the cortex tissue surrounding the infarct border. These findings strengthen the prior assumption that combination therapies can provide a greater benefit than single treatments alone, and open the door to new potential research with Cannabis-containing CB2 agonists in combination with CBD in the treatment of ischemic stroke and other diseases.

Acknowledgements: Department of Defense award W81XWH-14-1-0389 “Novel strategies to mitigate spinal cord injury and secondary inflammation following spinal cord injury (PI: SJW)

BEHAVIOURAL CHARACTERIZATION AND STUDY OF THE ENDOCANNABINOID SYSTEM OF A KNOCK-IN MOUSE MODEL OF DRAVET SYNDROME

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Introduction: Dravet Syndrome (DS) is a rare genetic epileptic syndrome caused by mutations in the human *SCN1A* gene encoding the α1 subunit of the voltage-gated sodium channel Nav1.1. The disease initiates in the first year of life usually associated with febrile seizures that progress to severe partial or generalized tonic-clonic seizures. They tend to become less frequent during adolescence and, in some cases, appear to remit in adulthood. Normally, DS is often accompanied by hyperactivity and behavioral disturbances that are common in childhood, but are not frequently seen in adulthood. On the contrary, patients exhibit many autistic like traits in adulthood. Current therapies for DS, mainly based on classic antiepileptic agents, are limited, but recent studies, using cannabidiol, have recruited new hopes for the treatment of seizures, and even for the long-term cognitive and motor deficits seen in DS patients. The aim of this study was to characterize a new DS conditional knock-in mouse model in relation with those behavioural, neurochemical and histopathological signs that better reflect the disease. Such model may be extremely useful for evaluating cannabinoid treatments.

Methods: Heterozygous conditional knock-in mice (B6(Cg) -Scn1atm1.1Dsf/J) carrying a missense mutation in *SCN1A* gene, A1783V, were crossed to Cre recombinase mice under the synapsin-1 promoter (CreB6.Cg-Tg(Syn1-cre)671Jxm/J), then generating offspring bearing the A1783V mutation neuronal population. Animals were subjected to different behavioural tests to analyze movement deficits (computer-aided motor activity, rotarod), emotional and cognitive impairment (elevated-plus maze test and Y-maze), and autistic-like traits (social interaction). Biochemical and histological analyses were carried out by qRT-PCR and immunolabeling, respectively. The study was concentrated on an age, postnatal day 25 (PND25), in mice equivalent to early ages in humans, when main features of this disease are evident.

Results: At PND25, DS mice showed hyperactivity reflected in a significant increase of total distance travelled, frequency of fast movements and rearing activity *versus* the controls. No difference was found in motor coordination using rotarod test. In the elevated-plus maze, the percentage of time spent in open arms by DS mice was higher than the controls,
suggesting a lower level of anxiety-like behaviour in DS mice. We also found that DS mice presented a reduced social behaviour, displaying significant less interaction time with novel unfamiliar partner compared to the controls. The neurochemical analysis revealed the existence of a decrease in CB_1 receptor levels and MAGL expression in the cerebellum as well as a significant reduction in MAGL expression in prefrontal cortex. No difference in the expression of the other endocannabinoid elements analyzed in various CNS areas. We also detected microglial and astrocyte recruitment and reactivity in the cerebral cortex, striatum and hippocampus of DS mice, using Iba-1 and GFAP immunolabeling respectively.

Conclusions: Our results revealed that DS mice present behavioral disturbances in parallel to certain endocannabinoid dysregulation and neuroinflammatory events, resembling the situation in humans. It appears a useful model to develop pharmacological studies with cannabinoids.

Supported by MINECO (SAF2015-68580-C2-1-R) and SANTANDER-UCM (PR26/16-20352)

CANNABIDIOL PREVENTS NEONATAL BRAIN HYPOXIA-ISCHEMIA-INDUCED LONG-TERM INCREASE OF SEIZURE SENSITIVITY IN RATS

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Background: epilepsy is a major long-lasting sequela from newborn hypoxic-ischemic encephalopathy (NHIE). The standard of care, therapeutic hypothermia (TH), has not been proven to reduce the incidence of NHIE-epilepsy. Cannabidiol (CBD) has neuroprotective effects in murine models of NHIE, as well as anticonvulsant properties. The aim is to study CBD's effects on NHIE-induced increased seizure sensitivity (SS) in rats as compared to TH.

Methods: unilateral HI brain damage was induced in newborn Wistar rats (7 days-old: P7) by exposure to hypoxia (10% FiO_2) for 112 min after left carotid artery electrocoagulation under anaesthesia. Thirty min post-HI, pups were randomly assigned after the end of HI either to normothermia (NT, 5 h at 38 ºC) or HT (5 h at 33.0-33.5 ºC), and vehicle (VEH) or CBD (GW Research Ltd, Cambridge, UK) 1 mg/kg single dose i.p. Non-HI pups receiving VEH remained as controls (SHM). Neurobehavioral assessment was carried out: at P14, negative geotaxis (motor coordination); at P37,Cylinder Rear Test (CRT, hemiparesis) and Novel Object Recognition (NOR, memory); at P67, Open Field Test (OF, movement and anxiety). At P14, P37 and P67, SS was determined as the dose of Pentylenetetrazol (PTZ) (infused i.v. in a solution of 10 mg/mL at 0.5 mL/min) necessary to induce seizures. At P67 brain histological damage was quantified by determining brain cortical neuron, astrocyte and microglia density NeuN, GFAP, Iba-1, respectively, by immunohistochemistry (IHC).

Results: seizure threshold at different ages and groups are shown in Table 1.

	NT			HT		
	SHM	HI+VEH	HI+CBD	SHM	HI+VEH	HI+CBD
P14	17,24 (1,81)	12,05 (1,29)	18,12 (2,99)	61,13 (4,41)	29,14 (7,73)	42,62 (11,85)
P37	52,87 (5,05)	48,09 (2,19)	65,95 (7,96)	63,36 (13,13)	49,51 (4,14)	63,2 (4,52)
P67	32,31 (2,19)	22,94 (3,49)	39,67 (3,46)	38,82 (4,4)	25,78 (2,78)	40,93 (3,85)

PTZ dose to induce seizures, in mg/kg. Results expressed as mean(SEM) from 8-12 experiments. (*) $p<0.05$ vs SHM+VEH+NT; (§) $p<0.05$ vs HI+VEH+NT; (#) $p<0.05$ vs SHM+VEH+HT; (¶) $p<0.05$ vs HI+VEH+HT, all by Mann-Whitney with Bonferroni's correction for multiple comparisons.

Juvenile and adult SS was increased by NHIE as shown by the reduced seizure threshold in HI+VEH+NT. Treatment with HT reduced SS at P14 and P37 but not at P67. By contrast, CBD administration to newborn rats just after the HI insult restored seizure threshold as assessed at every timepoint, an effect not enhanced by HT co-treatment. Seizure threshold at P67 correlated with motor impairment at P14 (Geotaxis vs. SS, Pearson's R=-0.37, p=0.03) andP37 (CRT vs SS, Pearson's R=-0.43, p=0.04). At P67, SS was related to anxiety (number of entries on open field vs. SS, Pearson's R=0.43, p=0.01) but not to motor impairment.IHC results revealed a correlation between SS and astrogliosis (GFAP), but not with neuron (NeuN) or microglia (Iba-1) density.

Conclusions: HIE in newborn rats led to increased SS in adulthood, being even more sensitive than in childhood and adolescence. This was not related to the brain damage observed in adults (P67), but to parameters reflecting the neonatal damage. Thus, increased SS in adulthood seems to be a sequela from NHIE rather than reflecting current adult brain damage. CBD reduced NHIE-induced increase in SS, likely due to its neuroprotective effects after HI. *Supported by grant from GW Research Ltd.*

NEUROPROTECTIVE EFFECT OF CANNABIDIOL IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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Parkinson's disease is a progressive and untreatable neurodegenerative condition. Administration of 6-hydroxydopamine (6-OHDA) into the striatum produces selective damage in the dopaminergic neurons of basal ganglia, microglial cell activation, and parkinsonism-like symptoms. Cannabidiol (CBD) is the main no-psychotropic agent of *Cannabis*. CBD has shown neuroprotective effects including in animal models of PD. However, has not been demonstrated the mechanisms underlying the neuroprotection evoked by CBD in the PD. Thus, since the PD is a neurodegenerative condition without effective pharmacological treatment to prevent the progression of the disease, we suggest that CBD could interfere with PD evolution. We used C57BL/6 mice submitted to the unilateral lesion of nigrostriatal pathway evoked by 6-OHDA injection into the striatum. CBD (i.p., 10, 30 or 60 mg/kg) was administered for 5 days (treatment started on the day of 6-OHDA injection into the striatum).

The dose of 10 and 60 mg/kg did not alter the dopaminergic loss in the nigrostriatal pathway. However, 30mg/kg of CBD reduced the microglial cell activation as well as the loss of both dopaminergic neuronal terminals in the striatum and the number of dopaminergic cells in the substantia nigra pars compacta. Moreover, CBD increased the ambulation of animals in the actimeter test, the number of touches with the right paw in the cylinder test and decreased the immobility time in the tail suspension test as well as decreased the oscillations ratio between the right and left side of the body of animals. 30mg/kg of CBD also improved the novel object recognition memory. Therefore, CBD protected the dopaminergic neurons of the nigrostriatal pathway and improved the motor deficits and the cognitive decline demonstrated by animals injected with the neurotoxin 6-OHDA.

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

A frase acima deve ser colocada nos agradecimentos do painel ou na apresentação oral.
Financial support: FAEPA, Prati-Donaduzzi, FAPESP.

LONG-LASTING NEUROPROTECTIVE EFFECTS OF COMBINING CANNABIDIOL SINGLE OR MULTIPLE DOSE AND HYPOTHERMIA IN HYPOXIC-ISCHEMIC RATS

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Background: The standard in newborn hypoxic-ischemic (HI) brain damage is therapeutic hypothermia (TH). Cannabidiol (CBD), which shows long-lasting neuroprotective effects in HI newborn rats, has demonstrated some additive neuroprotective effects to TH in a short-term study (6h) of HI newborn pigs.

Aim: To study whether CBD has long-lasting and sustained additive neuroprotective effects to TH in newborn rats and to compare the effects of administering CBD by single or multiple doses.

Methods: P7 HI Wistar rats (left carotid artery plus 112 min-long hypoxia [10% FiO_2]) were randomly assigned 30 min postHI to normothermia (NT, 5 h at 37 ºC) or hypothermia (HT, 5 h at 32-33,5 ºC) and i.p. vehicle (HVsd) or CBD (GW Research Ltd, Cambridge, UK) 1 mg/kg single dose (HCsd) or multiple doses (HCmd: i.p. 6 h, 24 h and 48 h postHI and then enteral for 10 day). Non-HI NT or HT pups remained as controls (SHM). A set of neurobehavioral (NB) tests was conducted a P14 negative geotaxis [coordination] and grasp test [strength]) and P37(Cylinder Rear Test, CRT [hemiparesis] and Novel Object Recognition, NOR [memory]).

Results: results from NB tests are shown in table 1.

	NT				HT			
	SHAM	**HI+VEH**	**HI+CBD^{sd}**	**HI+CBD^{md}**	**SHAM**	**HI+VEH**	**HI+CBD^{sd}**	**HI+CBD^{md}**
Geotaxis (sec)	5.0 (0.4)	8.3 (1.1)*	7.1 (0.7)*	9.9 (2.0)*	5.2 (0.4)	8.2 (0.8)*&	5.5 (0.4)	7.4 (1.0)*&
Grasp (points)	1.5 (0.1)	1.1 (0.1)*	1.1 (0.1)*	0.6 (0.2)*&	1.3 (0.1)	0.9 (0.1)$	1.1 (0.1)$	0.9 (0.2)$
CRT (%)	-5.7 (4.8)	19.4 (5.0)*&	3.3 (6.7)#	5.6 (6.8)#	9.3 (9.3)	33.9 (10.9)$§	8.0 (12.0)†	17.8 (6.3)†
NOR (%)	54.5 (4.2)	33.6 (5.8)*&	52.8 (7.4)#	47.0 (7.1)#	45.9 (5.2)	26.8 (4.9)$§	37.0 (6.2)†	43.5 (6.5)†

Results expressed as mean(SEM) from 8-12 experiments. (*) p<0.05 vs SHM+NT; (#) p<0.05 vs HI+VEH+NT; (&) p<0.05 vs HI+CBD^{sd}+NT; ($) p<0.05 vs SHAM+HT; (†) p<0.05 vs HI+VEH+HT; (§) p<0.05 vs HI+CBD^{sd}+HT all by ANOVA.

The HI insult led to reduced coordination and strength at P14 as well as to increased hemiparesis and memory impairment at P37. HT was associated with no protective effect. By contrast, CBDsd led to neuroprotective effects as shown by NB tests at P14 and P37. Administration of CBD multiple dose did not improve CBDsd effects at P37. CBDmd results were even worse than those of CBDsd in the grasp test. Noteworthy, enteral administration of VEH or CBD led to vagal spasms in HI rats, which could have been influencing the results of CBDmd. Combining CBD and HT rend no benefit to CBD alone, though co-administration of CBD and HT showed better results than HI+VEH+HT.

Conclusions: CBDsd led to sustained neuroprotective effects in HI rats. TH, the standard of treatment, rend no protection. Post-HI enteral multiple dose of CBD did not rent additional benefit but further studies using multiple i.p. are warranted to avoid the effects of enteral administration-induced vagal spasms occurring in a vulnerable post-HI period. *Supported by grants from PI 13/07122 and GW Research Ltd.*

ORAL Δ^9-TETRACANNABADIOL DECREASES WEIGHT AND INCREASES MRNA FOR PYY, INSULIN, AND PRO-INFLAMMATORY FACTORS IN OBESE MICE

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GPR119 is a G-protein coupled receptor known for its effects on glucose metabolism, implicating this receptor as a potential therapeutic target for type II diabetes. Previously, we found that THC is a low efficacy agonist at GPR119 in GPR119 transfected HEK cells, causing internalization and potentially initiating downstream signaling events. Additionally, we showed that obese mice treated via IP injections of THC lost significant amounts of weight. However, THC can also modulate the activity of various immune cells by activating CB1 or CB2 receptors, potentially altering inflammatory responses. Therefore, it is important to investigate the implications behind using THC to treat obesity through GPR119, due to confounding responses such as inflammation, which can occur despite lacking a direct and specific signaling pathway. The purpose of this study was to determine whether THC treatment through oral dosing in obese mice would promote results similar to those seen upon IP injections of THC, and to investigate the specific proteins involved in glucose tolerance by identifying mRNA. We hypothesized that oral dosing THC treatment of obese mice would affect metabolism and increase weight loss, as well as induce changes in mRNA for certain metabolic proteins.

Mice were fed a high fat diet until they reached 25% above average weight, at which point they were given their first oral dose of THC. A dose of 10 mg/kg of THC was administered orally on a daily basis and weights were measured daily. At the end of the 14-day treatment period, the pancreas, large intestine, and cerebellum tissues were harvested and analyzed for changes in metabolic marker mRNA using qRT-PCR.

We found that oral dosing of THC in obese mice potentiated the average weight loss (11.6% of their weight). Oral THC dosing also promoted upregulation of PYY in the large intestine, and insulin in the pancreas. In the cerebellum, we observed an increase of mRNA for Ptgs2, IL-1β, and GFAP while TNF-α mRNA levels decreased in THC treated groups. IL-1β and TNF- α are inflammatory cytokines, Ptgs2 is the gene that encodes for COX2, and GFAP is a protein that plays an important role in the function of the blood brain barrier. PYY activates hypothalamic Y2R receptors, thus increasing feelings of satiety, and insulin initiates the uptake of glucose into cells.

Oral treatment of THC in obese mice lead to weight loss, via activation of GPR119, and subsequent upregulation of PYY and insulin. Upregulation of inflammatory markers in the cerebellum could potentially be due to obesity related factors, THC use, or a combination of the two. Although the use of THC to treat type II diabetes through GPR119 may be a novel therapeutic option, it is important to first identify and understand how THC not only affects glucose metabolism, but how it affects other important physiological processes, when coupled with obesity.

IMPLICIT CANNABIS COGNITIONS PREDICT CANNABIS USE

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Cannabis initiation and use in adolescence is complex. As such, it is important that research examines not only the apparent consequences of adolescent cannabis use, but also the cognitions that precede and maintain use. Research suggests that substance use is influenced by both explicit and implicit cognitions (Wiers et al., 2002). Explicit cognitions may be influenced by current events while implicit cognitions may be based on affective experiences early in life (Rudman, 2004). As such, the two types of cognitions may differentially effect substance use. While much work has been done on explicit cognitions, the field is now exploring the role of implicit cognitions in adolescent substance use (Cameron, Brown-Iannuzzi, & Payne, 2012; Fulton et al., 2012; Payne, Lee, Giletta, & Prinstein, 2016).

Implicit cognitive tasks measure substance-related cognitions that are spontaneous and activated without causing the participant to engage in deliberate recall or introspection about the cause of their attitude (Ames et al., 2007). Implicit measures are less sensitive to the effects of social desirability, thus providing a different measure of attitude than explicit measures offer. Explicit reports are more likely to be influenced by self-presentation biases (Wiers & Stacy, 2006). Implicit measures also may help explain circumstances where behavior appears to be incongruent with explicitly held beliefs or attitudes (Wiers & Stacy, 2006). Collectively, studies utilizing implicit measures suggest that participants who respond with higher frequency of drug or alcohol related responses are more likely to report greater levels of substance use (Ames et al., 2007).

The present study examined the utility of an implicit cognitive measure to predict adolescent cannabis use longitudinally. To do so, implicit cannabis cognitions were measured at baseline and 6 months later. We recruited 535 eighth grade students from two school districts in British Columbia, Canada. Implicit cognitions were measured using the affect misattribution procedure (AMP; Payne & Lundberg, 2014). This procedure measured implicit reactions to visual stimuli through affective misattributions. The reliability and validity of this procedure is well-established with other stimuli, including alcohol-related images (Cameron, Brown-Iannuzzi, & Payne, 2012). To our knowledge, this study is the first to use the AMP to predict the trajectory of cannabis use in a large sample of Canadian adolescents. Attitudes toward substance use, social norms, and intention to initiate using cannabis were also measured.

Preliminary analyses indicate that implicit cannabis cognitions at baseline are positively related to having used cannabis in the past 12 months when queried at a 6-month follow-up. Further, these cognitions were positively related to an explicit measure of cannabis cognitions. These finding suggest preliminary evidence for the predictive ability of implicit measures on cannabis use. Comprehensive longitudinal results, implications, and directions for future research will be discussed.

NEUROPROTECTIVE EFFECTS OF THE PHYTOCANNABINOID CANNABIDIOLIC ACID (CBDA) COMPARED TO RILUZOLE IN TDP-43 TRANSGENIC MICE, A MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive and disabling disease characterized by progressive loss of upper and lower motor neurons, which produces muscle denervation, weakness and atrophy leading lastly to paralysis and death frequently by respiratory failure. In fact, 90% of patients are deceased within 3 years of diagnosis. At present, the only approved treatment for ALS is riluzole, an antiglutamatergic agent that increases survival by only 3-4 months. Edaravone is a mitochondria-acting agent recently approved by the FDA, based on the results obtained in a small clinical trial with early stage ALS patients, but its mechanism of action in ALS is still unknown. Due to these limited treatment options, cannabinoids have been proposed as a promising multitarget strategy for ALS. Phytocannabinoids, such as Δ^9-tetrahydrocannabinol or cannabinol, have been investigated as potential neuroprotective agents in animal models of ALS with promising results. The interest in phytocannabinoids for the treatment of ALS has now been extended to other minor plant-derived cannabinoids, such as cannabidiolic acid (CBDA), which may provide a different pharmacological profile compared to classic phytocannabinoids. In this study, we have investigated the neuroprotective properties of CBDA in Prp-hTDP-43(A315T) transgenic mice, an experimental model of ALS, and we compared these effects to those observed with riluzole. Mice were treated daily with several doses of CBDA (0.1, 1 and 10 mg/kg, i.p.) (GW Research Ltd, Cambridge, UK) or riluzole (5 and 10 mg/kg, i.p.) (Sigma Chemistry) from early to symptomatic stage (65^{th} to 90^{th} days of age) before being euthanized. Their neurological status (rotarod performance and limb clasping) was assessed weekly during the treatment period, and their spinal cords were used after autopsy for neuropathological evaluation. CBDA treatment showed neuroprotective effects in dose-dependent manner, with the dose of 10 mg/kg having the best therapeutic effect. We observed a significant improvement with CBDA in the motor impairment compared with TDP-43 transgenic mice treated with vehicle, which showed a progressive neurological deterioration. These positive effects correlated with a notable reduction in the loss of spinal motor neurons, as well as an attenuation in GFAP and Iba-1 immunoreactivity. By contrast, the treatment with riluzole caused a partial improvement (only significant in clasping) in the neurological status only at the dose of 10 mg/kg, followed by a clear attenuation of Iba-1 immunoreactivity (both doses), but no effects on astroglial reactivity (labelled with GFAP) and the motor neuron death. Our results demonstrate significant neuroprotective effects exerted by CBDA in TDP-43 transgenic mice, which were much more evident than those observed in these animals with riluzole, the classic treatment in ALS. These results provide robust evidence in support of a possible cannabinoid-based therapy in ALS, either alone or in combination with other neuroprotective agents, although additional research is still necessary to promote the translation of these results to patients.

Supported by MINECO (SAF2015-68580-C2-1-R), CIBERNED (CB06/05/0089) and GW Research Ltd.

THE SELECTIVE INHIBITION OF FAAH AMELIORATES COGNITIVE DECLINE, DEPRESSIVE-LIKE SYMPTOMS AND NEUROPATHOLOGICAL ALTERATIONS IN A MURINE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and alteration of other non-cognitive domains. Novel epidemiological data globally recognized the AD as the leading cause of dementia as well as one of the main causes of death.
Overwhelming evidence showed the primary role for the modulation of the endocannabinoid system in the pathogenesis of AD. To this regard, several studies highlighted the neuroprotective and anti-inflammatory effects associated to the increased tone of acylethanolamides, which could be potentially used as a novel therapeutic strategy for neurodegenerative disorders, such as AD.

The pharmacological inhibition of the enzyme fatty acid amide hydrolase (FAAH) can increase the endogenous tone of acylethanolamides, such as anandamide. Currently, it is well known that anandamide increase is able to produce cognitive improvements, as well as anxiolytic- and antidepressant-like effects in rodents. Nevertheless, the phase I clinical trial with BIA 10–2474, a non-selective FAAH inhibitor, has been discontinued due to its off−target effects, which caused severe adverse effects.

Therefore, in our project we tested the hypothesis that a chronic treatment with the highly selective FAAH inhibitor PF-3845 (K_i = 0.23 μM) could exert beneficial effects on the onset and/or the progression of the neurofunctional alterations found in the triple transgenic mouse model of AD (3×Tg-AD). 3×Tg-AD mice develop a progressive and age-related neuropathology characterized by cognitive decline and depressive-like symptoms associated to amyloid-β and tau pathology. This complex phenotype starts appearing around 5-6 months of age and is fully expressed during the old age (10 to 12 months).

To this aim, both young ("pre-symptomatic" at 4 months of age) and old ("symptomatic" at 10 months of age) male 3×Tg-AD mice were treated every other day with PF-3845 (10 mg/kg, s.c.) or vehicle (saline, tween80 and PEG, 2 ml/kg, s.c.) for two months. At the end of the treatments, we tested our hypothesis following an integrated approach, involving behavioural (tests for cognitive and depressive-like alterations), biochemical and immunohistochemical analyses (for the neuropathological markers and BDNF analyses), as well as neurochemical evaluation (HPLC analysis of monoamine levels).

As expected, PF-3845 treatment was able to improve spatial and recognition memory, and to ameliorate the depressive-like symptoms in both young and old 3×Tg-AD mice. Moreover, PF-3845 treatment (i) reduced Aβ and tau pathology in the frontal cortex and hippocampus, (ii) increased the hippocampal expression of BDNF and (iii) modulated the monoamine levels in both brain regions.
Overall, PF-3845 treatment was efficacious not only in preventing the onset of the neurofunctional alterations, but also to partially restore these alterations in old symptomatic mice, thus suggesting that the selective FAAH inhibition may still represent a promising target for the development of novel and efficacious anti-Alzheimer's therapies.

Acknowledgements: Funded by MIUR-PRIN 2015 prot. n. 20153NBRS3_003

IMPACT OF THE ENDOCANNABINOID SYSTEM MODULATION ON ADOLESCENT BRAIN MATURATION

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Adolescence is a time of important neurobiological and behavioral changes, but is also the period in which several mental illnesses emerge, included psychosis and mood disorders, as well as substance abuse especially Cannabis consumption.

In this time window, the brain undergoes intensive processes of neuronal refinement especially in cortical regions. Adolescent brain maturation involves a thinning of the gray matter (GM – it contains the cell bodies, dendrites and axon terminals of neurons) as the result of synaptic pruning processes, through which "redundant" synapses overproduced in the early years of life are being eliminated. In the meanwhile, volumes of white matter (WM – it is made of myelinated axons) increases, improving neural transmission and enhancing brain-regional connectivity and cognitive function. Thus, alterations in synaptic refinement, and/or in myelination, during this sensitive period could confer a vulnerability to psychiatric diseases.

Remodeling of the different components of ECS is also present in adolescence. So far, many works have been focused on the adolescent brain maturation, but the involvement of ECS in the adolescent brain refinement remains to be elucidated.

Our goal is to thoroughly investigate the role played by the endocannabinoid signaling on several markers related to plasticity and myelination during the early and middle adolescence. To clearly depict each step of adolescent brain maturation, we treated adolescent female rats for 5 days (from 28 to 32 PND; from 33 to 37 PND; from 38 to 42 PND) with three different modulators of the ECS. Specifically, we administered (i.p.) the antagonist of CB1 receptor AM251 (0.5 mg/kg), the FAAH enzyme inhibitor URB597 (0.3 mg/kg), and the selective inhibitor of MAGL JZL184 (8 mg/kg). In the Prefrontal cortex, western blot analyses were performed to investigate the protein levels of Synaptophysin, PSD95, SAP102, GluN2A and Glu2NB (markers of synaptic refinement) and MOG and MBP (markers of myelination). From 28 to 32 PND, URB597 administration significantly increased Synaptophysin, PSD95 and SAP102, whereas JZL184 administration significantly increased PSD95, GluN2A and MOG levels. From 33 to 37 PND, AM251 administration significantly increased Synaptophysin and PSD95; URB597 administration significantly increased Synaptophysin, PSD95 and GluN2A, whereas JZL184 administration significantly increased MBP and MOG levels**.** Finally, from 38 to 42 PND, both AM251 and URB597 administration significantly decreased GluN2B, whereas only AM251 increased MBP levels.

As a whole, ECS modulation seems to promote synaptic strength by the increase of markers associated with synapse maturation during early adolescence, whereas it leads to a decrease of markers associated with immature synapses, probably boosting pruning events in the middle adolescence. The effect of ECS modulation on myelination seems to promote these events even if acting by different component. Thus, these preliminary results seem to suggest that the ECS components play an important role in the adolescent brain refinement in a very time-specific manner.

Acknowledgements: PP has a fellowship provided by Zardi-Gori Foundation.

THE EFFECTS OF PHYTOCANNABINOIDS ON ASTROCYTES EXPOSED TO OGD/R INJURY

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Background and purpose: Cannabidiol (CBD) is neuroprotective against ischaemia/reperfusion (I/R) injury *in vitro* and *in vivo*. This effect is mediated by the serotonin 1A receptor (5-HT1$_A$), regulating intracellular Ca^{2+} through the mitochondrial Na^+ /Ca^{2+} exchangers (NCX) and enhancing mitochondrial bioenergetics by optimising glucose metabolism, thus maintaining functional cellular homeostasis. It is possible that other phytocannabinoids are also neuroprotective in these ways. Therefore, the aim of this study was to assess how cannabigevarin (CBGV), cannabidivarin (CBDV) and cannabichromene (CBC) affect astrocyte viability in an oxygen glucose deprivation/reperfusion (OGD/R) model of I/R injury. The effects of each phytocannabinoid were assessed when given before (prophylactically) and after (therapeutically) the insult.

Experimental approach: Confluent human astrocytes (n=9-12, over 3 separate experiments) were exposed to OGD conditions (0% oxygen & 0% glucose) for 8hrs using anaerobic pouches. Cells were treated with phytocannabinoids (supplied by GW) at 100nM, 1µM and 10µM immediately before or after OGD. Following OGD, cells were returned to normoxic conditions (glucose & 20% O_2, 5% CO_2). Lactate Dehydrogenase (LDH) activity was measured in media samples at 0h, 8h (post-OGD), 16h, 40h, 64h, 88h, 112h, 136h and 160h. Data obtained from 0-16h was expressed as percentage change from vehicle and compared to vehicle using a 2-way ANOVA. Data obtained from 16-160h was expressed as area under the curve (AUC, nmol/50µl/h, an indicator of the secondary damage of OGD) and compared to vehicle using ANOVA (data was normally distributed).

Key results: 8h OGD increased LDH immediately post-OGD (t=0-16h). Following reperfusion, a secondary increase of LDH occurred at later time-points (t=16-160h). CBD (F (6, 70) = 1.198) administered prophylactically reduced the increase in LDH immediately post-OGD (i.e. the damage induced by ischaemia) (post hoc analysis showed 10µM decreased LDH by 32%, p<0.0419). Prophylactically, CBD (F (3, 44) = 1.308) also augmented LDH levels compared to vehicle (AUC of LDH over 16-160h) (100nM p=0.0311; 1µM p=0.0026; 10µM p=0.0040). CBD administered immediately after OGD did not alter LDH content at any time points. CBC (F (6, 88) = 0.7838) administered prophylactically decreased the post-OGD increase of LDH by 24% at 1µM (p=0.0376) but didn't affect the secondary damage of OGD. CBC (F (3, 34) = 2.783) administered immediately post-OGD reduced the secondary damage of OGD (AUC over 16-160h) but only at 10µM (p=0.0419). CBGV (F (6, 70) = 7.655) administered prophylactically decreased LDH immediately post-OGD by 13% (1µM, p=0.0045) and 47% (10µM, p=00001), but didn't affect the secondary damage of OGD. However, CBGV (F (3, 44) = 5.61) administered immediately following OGD reduced the secondary damage over 16-160h at all concentrations (100nM p=0.0077; 1µM p=0.0015; 10µM p=0.0142) (See Figure 1). CBDV (F (6, 88) = 2.155) administered prophylactically further increased LDH immediately post-OGD (0-16h) (100nM, p=0.0005). pre administered CBDV also reduced the AUC over 16-160h (1µM, p=0.0023). CBDV administered after the OGD did not alter LDH content at any time points.

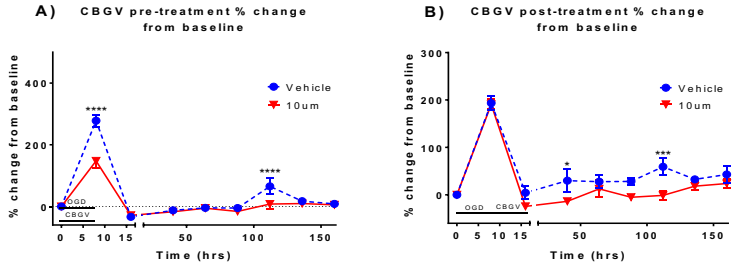

Figure 1. The effects of CBGV pre- (A) or post- (B) treatment on human astrocytes after 8 h oxygen/glucose deprivation. LDH data is expressed as mean (±S.E.M) % change from baseline, and was analysed by 2-way ANOVA.

Conclusion

All of the phytocannabinoids tested reduced either the acute or secondary damaging effects of OGD in human astrocytes to varying degrees. CBD and CBDV were only effective at reducing cellular damage when given prophylactically. CBC and CBGV were effective when administered either before or after OGD, however CBGV seemed better able to attenuate LDH increases in this model of ischaemia/reperfusion injury. Further work is required to establish the molecular targets of these compounds, effects of repeated treatment, and establish any potential additive effects or synergy between compounds.

POTENT INHIBITION OF ENDOCANNABINOID REUPTAKE AS A NOVEL PHARMACOLOGICAL STRATEGY WITH POTENTIAL TRANSLATIONAL APPLICATION IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is considered an autoimmune disease, characterized by progressive neurodegeneration associated with demyelination and excitotoxicity. Although several advances in the treatment of MS have been made, the current treatments do not efficiently control the disease progression and can be linked to serious adverse effects. Cannabis has been used for decades to mitigate MS-associated symptoms, while only recently the equimolar combination of delta-9-tetrahydrocannabinol (THC) and cannabidiol was approved for controlling MS-associated spasticity. Despite the perceived effects on slowing MS progression in some patients taking cannabis, the THC synthetic analogue dronabinol did not show significant improvement on neurological deterioration in a 3-year clinical trial [1]. However, preclinical evidence support the role of the endocannabinoid system (ECS) as a key target to treat neuroinflammatory and neurodegenerative diseases such as MS.

Alternative strategies to indirectly activate the ECS have been developed. These approaches act mainly through the inhibition of the main endocannabinoid (EC) degrading enzymes (fatty acid amide hydrolase, FAAH and monoacylglycerol lipase, MAGL), thus raising the level of anandamide (AEA) or 2-arachidonoyl glycerol (2-AG), the two major ECs. Although these treatments showed some positive results in mouse models of MS, they might have some limitations in clinical settings for chronic use. Our approach is based on a novel class of compounds acting as selective EC reuptake inhibitors (SERIs). SERIs simultaneously increase the levels of AEA and 2-AG with an intrinsic self-limiting capacity that can prevent an EC overflow, especially during chronic treatment. The most potent SERI WOBE437 was used to uncover a novel type of pharmacological modulation of the ECS [2]. *In vivo*, WOBE437 showed anti-inflammatory, anxiolytic and analgesic effects in different animal models [2]. Hence, our aim is to investigate the role of SERIs as potential disease-modifying agents for MS, using a mouse model of experimental autoimmune encephalomyelitis (EAE). Our results showed that post-onset daily treatment with WOBE437 significantly reduced the severity of EAE symptoms and accelerated the recovery. The role of CB1 and CB2 receptors on WOBE437 effects is being evaluated along with the modulation of ECs and related lipids in brain and the number of infiltrated cells into the CNS. SERIs have the potential to be an innovative and effective therapeutic option for slowing MS progression, by counteracting the two main features of the disease, chronic inflammation and excitotoxicity.

References:
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EXPLORING THE RELATIONSHIP BETWEEN GENDER AND THC LEVELS FOLLOWING AD LIBITUM CANNABIS INTOXICATION

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Legal access to medical and recreational cannabis has surged in the United States this decade, alongside a substantial rise in the availability and popularity of high-potency products. Yet the pace of scientific research has not matched these trends, and most extant research to inform public health and policy has limited ecological validity, due to controlled administration and dramatically lower potency of most tested products. Furthermore, the current literature has left many basic questions unanswered, which would inform the public's consumption choices, as well as health and legal guidelines. For instance, in contrast to alcohol use, research has thus far provided only a cursory understanding of how demographic variables such as gender may influence cannabis intoxication. Current information points to gender differences in development of cannabis use disorder (CUD), progression to CUD, and subjective effects during intoxication. The present study aims to enrich this literature by using an ecologically valid research design to explore how gender is related to quantity of *ad libitum* cannabis consumption, and THC concentration in the blood after administration.

Current cannabis users were recruited from Boulder, Colorado, where adults have legal access to recreational cannabis. Our sample's mean age was 29.8 (SD=11.3), with 39 women and 51 men. Participants reported using cannabis 4.9 days per week on average (SD=2.5). The cannabis participants used was either in flower (16-24% THC) or concentrated oil form (70-90% THC), and they were asked to use it exclusively and *ad libitum* for five days prior to an acute administration session, which occurred in a mobile pharmacology lab that was driven to participants' homes. The session included measures before and immediately after *ad libitum* self-administration (in the participant's home) of dispensary-purchased cannabis. A scale was provided for participants to record product weight before and after self-administration, to assess quantity of cannabis consumed. Blood was collected at both timepoints and analyzed for plasma THC levels. Participants' self-reported ratings of intoxication, gender, and weight were also collected. There were no gender differences in quantity consumed ($p>.8$) or subjective intoxication ($p>.8$). Mean post-administration plasma THC levels, however, were 98.2 ng/ml for women (SD= 108.9), and 174.9 ng/ml for men (SD= 140.6; $p<.005$). Using multiple regression to control for participant weight, self-reported amount of cannabis product used, pre-administration plasma THC levels, and cannabis product type (flower vs. oil), post-administration plasma THC levels were 60.2 ng/ml higher in men than women ($p<.01$). These results imply that even when using the same amount of cannabis and controlling for body weight, women are either in-taking a lower dose of cannabinoids (e.g., due to shorter length of inhalation), and/or that there are gender differences in metabolism of THC. These findings suggest that there may be gender-based differences that impact cannabis consumption and intoxication. Further, gender differences in cannabis exposure and intoxication may differ from patterns seen, for example, in alcohol, prompting a need for further investigation in order to inform relatively safe vs. risky levels of use in male and female users.

Acknowledgements: Funded by NIDA (Hutchison) and the state of Colorado (Bidwell)

THE ORPHAN RECEPTOR GPR6: HOMOLOGY MODEL CONSTRUCTION

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G protein coupled receptor 6 (GPR6) is a cannabinoid-related Class A GPCR orphan receptor, with high abundance in the central nervous system and high constitutive activation of adenylyl cyclase rivaling the levels of cAMP produced by full ligand activation of other GPCRs. GPR6 has been shown to recognize several phytocannabinoids, including cannabidiol and cannabinoid antagonists, including SR144528. The orphan receptors GPR6, GPR3 and GPR12 share more than 50% identity and 65% similarity at the amino acid level. They belong to the GPCR Class A MECA cluster of receptors (melanocortin receptors (MCRs), endothelial differentiation G-protein coupled receptors, cannabinoid receptors (CNRs), and adenosine binding receptors (ADORAs)).

As demonstrated by different research groups, GPR6 represents a possible target for the treatment of different pathological conditions such as Parkinson's disease, Alzheimer´s disease, Huntington's Disease, or cell survival. Patents from many different pharmaceutical companies have claimed the use of GPR6 modulators for the treatment of several neurological conditions.

The goal of the current study is to develop a human GPR6 homology model that helps us to elucidate the structural determinants governing ligand-receptor interactions. The X-ray crystal structure of the Sphingosine-1-phosphate receptor 1 (S1P1) was used as a template. This GPCR shares 33% homology (similarity) with the GPR6 sequence. Some important sequence differences between S1P1 and GPR6 in TMHs 1,2,4,6 and 7 were identified, and the Conformational Memories (CM) technique was used to explore the flexibility introduced by helix bending residues in each of these helices. CM provides a set of low-free energy conformations from which a new helix conformation for each helix was chosen. The extracellular and intracellular loop geometries were calculated using Modeller v9.1. The resultant model was then energy minimized using a standard protocol. The model was then embedded in a fully hydrated lipid bilayer environment for equilibration of the model via MD simulation. An equilibrated bundle with low root mean-square deviation (RMSD) was chosen to be used for the docking studies.

The development of the GPR6 active and inactive state models will enable the rational design of novel GPR6 ligands which may serve as research tools for further understanding the biological role of this orphan receptor. [Support: NIDA KO5 DA021358]

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

MAGL INHIBITION ATTENUATES PAW INFLAMMATION AND FUNCTIONAL DEFICITS CAUSED BY COLLAGEN-INDUCED 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 endocannabinoid system is a putative target for RA treatments, and cannabinoid drugs demonstrate anti-inflammatory and analgesic efficacy in animal models of acute peripheral inflammation and pain. The present experiment used a mouse model of collagen induced arthritis (CIA) to test the hypothesis that MAGL inhibition (i.e., 16 x daily 8 or 40 mg/kg JZL184) attenuates the progression of inflammatory arthritis. Male DBA1/J mice were subjected to CIA, and paw thickness and clinical scores were assessed daily. Novel paw function assays (e.g., grip strength and balance beam) were also used to determine arthritis severity and treatment efficacy. Paws were harvested, and cytokine and chemokine levels were quantified by ELISA. JZL184 (40 mg/kg) treatment significantly attenuated CIA-induced paw swelling, clinical severity, and functional deficits. JZL184 also partially attenuated the CIA-induced increase in paw levels of TNF-α, IL-1β, IL-6, IL-17, and IL-10. These data suggest that JZL184 has anti-inflammatory efficacy to reduce the morphological and behavioral effects of CIA.

ACUTE EFFECTS OF CONCENTRATED CANNABIS ON MOTOR CONTROL AND TIMING: SMARTPHONE-BASED MOBILE ASSESSMENT

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As of 2018, Canada has legalized access to cannabis at the national level while 63% and 21% of the U.S. population lives in a state with legal access to medical and recreational cannabis, respectively. In this legal context, sales of concentrates with tetrahydrocannabinol (THC) potencies up to 90% have increased dramatically, sparking concerns about the impact of these products on public health and safety. Acute effects of concentrated cannabis (often inhaled by "dabbing" or vaping) on motor function have not been described. To assess acute impairment under the influence of concentrates, we developed a motor battery measuring general and driving-related neuromotor function. Experienced users (age: 29±12 years; dabbing frequency: 17±2 days/month) were assessed on the motor battery in a mobile laboratory before, immediately after, and 1 hour after self-administration of highly concentrated cannabis, ranging from 70-90% THC (N=36; 14 females, 22 males). A smartphone was attached to the hip, wrist, or ankle of the subject and tri-axial acceleration was measured at 100 Hz. Standing balance was measured with eyes open, with eyes closed, and with the head tilted back for 30 seconds. The standard deviation of acceleration was measured in anterior-posterior (AP) and medial-lateral (ML) directions. To calculate finger tap rate, each subject tapped the smartphone as rapidly as possible for 20 seconds and the time of each deflection peak was determined. Reaction time and peak acceleration was measured from 10 repetitions of rapid vertical leg withdrawal and 10 rapid horizontal arm movements (punch).

After use of concentrated cannabis, postural stability deteriorated in both AP and ML directions and average and peak finger tapping rate and peak arm punching acceleration slowed (ps < 0.05), compared to before cannabis use. Average arm punching reaction time and rapid leg withdrawal reaction time and peak acceleration were unchanged (ps > 0.05). Performance in standing balance, finger tapping, and arm punching changed over time (before, immediately after, or one hour after smoking) and by condition (eyes open, closed, or with the head tilted back), suggesting that recovery time from intoxication and proprioception influence concentrated cannabis motor effects. Results encourage further research on the impact of concentrated cannabis on driving ability and for public health applications in roadside testing. Data collection is ongoing and results from the updated sample will be presented.

Acknowledgments: Funded by the Department of Public Health and Environment - State of Colorado: 17 96947 (Bidwell) and DA039707 (Hutchison).

MODULATION OF ENDOCANNABINOID RECEPTORS REDUCES IMMUNE RESPONSES IN EXPERIMENTAL SEPSIS

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Sepsis is defined as a dysregulated immune response to an infection. Following an initial infection, host response results in excessive release of inflammatory mediators, causing multi-organ dysfunction, failure and death. Recently, the endocannabinoid system has emerged as a potential therapeutic target in sepsis due to its immune modulatory functions. We have previously demonstrated that activation of the CB2R significantly suppressed immune hyper-activation and improved intestinal microcirculation in experimental sepsis. In addition, activating the CB2 pathway through the enzyme inhibitors URB597 and JZL184 showed similar results. However, in CB2 knockout mice, the suppressed immune response and preserved intestinal microcirculation were also observed in the presence of JZL184 and simultaneous CB1R inhibition, suggesting evolvement of other mechanisms. Since GPR55 has been suggested to be involved in cannabinoid-related immune-modulation, we investigated the role of GPR55 in our experimental model. We were able to show that inhibition of GPR55 using a selective antagonist, CID16020046, or synthetic antagonist, O-1918, significantly reduced leukocyte-endothelial adhesion and improved intestinal microcirculation. In addition, the inhibition of GPR55 reduced inflammatory cytokine secretion but did not suppress neutrophil migration in vitro. These data suggest that CB2R activation and GPR55 inhibition may be novel therapeutic targets for attenuating the early acute phase of hyperinflammation during sepsis.

Acknowledgement: Funded by the Nova Scotia Health Research Foundation.

CANNABINOIDS EFFECTIVELY BLOCK PROLIFERATION OF PAEDIATRIC MEDULLOBLASTOMA CELLS *IN VITRO*

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Tumours arising in the central nervous system are the major cause of childhood cancer deaths, and specifically medulloblastoma is the most common malignant brain cancer of childhood. The most effective therapy for medulloblastoma is a combination of surgery, followed by radiotherapy and high dose cytotoxic chemotherapy. Although overall survival rates for medulloblastoma patients exceed 75%, these rates have not improved for decades, and remain at a level well below that of other childhood cancers. Moreover, devastating long-term sequelae including developmental defects, psychosocial deficits and secondary tumours are frequently encountered by survivors. Thus, there is an urgent need to identify more effective therapeutic strategies for MB that have potential to improve survival rates and reduce treatment-related toxicity.

A large body of evidence has demonstrated that plant-derived, endogenously-produced and synthetic cannabinoids exert anti-tumour actions in different cancer types such as breast cancer, melanoma, lymphoma and adult brain tumours to name a few. Moreover, it has been shown that cannabinoids can improve the effect of chemotherapy and RT in preclinical glioblastoma models. Even though there is growing evidence about the anti-tumour properties of cannabinoids in adult cancers, there is very little data about their effect on paediatric tumours. In particular, there is no existing data in paediatric brain tumour models. In this context, we aimed to determine if cannabinoids have anti-tumour efficacy on paediatric brain tumours.

Here we show that several different paediatric brain tumour types express both cannabinoid receptors, CB1 and CB2. We further investigated the effects of cannabinoids in medulloblastoma cells and show that Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) reduce the viability of these cells; an effect that is mediated, at least in part, by CB2. Additionally, drug interaction assays show that the combination of cannabinoids with conventional chemotherapeutics enhances the anti-proliferative effects in these cells.

Taken together, these results suggest that cannabinoids could be an interesting therapeutic tool for the management of children with medulloblastoma. Current work is investigating the therapeutic potential of these agents using preclinical models.

Acknowledgements: funded by Zelda Therapeutics Pty Ltd

MODULAR SYNTHESIS OF NOVEL CANNABINOID LIGANDS BASED ON SUBSTITUTED COUMARINS AS CB_1, CB_2, GPR55 AGONISTS AND ANTAGONISTS

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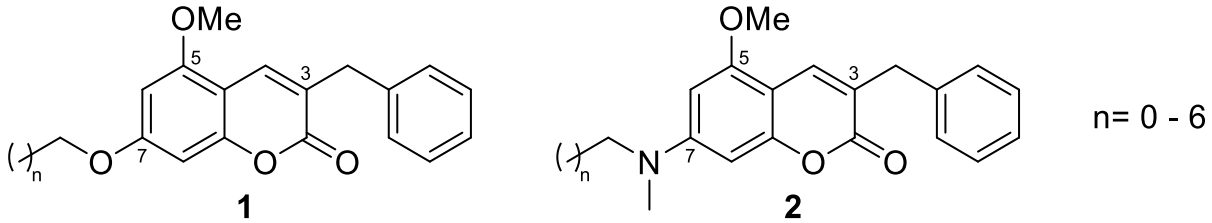
Coumarines are very common and wide spread natural motifs which find applications in medicinal chemistry. In previous investigations in our group was found out, that besides the general biological activities, modified coumarines also show biological activities against the cannabinoid receptor system. This led to the establishment of a modular microwave supported synthesis for modified coumarines (Braese et al., Eur. J. Org. Chem. 6 (2007) 943-952). The synthesis is starting from derivatized salicylaldehydes which are coupled with derivatized cinnamaldehydes in the present of an *N*-heterocyclic carben (NHC) as catalyst via a microwave supported condensation reaction. With this synthesis as tool further investigations led to the establishment of a library of substituted 3-benzylcoumarines. In additional investigations the diversity of our library was extended by the 3-alkylcoumarin moiety (Hurrle, PhD thesis, KIT, unpublished yet). These structure moiety is easily accessible via Perkin-reaction by condensation with acetic anhydrides

The biological activity validation against receptor type and specific receptor interactions is proven in different *SAR*-studies by our cooperation partners. The first assays were done by the group of C. Müller at the PharmaCenter Bonn, Germany. In a radioligand-binding-assay and the *β*-Arrestin-Recruitment-Assay our substances were tested on their activity against CB_1/CB_2 and GPR55/GPR18. Additional their effect as agonist respectively antagonist and their potency were tested (Braese et al., Biorg. Med. Chem. 17 (2009) 2842-2851; Braese et al., J. Med. Chem. 56 (2013) 4798-4810; Glaeser, PhD thesis, KIT, 2014). The results of this assays led to first trends about the different chemical structures and their corresponding biological effects.

In our latest investigations we extended the diversity of our lead structures by the introduction of heteroatoms in the side chain of position 7 and completion of the corresponding 3-benzylcoumarin library **1**. For this purpose, we initially developed two different synthesis strategies for the oxygen (O) respectively nitrogen (N) substituted salicylic aldehydes. These synthesis routes are characteriezed by the simplicity by which the introduction of the different alkyl chain lengths is achieved.



CHRONIC CANNABINOID AND TYPICAL ANTIPSYCHOTIC TREATMENT REDUCES CANNABINOID RECEPTOR TYPE 1 (CB_1) AND THE DOPAMINE RECEPTOR TYPE 2 (D_2) HETEROMER EXPRESSION IN THE GLOBUS PALLIDUS OF C57BL/6J MALE MICE

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The cannabinoid receptor type 1 (CB_1) and the dopamine receptor type 2 (D_2) are co-localized on medium spiny neuron terminals in the globus pallidus where they play an important role in modulating voluntary movement. Physical interactions between the two receptors (heteromerization) have been shown to alter receptor coupling and signaling in cell culture. The main objectives of the current study were to examine whether CB_1 and D_2 heteromers can be detected in the globus pallidus of C57BL/6J mice and to determine whether CB_1/D_2 heteromer levels are altered following chronic treatment with cannabinoids and antipsychotics alone or in combination. By using *in situ* proximity ligation assays, we observed CB_1/D_2 heteromer-specific signals in the globus pallidus of C57BL/6J mice. An increase in CB_1/D_2 heteromer-specific signal was observed in the globus pallidus of C57BL/6J mice following chronic CP55,940 treatment. In contrast, treatment with the typical antipsychotic haloperidol reduced CB_1/D_2 heteromer-specific signals while the atypical antipsychotic olanzapine treatment did not affect CB_1/D_2 heteromer-specific signals. Chronic co-treatment with CP55,940 and haloperidol resulted in CB_1/D_2 heteromer-specific signals similar to those observed in the haloperidol-treated group. Chronic co-treatment with CP55,940 and olanzapine resulted in a similar distribution of heteromers as seen in the CP55,940-treated group. The alteration in CB_1/D_2 heteromer-specific signals following persistent ligand exposure was due to changes in the affinity of CB_1 and D_2 to form heteromers and was not due to changes in CB_1/D_2 protein expression or receptor co-localization. Chronic exposure to cannabinoid and antipsychotics alone or in combination altered CB_1/D_2 heteromerization and affects movement. Overall, drugs that target CB_1 and D_2 receptors must be considered in the context of their effects on their cognate receptors and for actions within allosteric heteromeric complexes. Pharmacodynamic drug-drug interactions are likely.

Acknowledgements: Funding provided by a grant to MEMK from NSERC. AMB was supported by a King AbdulAziz studentship. RBL was funded by a CIHR postdoctoral fellowship.

A CHEMICAL GENETICS STRATEGY FOR SUBTYPE-SELECTIVE INHIBITION OF DIACYLGLYCEROL LIPASES

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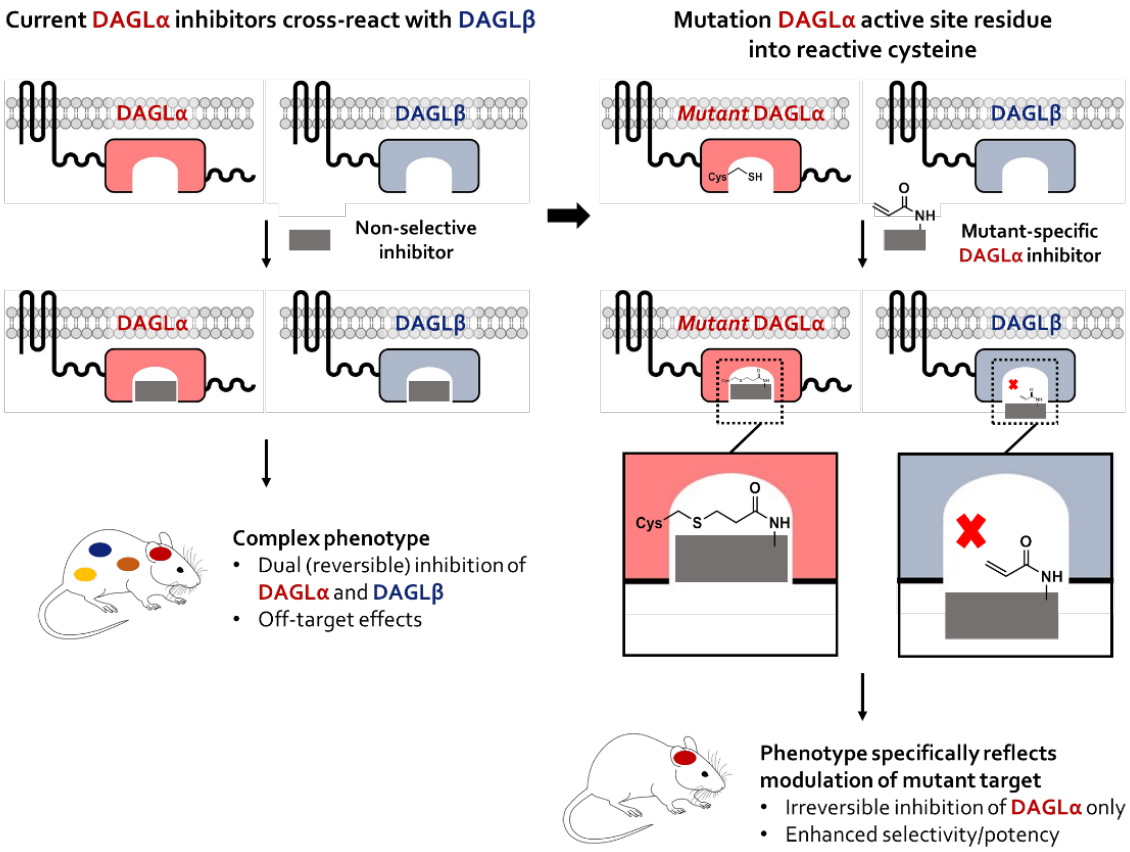
The endocannabinoid 2-AG is biosynthesized by diacylglycerol lipases (DAGLs) α and β in the brain, but the specific functions of these two related hydrolases remain unclear. This is in part caused by a lack of subtype-selective inhibitors. Here, we present a chemical genetics strategy to address these selectivity issues. Using homology model directed design, we identified catalytically active DAGLα mutants harboring reactive cysteines in the active site and in conjunction synthesized inhibitors with electrophilic traps to irreversibly react with these cysteines. This led to the discovery of WEN014 as a potent, irreversible inhibitor of DAGLα^{L651C}, but not wild-type DAGLα and DAGLβ.



INVESTIGATING SYNAPTAMIDE IN IMMUNOLOGICAL CONTEXT

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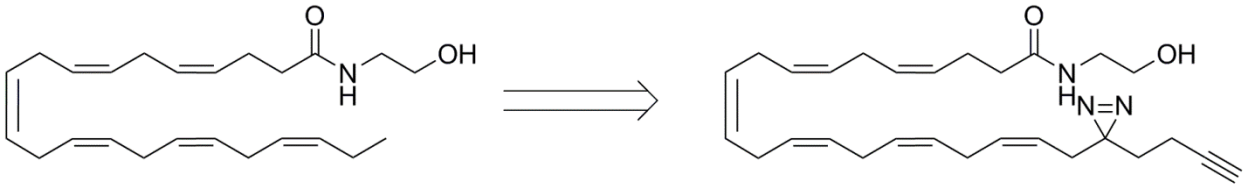
Dietary ω-3 fatty acids (ω-3 FAs) are largely recognized in having cardiovascular and anti-inflammatory beneficial effects. *N*-docosahexaenoylethanolamine, or synaptamide, is the ethanol amide derivative of docosahexaenoic acid (DHA, 22:6N-3), a dietary ω-3 FA. Synaptamide is known to have a role in neurogenesis, neuritogenesis and synaptogenesis (Lee et al., Nat. Commun. 7 (2016) 13123). Moreover, synaptamide has been shown to potently inhibit macrophage activation (Meijerink et al., Br. J. Pharmacol. 172 (2015) 24–37).

In physiological conditions synaptamide is mainly present in the central nervous system (CNS) at concentrations comparable to those of anandamide (AEA). Even though synaptamide lacks affinity to cannabinoid receptors (Meijerink et al., Br. J. Pharmacol. 169 (2013) 772–83), its epoxidative derivatives act on both endocannabinoid and epoxyeicosanoid signaling (McDougle et al., Proc. Natl. Acad. Sci. 114 (2017) 6034–43).

Considering the pleiotropic nature of synaptamide and its metabolites (Alhouayek et al., BBA Mol. Cell. Biol. Lipids 1862 (2017) 474–84; Yang et al., J. Biol. Chem. 286 (2011) 31532–41), we developed a photoaffinity lipid probe in order to map the synaptamide-interacting proteins in complex proteome landscapes. Once confirmed that our lipid probe possesses the same biological effects of synaptamide, we went on to characterize the possible molecular pathways behind its therapeutic anti-inflammatory potentials.



INFLUENCE OF CANNABIS USE ON CONSCIOUS SEDATION FOR TRANS-OESOPHAGEAL ECHOCARDIOGRAPHY

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Background: The number of countries and jurisdictions legalising cannabis for medicinal or recreational uses continues to increase. At the same time there is evidence linking cannabis use to stroke at a younger age. In view of the possible implications of cannabis use in clinical practice it was decided to determine if self-reported cannabis use had an influence when undertaking Trans-Oesophageal Echocardiography (TOE). We report our single-centre experience.

Method: A retrospective audit of 221 consecutive files of TOEs performed from October 2015 to October 2017 was undertaken. Data was obtained from patient questionnaires and medical records. All TOE referrals for stroke work-up and Infective Endocarditis (IE) investigation were examined in detail.

Results: There were 162 TOE referrals for either stroke work-up or infective endocarditis investigation during the period audited. There were 20 patients (14%) who self-reported cannabis use. The remaining 142 TOE referrals were referred to as being for cannabis non-users. The average age for stroke work-up or infective endocarditis investigation in cannabis users was significantly younger than that of non-users (42.8 vs 63.7 years; $p<0.0001$). Cannabis users reported a higher rate of intravenous drug use of other drugs of dependence than non-users (50% vs 2.1%). The use of specialist anaesthetic support was not statistically different for users (65%) than for non-users (51%). In those undergoing conscious sedation in the cardiology department, cannabis users had a higher requirement for midazolam than non-users (5.1 mg vs 3.9 mg; $p=0.0466$) but the dose requirements for fentanyl were similar (72.5 μg vs 68.8 μg).

Conclusions: The results of the present study show that self-reporting cannabis users are referred for TOE for stroke work-up and infective endocarditis investigation at a much younger age than non-users. Further investigation is required to confirm if this a consequence of cannabis use or possibly other drug use. In addition the apparent difference in drug requirements for conscious sedation in cannabis users may need to be considered in clinical practice. However due to the limited numbers and the self-reported cannabis use further work is required.

EVALUATION OF THE CB_2 PET RADIOTRACER ^{18}F-FC024 IN THE *h*CB_2-AAV RAT MODEL OF HUMAN CANNABINOID TYPE 2 RECEPTOR LOCAL OVEREXPRESSION

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Upregulation of the cannabinoid type 2 receptors (CB_2R) unveils pathological processes such as neuroinflammation in multiple sclerosis, Alzheimer's or Parkinson's diseases. Positron emission tomography (PET) is a highly sensitive and translational molecular imaging technique allowing *in vivo* quantitative detection of radiotracers. **^{18}F-FC024** has recently been described as a promising fluorine-18 labeled radiotracer for visualization of the CB_2 receptor with PET. In the course of translating this tracer to clinical trials, we report herein both *in vitro* autoradiography and an *in vivo* microPET imaging study in a rat model with local adeno-associated viral (AAV)-induced overexpression of *h*CB_2. Female Wistar rats were stereotactically injected with an AAV vector serotype 2/7 encoding *h*CB_2 (D80N) under control of a CaMKII promoter, in the right striatum. A control AAV2/7 vector expressing the enhanced green fluorescent protein (eGFP) under control of a CaMKII promoter was injected in the left striatum. The rat model was validated for CB_2 expression by injection of the well-characterized CB_2R inverse agonist radiotracer ^{11}C-NE40.

Autoradiography study confirmed an 8-fold increase of the signal in the right relative to the left striatum of *h*CB_2-AAV rat brain sections. A 91% reduction in the signal was observed in right striatum of *h*CB_2-AAV rats when blocking with NE40 (100 µM). microPET imaging showed that **^{18}F-FC024** efficiently crosses the blood brain barrier (BBB) and binds to the *h*CB_2R in the ipsilateral right striatum of *h*CB_2-AAV rats with a 4-fold higher SUV compared to the CB_2R reference tracer ^{11}C-NE40. Time-activity curves showed uptake and fast wash-out of the tracer in the contralateral left striatum whereas a maximal uptake (SUV=6) was reached after 30 min *p.i.* in the ipsilateral region, underlining strong binding of **^{18}F-FC024** to the *h*CB_2R. Autoradiography studies with brain slices of control rats showed 27% blocking effect from coincubation with NE40 (100 µM) indicating tracer binding to rat brain CB_2R. Self-blocking experiments with **FC024** and blocking experiments with a CB_1-selective compound still need to be performed to evaluate a potential off-target binding. **^{18}F-FC024** is therefore a very promising tracer to image the CB_2R *in vivo* in the brain with PET. Both a brain radiometabolite analysis study and PET experiments in non-human primates are currently ongoing to fully characterize this tracer before performing a first-in-man study in patients with neurodegenerative diseases.

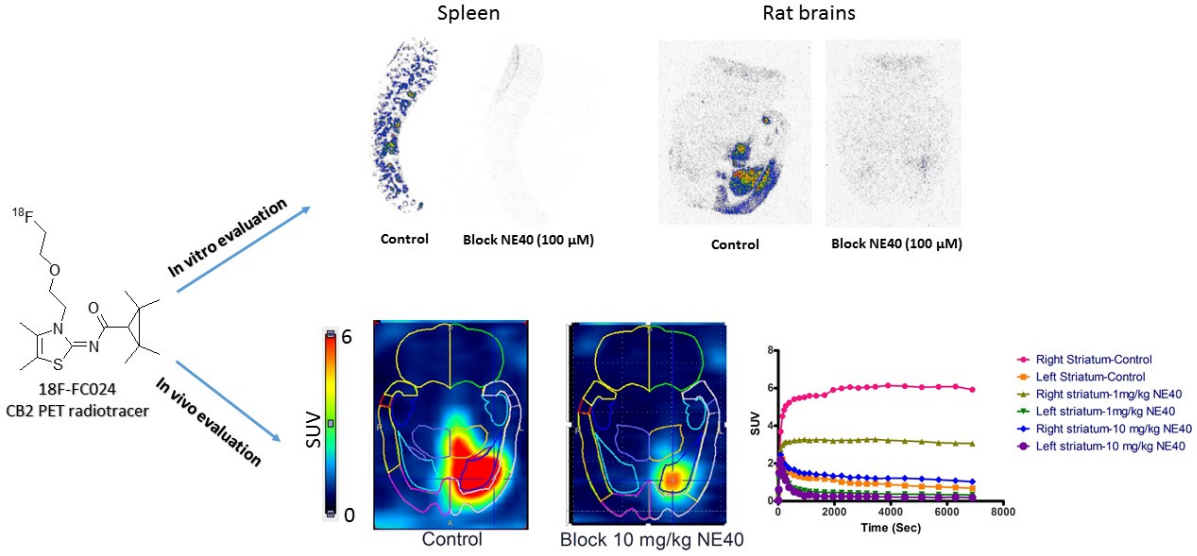

Figure 1: 18F-FC024 developed as a specific PET radiotracer for in vitro and in vivo in a rat model with local over expression of hCB2.

The authors thank the INMiND consortium (HEALTH-F2-2011-278850) for financial support.

CANNABINOID RECEPTOR-MEDIATED CHANGES OF MITRAL CELL ACTIVITY THROUGH MODIFICATION OF SYNAPTIC INPUT IN THE MAIN OLFACTORY BULB

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The endocannabinoid (eCB) signaling system has been functionally implicated in many brain regions but our understanding of the role of cannabinoid receptor type 1 (CB_1) in olfactory processing remains limited. Cannabinoid signaling is involved in the regulation of glomerular activity in the main olfactory bulb (MOB). However, the cannabinoid-related circuitry of inputs to mitral cells in the MOB has not been determined.

Using a combination of anatomical and functional approaches we have explored this question. CB_1 was present in periglomerular processes of a GAD65-positive population of interneurons but not in mitral cells. We detected eCBs in the mouse MOB as well as the expression of CB_1 and other genes associated with the cannabinoid signaling system in the MOB. Patch-clamp electrophysiology in mouse brain slices demonstrated that CB_1 agonists evoked membrane depolarization, increased action potential firing, and an inward current in mitral cells, while CB_1 antagonists reduced firing and evoked an outward current. CB_1 effects on mitral cells were absent in subglomerular slices in which the olfactory nerve layer and glomerular layer were removed, suggesting the glomerular layer as the site of CB_1 action. We previously observed that GABAergic periglomerular cells show the inverse response pattern to CB_1 activation compared to mitral cells, suggesting that CB_1 indirectly regulates mitral cell activity as a result of cellular activation of periglomerular cells. This hypothesis was supported by the finding that cannabinoids modulated synaptic transmission to mitral cells.

We conclude that CB_1 directly regulates periglomerular cell activity to control GABA release and, in turn, regulates mitral cell activity with potential effects on olfactory threshold and behavior.

DO GLIOBLASTOMA MULTIFORME (GBM) CULTURES EXPRESS CANNABINOID RECEPTORS AND ARE THEY INVOLVED IN REGULATING GBM FUNCTIONS?

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The goal of this study was to understand whether human glioblastoma multiforme (GBM) cultures are regulated by cannabinoid ligands. The GBM cultures were developed from tissue resected from patient's tumours by Auckland Cancer Research Society Centre (ACRSC) under the Directorship of Professor Bruce Bageley. The main aim of this research was to investigate whether the GBM cultures expressed detectible levels of cannabinoid receptors and assess their involvement in regulating cell viability, signalling and cytokine secretion. A broad range of cannabinoid ligands were assess for their effects on the viability of 8 different patient-derived GBM cultures. In brief, cytotoxic effects were observed in some GBM lines for some cannabinoids (including THC, HU210, HU308, CBD, CP55,940, SR1 and WIN55,212), but these were typically observed at or above 10 μM. Importantly, there was no detectible cytotoxicity within the nM range of any of the ligands tested. This was assessed temporally for at least 7 days to observe acute cytotoxicity as well delayed responses. The high concentrations of ligands implied that the cytotoxic mechanisms were independent of expected cannabinoid receptor pharmacology. Of the 8 GBM lines investigated so far, 3 lines have demonstrated CB1 mediated activation of pERK signalling. This was induced by 100 nM CP and completely blocked by 1 μM SR1, peaking at 10 minutes and, demonstrating involvement of CB1. Further pharmacological analysis demonstrated EC50 values between 1nM and 5nM for HU210 and CP across the different responsive GBM lines for pERK activation. The presence of CB1 mRNA has been confirmed in these 3 lines by PCR and we have obtained promising results supporting receptor expression using immunocytochemical staining of the cells. Current work is focusing on whether these cannabinoids can regulate the secretory cytokine profile of the GBM cells. Cytokines are potentially important in controlling the tumour microenvironment, particularly with regard to manipulation of infiltrated immune cells and vasculature permeability. Our pilot data has shown that the GBM lines secrete an abundance of MCP-1, IL-8 and VEGF. This is very interesting as monocytes and macrophages express the chemokine receptors for MCP-1 and IL-8 and these cells may contribute to the myeloid-derived suppressors cells present in the tumour microenvironment. VEGF is of known importance for the regulation of vascular permeability. Current experiments are investigating the regulation of these cytokines using the ligands at pharmacologically appropriate concentration (in the nM range) to ascertain whether cannabinoid ligands regulate these cytokine in either a cannabinoid receptor dependent or independent manner.

DISCOVERY OF SELECTIVE INHIBITORS FOR Ca^{2+}-INDEPENDENT N-ACYLTRANSFERASES

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Phospholipase A1 / Acyltransferases 1-5 (PLA/ATs) are Ca^{2+}-independent enzymes that produce N-acylphosphatidylethanolamines (NAPEs), which are the central precursors for the formation of *N*-acylethanolamines (NAEs), including the endocannabinoid anandamide. Little is known about the regulation of NAPE formation and it is unclear to what extent anandamide production via PLA/ATs is responsible for distinct cannabinoid CB1 receptor mediated biological processes. Selective inhibitors of PLA/ATs may contribute to a more fundamental understanding of the physiological role of NAEs and anandamide and may serve as potential drug candidates for the treatment of obesity and neurodegenerative diseases. Currently, there are no selective inhibitors available for the study of PLA/ATs .

Here, we report the discovery that lipase-tailored activity-based probe MB064 also reacts with the catalytic cysteine of PLA/ATs [1,2]. Using MB064 we identified the first selective PLA/AT inhibitor (LEI301) in a competitive activity-based protein profiling assay. Targeted lipidomics revealed that LEI301 could reduce anandamide levels, but not 2-AG, in U2OS cells overexpressing PLA/AT-2 or PLA/AT-5. In conclusion, we have developed LEI301 as a selective and cellular active PLA/AT inhibitor, which may help to elucidate the biological function of PLA/ATs and NAEs.

[1] Baggelaar et al., J. Am. Chem. Soc., 2015, 137, 8851
[2] Baggelaar et al., ACS Chem. Biol., 2017, 12, 852

PHYTOCANNABINOIDS PROFILES IN MEDICAL CANNABIS CONSUMED BY CHRONIC PAIN PATIENTS

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Health Canada has provided access to medical cannabis to patients in Canada for almost 19 years to date, the typical practice by patients has been to self-titrate medical cannabis in order to decide on the strain and dose which provides pain reduction. The majority of the patients consume medical cannabis use it to treat chronic pain conditions. In the past 5 years, medical cannabis can be directly procured by patients from licensed producers. In an ongoing clinical study at University Health Network, Toronto, Canada, we collected cannabis samples of medical cannabis and medical cannabis derived products from chronic pain patients, and conducted in-depth chemical analyses in order to develop an understanding and any underlying relationships between medical cannabis chemistry, and cannabinoids receptor responses.

We evaluated the qualitative and quantitative profiles for phytocannabinoids in the medical cannabis consumed by pain patients (protocol approved by the REB, University Health Network). These cannabis samples were extracted and were then evaluated for their activities at the CB1 and CB2 receptors. Phytocannabinoids profiling includes quantitative profiling of Δ^9-THC, CBD, Δ^9-THCA, and CBDA. In general, these extracts activated CB2 receptor better than CB1 receptor, despite significant differences in their Δ^9-THC and CBD profiles. This is an attempt to understand patients' preferences via a chemical scope, and the biochemical profiles associated with the patients preferences. A pattern appears to emerge from the chemical profiling, and their corresponding cannabinoid receptors responses. This presentation will detail the chemical compositions, and structure-activity relationships with respect to the receptor activities, and will discuss common and differential properties.

Acknowledgements: Funded by CannScience Innovations, Inc. (#410003527), Scientus Pharma, Inc. (#410008630), Ontario Innovation Trust and Canada Foundation for Innovations (#CFI 32350).

PHYTOCANNABINOIDS PROFILES IN MEDICAL CANNABIS CONSUMED BY PATIENTS DIAGNOSED WITH PTSD

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Post-traumatic stress disorder (PTSD) is a complex disorder involving neuropsychological, cognitive and psychiatric components. Many patients in Canada, including veterans, consume medical cannabis as a treatment of choice to gain relief and engage in daily activities. Typical practice in medical cannabis consumption has been to self-titrate the medication by the patient, and decide on the strain and dose. In a recent clinical study initiated at the Centre for Addiction and Mental Health and University Health Network, we collected cannabis samples from already known users of cannabis for the treatment of PTSD and conducted in-depth chemical analyses of the cannabis and cannabis-derived products consumed by these patients.

We collected 21 cannabis samples from 14 PTSD patients, and evaluated the qualitative and quantitative profiles for phytocannabinoids (study approved by the REBs at CAMH and UHN, Toronto). Diagnostic information on the severity of the PTSD condition was collected during an interview with each patient, along with other medications consumed by the patients. Medical cannabis samples were extracted and evaluated for their phytocannabinoids content, including quantitative profiling of Δ^9-THC, CBD, Δ^9-THCA, CBDA, CBGA, and CBG. A pattern appears to emerge from the chemical profiling, and higher concentrations of Δ^9-THC was identified in the medical samples collected from the patients. Details of the chemical compositions of the medical cannabis samples, patients' disease severity and concomitant medications profiles will be presented.

Acknowledgements: Funded by CannScience Innovations, Inc. (#410003527), Scientus Pharma, Inc. (#410008630), Ontario Innovation Trust and Canada Foundation for Innovations (#CFI 32350).

SAFETY ANALYSES OF MEDICAL CANNABIS PRODUCTS IN THE CANADIAN ACCESS TO CANNABIS FOR MEDICAL PURPOSES REGULATIONS

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Canada's *Access to Cannabis for Medical Purposes Regulations* (ACMPR) enacted in 2016, provide a unique opportunity for healthcare practitioners and clinical researchers to conduct impactful pharmacovigilance research within a well-controlled regulatory framework. The current program provides access to a variety of medical cannabis products produced and sold by Licensed Producers (LPs) with strict quality standards and requirements for identification of THC and CBD concentrations. Some LPs provide analyses of tertiary cannabinoids and terpene concentrations. Understanding the safety of the hundreds of medical cannabis products available in the ACMPR requires a robust monitoring via a database system. Santé Cannabis, a leading specialized medical cannabis clinic with locations in Quebec, Canada has collected a database of side effects and adverse events reported by approximately 3000 medical cannabis patients followed at the clinic. Side effect information, including severity classification, causal relationship, event outcome and specific product and dose information were also included in this database.

At initiation of medical cannabis treatment, all Santé Cannabis patients are instructed to identify and report any side effects or adverse events and are provided with a log sheet to record details. In total, at unscheduled and scheduled follow-up, 935 patients (31%) reported at least one side effect between November 2014 and January 2018. The vast majority of side effects were classified as mild, and were tolerated by the patient. Almost all of these patients continued with the medical cannabis treatment. In total, 71 adverse events of moderate (67) or severe (4) classification were identified, from 68 individual patients. Adverse events are significantly more likely to occur for patients using medical cannabis oil (oral administration, 59) versus dried cannabis (inhalation, 15). Risk of moderate or severe adverse events were significantly affected by patient error (patient taking a larger dose than was recommended), with 23 of such events recorded. THC concentration relative to CBD significantly impacts risk of side effect with 1:1 THC:CBD products reported most frequently (36) and THC-rich cannabis products (23) compared to CBD-rich products (12). An analysis of the outcome of the adverse events, most (50) ceased treatment as the effect was not resolved by change in product or dosage. Only 18 patients continued medical cannabis treatment without recurrence with the following adjustments; down-titration to lower dosage of same product (8); change in product to lower THC:CBD ratio (4); discontinued treatment temporarily and restarted on a lower dosage (3); discontinued temporarily while concomitant medications were down-titrated (3). Considering these differences in frequency of reported side effects between the oral administration and inhalation observed in clinical practice, the prescribing protocols, dose titration schedules and education program provided by Santé Cannabis have been adapted over time to improve dosing control, limit patient errors and improve persistence and adherence to medical cannabis treatments. As a result, a year over year reduction of moderate-severe adverse events has been recorded since 2015.

AROMATIC ANALGESIA – COMPARING THE TERPENE CONTENT OF MEDICAL CANNABIS PRODUCTS WITH PATIENT PREFERENCE FOR THE TREATMENT OF PAIN

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Significant research has been undertaken to understand how cannabinoids such as Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) can be effective analgesic substances. Herbal cannabis products contain an "entourage" of minor cannabinoids and terpenes besides THC and CBD which may contribute to their medical activity (Russo, 2011). Little research exists to describe how different terpenes might alter the effects of herbal cannabis in humans. In this study medical cannabis patients report preferences for certain cannabis products for the treatment of pain, which may be due to different terpene concentrations.

Medical cannabis patients of Whistler Medical Marijuana Corporation (WMMC) were surveyed about the effectiveness of different cannabis products for pain management, identifying which products they found most effective or least effective. Over three quarters of the total respondents reported pain as a symptom for which they use medical cannabis (76.7%; n=365). Patients rated the effectiveness of cannabis for the treatment of their pain on a rating scale from 1 to 10 with an average score of 7.7 and median of 8 (93/354 responses). Using the top five cannabis products rated most or least effective for treating pain, weighted average terpene and cannabinoid profiles are created for the most and least effective chemotypes. Using these average chemotypes, a correlation factor is calculated for each cannabinoid and terpene on a scale of -100 to 100. Negative numbers represent a correlation with less effectiveness for pain, and positive numbers with more effectiveness and the distance from zero indicates the strength of the association. Myrcene is the most abundant terpene in both groups, accounting for 22% of the total terpene content in both averages, creating a neutral association (Score: 1.0 ± 25.3). The second most concentrated terpene in the most effective group is trans-nerolidol accounting for 17% of the total (4% in least effective) creating a positive correlation with pain efficacy (score: 61.6 ± 25.3). The second most abundant terpene in the least effective group is terpinolene, accounting for 13% of the total (<1% in the most effective) creating a strong negative correlation (-88.7 ±41.1). There are statistically significant differences in the terpene content of cannabis products patients categorize as less or more effective for pain management, and these should be further investigated to create cannabis products optimized for the treatment of pain.

Acknowledgements: The authors would like to thank the WMMC patients who took the time to complete this survey. The survey was funded by WMMC and the analysis of the data was conducted by Whistler Therapeutics.

A NOVEL MICRODOSING SYSTEM TO FILL VAPOR CHIPS FOR THE SYQE MEDICAL INHALER WITH GROUND CANNABIS

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Pulmonary delivery of cannabis flos is the most effective administration route due to the achievable drug serum levels and the rapid onset. Patients prefer vaporization rather than smoking and appreciate the ease of titrating the dose by inhalation. However, up to date there are only very few projects to provide patients with convenient portable inhalers for controlled delivery. One remarkable development is the Syqe® inhaler, containing so called vapor chips with pre-metered doses of cannabis flos for precise and reproducible administration (E.Eisenberg et al., Journal of Pain & Palliative Care Pharmacotherapy (2014) 1-10).

The shape of the vapor chip's powder chamber (approx.. 10×6mm) is completely different from typical medical inhaler containers, like capsules (∅ 5-6mm) or blister pockets (typically 4×6mm), as the depth of the chamber is ≤ 1mm. This space has to be uniformly filled with 10mg to 15mg ground cannabis flowers, to make sure that the material is evenly and reproducibly extracted during the inhalation procedure. In addition the uniformity of the dosed mass has to comply with the pharmacopoeial requirements.

The ground cannabis powder consists of irregularly shaped particles with a D_{10} of 70µm and has a bulk density of approx. 0.3 g/ml. Despite the large particle size compared to typical powders for inhalation, the flowability is rather poor due to the resin content and the low density. This could be demonstrated by testing the powder with a FT4 powder rheometer (Freemantechnology, Welland, Malvern UK) and comparison with typical lactose carriers used in dry powder inhalers. Ground cannabis has a flowability similar to micronized lactose with a D_{50} of 2,7µm (basic flow energy 120 mJ) while the compressibility is even higher (38% at 15 kPa).

In a first approach the powder was dosed with standard methods which are used to fill dry powder inhalers. Dosator or vacuum dosator showed poor dosing accuracy, the membrane filler (M. Weigel, EP 2 195 244 (2012)), used to fill blister cavities to the rim, failed due to poor powder flowability. Using the one-sided sealed vapor chip directly as "vacuum dosator" (M. Lober, EP 2 995 209 (2016)) to suck the powder into the chip led to high dosing variance due to inhomogeneity of the thin powder layer to be prepared. The vacuum drum system (K.Seyfang, H.Steckel, TechnoPharm 3 (2013) 304-311) could dose the cannabis powder with very good accuracy (RSD < 1,5%), but produced cylindrical monolithic powder portions. Unfortunately, subsequent distribution of the powder dose by vibration did not work, due to the auto-granulation effect leading to agglomerates and inhomogeneous distribution inside the vapor chips.

Therefore a new dedicated dosing system has been developed, consisting of a drum dosing unit to precisely pre-meter the cannabis powder and a distribution unit, where the powder is pneumatically transferred into the vapor chips. Dedicated design of the transfer unit and control of air flow assures uniform powder distribution. After a compaction step the vapor chip is finally sealed and assembled into cartridges. The pilot machine fills and seals 8 vapor chips in parallel in one machine cycle, with a dosing accuracy of ≤ 1.5%.

SHORT-TERM INHIBITION OF ABHD6 ALLEVIATES SELECT BEHAVIORAL SYMPTOMS IN THE HDHQ200/200 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's Disease (HD) is a devastating inherited autosomal dominant neurodegenerative disease characterized by progressive deterioration of motor, cognitive and psychiatric functions with no known cure and few palliative treatment options available. The HdhQ mouse model was developed by inserting pathogenic-sized CAG repeats into the *Hdh* gene, the mouse homolog of the HD gene. Our longitudinal studies on behavioral phenotypes and pathology of the HdhQ200/200 model, a mouse line containing 200 CAG repeats, revealed disease onset as early as 6 months of age and end stage at approximately 12 months of age. Using *in vivo* calcium imaging, we examined pathophysiological abnormalities and found pronounced alterations in activity-dependent calcium dynamics in medium spiny neurons of the striatum in response to cortical stimulation in 8-month-old HdhQ200/200 female mice compared to wild-type females. This indicates prominent aberrant calcium handling and hyperexcitability in these neurons. Previous work has suggested that continuous cannabinoid treatments may alleviate behavioral and pathological deficits seen in other HD models. We tested KT-182, a novel ABHD6 inhibitor, at the age of 10 months to examine if inhibiting ABHD6 activity and increasing select lipid signals, including 2-AG, was sufficient to rescue select behavioral symptoms. One week of continuous infusion of KT-182 through mini-osmotic pumps showed indication of alleviating spontaneous dark phase locomotor and grip strength abnormalities at 10 months of age in female HdhQ200/200 mice. Our next set of experiments will determine if short-term ABHD6 inhibition can also reduce hyperexcitability in the striatum in the HdhQ200/200 mouse model. Our study will disclose if cannabinoid manipulation through ABHD6 inhibition can attenuate behavioral and pathophysiological changes in the HdhQ200/200 mouse model.

Acknowledgements: Funded by NIH grants DA026430 and MH104450.

POSITIVE ALLOSTERIC MODULATION OF CB1 RECEPTOR SIGNALING TO LOWER INTRAOCULAR PRESSURE

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Nearly half a century has passed since the demonstration that marijuana and its chief psychoactive component Δ^9-THC lower intraocular pressure (IOP). Elevated IOP remains the chief hallmark and therapeutic target for glaucoma, a condition that places millions at risk of blindness. It is likely that Δ^9-THC exerts much of its IOP-lowering effects via activation of CB_1 cannabinoid receptors. However the initial promise of CB_1 as a target for treating glaucoma has not thus far translated into a credible therapeutic strategy. We have recently shown that blocking monoacylglycerol lipase (MAGL), an enzyme that breaks the endocannabinoid 2-AG, substantially lowers IOP for 8 hours (Miller et al., 2016). Another strategy to lower IOP is to develop CB_1 receptor positive allosteric modulators (PAMs), ligands that bind to CB_1 receptors and enhance the actions of orthosteric CB_1 agonists, and are optimized for topical application to the eye.

We have developed and tested structural variations of 2-phenylindoles of CB1 receptor PAMs, using a combination of functional assays (cAMP, β-arrestin, GTPγS etc.), and electrophysiology in autaptic hippocampal neurons, a well-characterized model of endogenous cannabinoid signaling. We additionally investigated the ability of these compounds to modulate IOP in normotensive mice. The IOP measurements were taken with rebound tonometer at baseline (time 0), and at several time-points following drug administration. We now report that GAT592 and GAT1102 each exhibit PAM-like signaling in neurons, although each also exhibits signs of agonism suggesting that these are ago-PAMs. Topical administration of GAT592 (0.25%) alone lowers IOP at 1 hour after administration. When co-applied with a subthreshold concentration of WIN55,212-2 (0.25%) GAT592 significantly lowers IOP in normotensive mice at 1, 6 and 12 hours after administration. Administration of GAT1102 (0.25%) with a subthreshold concentration of WIN55,212 also significantly decreases IOP at 6 and 12 hours following administration .

Our results suggest that enhancement of CB_1 signaling with CB1 PAMs continues to be a promising approach to lower IOP in a murine model and merits further study in other model systems.

Acknowledgments: This work was supported by grants from the National Institute on Drug Abuse, DA021696 (KM), and the NEI, EY24625 (AS), EY24717 (GT)

INFLAMMATORY PAIN-INDUCED CHANGES IN CB1 RECEPTOR IN THE MIDBRAIN PERIAQUEDUCTAL GRAY

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The periaqueductal gray (PAG) mediates the antinociceptive properties of analgesics, including opioids and cannabinoids. Administration of either opioids or cannabinoids into the PAG induces antinociception. However, most studies characterizing the antinociceptive properties of cannabinoids in the PAG have been conducted in naive animals. Few studies have reported on the role of CB1 receptors in the PAG during conditions which would prompt the administration of analgesics; namely, in chronic pain. In this study, we used the CFA model to characterize CB1 receptor expression and function during persistent inflammatory pain. Using cellular fractionation and western blot analysis, we demonstrate that 48 hours after CFA injection, there is a significant upregulation in the expression of synaptic CB1 receptors. We confirmed this change in CB1 expression using quantitative PCR to measure CB1 mRNA after the induction of inflammatory pain. To assess whether this protein upregulation induces a functional change, we measured the anti-hyperalgesic action of WIN 55,212-2 microinjected directly into the PAG. Interestingly, the anti-hyperalgesic effect of WIN was more pronounced in male rats. By studying the potency of WIN during conditions in which analgesics would normally be administered, we have unveiled a novel compensatory change in the descending pain pathway during persistent pain. Opioids are still the most commonly prescribed analgesics for chronic pain. Because there are neuroanatomical and functional interactions between PAG CB1 and mu-opioid systems, these pain-induced changes provide critical insight into the analgesic efficacy of these drugs during persistent pain, and may aid the development of novel pharmaco-therapies.

Acknowledgements: This work was funded by NIDA K99 DA041467

DIFFERENTIAL ACUTE EFFECTS OF CANNABIS USE AND WITHDRAWAL SYMPTOMS BY USER TYPE AND AGE

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The rapidly evolving legal and medical culture surrounding cannabis has precipitated a corresponding change in the demographic of cannabis users. Specifically, stigma appears to be decreasing and the percentage of the aging population using cannabis is rising substantially. There have been few recent attempts to describe the experience of being "high", specifically for the purpose of educating naïve medical or older users in clinical settings. As such there is curiosity among cannabis naïve individuals about what it feels like to be "high," with many seeking to understand 1) what they can expect to feel or experience when using cannabis, 2) what withdrawal symptoms might be expected when discontinuing use, and 3) whether other users consider it "addictive." Therefore, the goal of this study was to survey current cannabis users on the acute effects of being 'high', as well as their subjective experiences of withdrawal and their beliefs around the addictive potential of cannabis. We further sought to contrast these effects across different user types (medical vs. recreational vs. mixed users) and age groups (18-29 [young], 30-49 [middle-aged], 50+ [older]).

A sample of 2905 cannabis users responded to the survey. Respondents were asked to "Please tell us about your experience immediately after cannabis use. What is your experience of 'being stoned'? Check all that apply", of 41 possible acute effects. They were asked about withdrawal symptoms experienced upon discontinuation of use: "Do you experience any of the following symptoms during the 72 hours after interrupting routine use? Check all that apply" of 14 possible responses. Finally, they were asked "In your opinion, do you believe that cannabis is addictive?" The most commonly reported acute effects were feeling more calm/peaceful (79.7%), a desire to eat (72.7%), feeling more creative (72.4%), dry mouth (63%), and feeling less anxious or fearful (56.7%). Comparisons of different user types revealed that medical users reported fewer undesirable cognitive (e.g., memory), psychological (e.g., paranoia), and physiological (e.g., dry mouth) effects than recreational or "mixed" users. Similarly, older individual (50+ years) reported fewer undesirable cognitive, psychological and physiological effects than middle-aged and younger individuals. The most commonly reported withdrawal symptoms were irritability (33.7%), insomnia (30.3%) and anxiety (22.7%). Significantly more medical and mixed users reported experiencing these withdrawal symptoms than recreational users. Younger individuals were also more likely to report withdrawal symptoms than older individuals. 16.7% reported experiencing trouble stopping cannabis use, corresponding with 17% believing that cannabis is addictive. Significantly more younger cannabis users reported trouble stopping cannabis than older individuals and significantly fewer older individuals reported believing cannabis is addictive than middle-aged and younger individuals. We suggest that medical users may either be divorced from the idea of the negative side effect profile or find that the enhanced quality of life shifts benefit/risk profile to an acceptable one. Older people may already be experiencing aging effects such as short term memory loss, and thus be more tolerant to some cannabis acute effects.

PATIENT REPORTED OUTCOMES AFTER THE INITIATION OF MEDICAL CANNABIS – AN OBSERVATIONAL STUDY

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Background: Medical cannabis use has gained increasing popularity in Canada and has been used for numerous indications including but not limited to pain, sleep, mood, anxiety, nausea, and seizures. There is currently a lack of understanding of consumers of cannabis and of long-term, large-scale studies assessing the role of cannabis in pain, sleep, and function. Therefore, we undertook an observational study to characterize the effects of smoked, inhaled, or orally consumed cannabis on participants from Canadian Licensed Producers.

Methods: The study has research ethics board approval (REB#15040). One thousand participants were recruited from 23 Canadian Cannabis Clinics in Ontario. Patients had to be English-speaking, 25 years or older, and were prescribed cannabis for a chronic condition. Exclusion criteria was based on Health Canada consumer guide for cannabis. Participants were surveyed based on the IMMPACT recommendations along with assessments for neuropathic pain and opioid consumption. Surveys were administered online at baseline, 3 and 6 months. Enrollment period was between July 2015 and Oct 2017.

Results: A total of 1000 patients were enrolled and completed the baseline survey. Females accounted for 60.7% of those enrolled. White/Caucasian made up of 92.1% of those enrolled. Pain was indicated as a reason for seeking cannabis use for 87.1% of patients. The most common pain types were neuropathic pain (30.7%), whole body pain / fibromyalgia (26.6%), and arthritis pain (25.0%). Cannabis oil (56.5%) was favoured slightly more than dried cannabis (40.0%) at 6 months. Inhalation was the most common form of cannabis administration (63.9%). A significant majority of patients used less than 3g of cannabis per day (84.7%). In patients taking opioids at 6 months, 66.1% of those patients indicated a reduction in opioid dose since initiating cannabis.

Conclusion: Patients predominantly sought cannabis for treatment of pain in Cannabis Clinics in Ontario. A reduction in opioid consumption in the majority of patients suggests that cannabis may be useful in the clinic to help with opioid weaning.

Research funded by CanvasRx Inc., Ontario, Canada

POSITIVE ALLOSTERIC MODULATION OF CB_1 CANNABINOID RECEPTOR SIGNALING ENHANCES ANTI-ALLODYNIC EFFECTS OF MORPHINE AND ATTENUATES MORPHINE TOLERANCE

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Opioid treatment has been a mainstay for treating chronic pain disorders, representing a critical treatment in the analgesic ladder of the World Health Organization. While effective, their use can result in a number of unwanted side-effects including incomplete efficacy, constipation and overdose liability. Cannabinoids enhance the pain-relieving effects of opioids in preclinical studies and dampen unwanted side-effects resulting from excessive opioid administration (i.e. withdrawal).We recently reported that a CB_1 positive allosteric modulator (PAM) exhibits antinociceptive efficacy in models of pathological pain and lacks the adverse side effects of direct CB_1 activation (Slivicki et al. (2017) *Biol Psychiatry)*. In the present study, we evaluated whether a CB_1-PAM would enhance morphine's therapeutic efficacy in an animal model of chemotherapy-induced pain and mitigate unwanted side-effects associated with chronic opioid administration. On day 21, mice receiving chronic treatments were also challenged with the opioid antagonist naloxone to precipitate opioid withdrawal. In neuropathic mice, both the CB_1-PAM GAT211 and the opioid-analgesic morphine reduced paclitaxel-induced hypersensitivities to mechanical and cold stimulation in a dose dependent manner. Isobolographic analysis revealed that GAT211 and morphine resulted in antinociceptive synergism. Tolerance developed to the anti-allodynic effects of morphine administered alone, but not to the anti-allodynic effects of GAT211. Furthermore, a behaviorally inactive dose of GAT211 attenuated tolerance to anti-allodynic effects of morphine throughout the chronic dosing period. No differences in opioid withdrawal signs were observed in morphine and GAT211-treated groups suggesting that the CB_1-PAM did not enhance opioid dependence. We also compared the impact of GAT211 treatment on the dose response curve for morphine to produce antinociception in the tail-flick test in the absence of the neuropathic pain state. The dose response for morphine antinociception was determined before and after chronic treatments with vehicle, GAT211, morphine, or GAT211 + morphine in combination. GAT211 prolonged antinociceptive efficacy of morphine, although tolerance developed by day 6 of chronic dosing. Co-administration of GAT211with morphine also reduced the right-ward shift of the dose-response for morphine to produce antinociception. Our results suggest that a CB_1-PAM may be beneficial in enhancing and prolonging the therapeutic properties of opioids while potentially sparing unwanted side-effects such as dependence and tolerance which occur with repeated opioid treatment.

Supported by DA041229, CA200417, DA042584 (to AGH) and EY024717 (to GAT). RAS is supported by T32DA024628 and the Harlan Research Summer Scholars Program.

DEVELOPMENT OF CANNABIS CHEMOVARS FOR WHOLE PLANT MEDICINES BASED ON DATA FROM LABORATORY ASSAYS TO IMPROVE EFFICACY

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Introduction: Whole plant medicine is often touted as more efficacious than pure, single-compound, silver bullet preparations. This concept is based on the assumption that synergistic effects arise from the co-administration of certain compounds that improve efficacy, but do not present themselves when each compound is administered separately.

Whole-plant products should not be viewed equally nor be considered better than single-compound options merely because they are whole-plant products. To be credible, "synergistic effects," and related concepts of "entourage effects" and "symphonic effects" need to be based objectively on verifiable inputs and outputs, namely: (i) specified compounds, (ii) specified concentrations, and (ii) measureable effects stemming from co-administration of the specified compounds at various ratios. Whether in the medical-use or adult-use context, this approach will allow healthcare practitioners and consumers alike to benefit from an objective, evidence-based approach to comparing and selecting whole-plant cannabis products. This approach was used to proactively guide the breeding programs and research and development initiatives presented herein.

Traditionally, naturally occurring plants and plant-based products are screened in laboratory assays to identify prospects for therapeutic use. Herein, we employ data from laboratory assays that identify objectively measurable synergistic effects stemming from the combination of single-molecule compounds and how such data can be used to guide plant breeding in order to create plants that contain specified synergistic combinations of compounds in specified concentrations designed to improve efficacy and user experience.

Methods: Efficacy of various ratios of cannabidiol (CBD) and β-caryophyllene (BCP) to inhibit cellular inflammation induced by bacterial LPS in murine macrophages were compared based on the percent of inhibition. Marker-assisted breeding methods were initiated to create plant material containing ratios identified by the in-vitro assays to be of particular interest. Cannabis samples stemming from the breeding program were analyzed using the techniques of Giese et al., 2015. Reporting of analytical results was effected with PhytoFacts®.

Results: Unique chemovars were bred using a sophisticated breeding program targeting CBD and BCP production in plants that were informed by concentrations that demonstrated measurable synergistic effects in macrophage assays. CBD and BCP each individually exhibited a range of anti-inflammatory effects in a macrophage model with characteristic dose-response patterns (range: 0.0001 – 100 μM). Co-administration of the two compounds in the macrophage model produced enhanced effects as compared to each compound alone, with the level of enhancement impacted by both the absolute and relative concentration levels of each compound. Of particular interest, the inhibition produced by certain CBD concentrations increased by up to 10x when co-administered with certain concentrations of BCP. Targeted breeding efforts driven by these data resulted in more than a 6x increase in plant BCP biosynthesis pushing the concentration from 1.5 mg/g to over 10 mg/g.

Conclusions: Bioprospecting and sophisticated breeding techniques were used along with analytical assays to produce desired chemical profiles in cannabis plants that have objectively measurable greater synergistic effect than naturally occurring cannabis plants, cannabis found in the marketplace, and cannabis commonly used in research.

CANNABINOID AND TERPENE FORMULATIONS ELICIT DISTINCT MOOD EFFECTS

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Inhaling vaporized marijuana concentrate elicits a feeling of euphoria that sometimes includes nuanced states of mind such as creativity, relaxation, and stimulation. We investigated marijuana's potential to evoke several moods by using a visual analog scale (VAS) to subjectively and repeatedly score mood effects before, and at two time points after, subjects inhaled vaporized mixtures of terpenes and chromatographically purified cannabinoids. Delta from baseline mean VAS scores (student's t-test, p-value ≤ 0.001) from a double-blinded, randomized observational study suggested that each of two tested formulations elicited distinct mood effects. Statistically significant scores suggested that each formulation could be described by euphoric VAS adjectives, but adjectives describing stimulating mood effects were more abundant for one formulation, whereas the second formulation was characterized by VAS adjectives that describe relaxation. The observed contrast between relaxing and stimulating formulations was sustained by submitting VAS scores to two-sided student's t-testing, which differentiated (p-value ≤ 0.05) the two formulations on the basis of stimulation or relaxation. Our results point to agreement among a population of subjects on the mood effects elicited by cannabinoid and terpene formulations, and also to differences in moods elicited by each formulation. Together, our results encourage further formulation of terpenes and purified cannabinoids for eliciting other user-desired effects.


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ICRS2018
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ISBN 978-604-0-00008-8