32ND ANNUAL SYMPOSIUM OF THE INTERNATIONAL CANNABINOID RESEARCH SOCIETY

GALWAY
IRELAND

JUNE 25-30, 2022

32ND ANNUAL

SYMPOSIUM OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

GALWAY

JUNE 25 – 30, 2022

Symposium Programming by Cortical Systematics LLC

International Cannabinoid Research Society
Research Triangle Park, NC
USA

ISBN: 978-604-0-00069-9

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

Author(s), Abstract Title (2022) 32nd 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.

ICRS2022 GOLD Sponsors

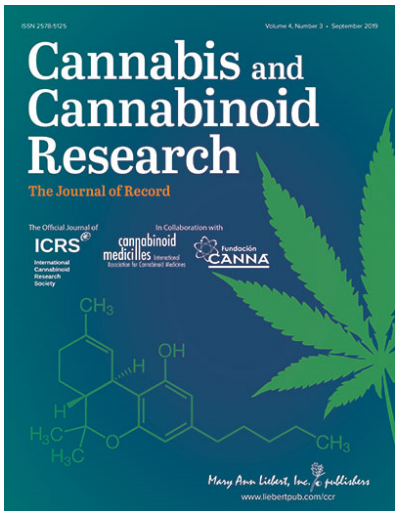
Fáilte Ireland

MEET IN IRELAND

bedrocan®

Cannabinoid Education 360°

Developed by Jazz Pharmaceuticals



Mary Ann Liebert, Inc.

SILVER Sponsor

SciSparc

Cayman CHEMICAL

BRONZE Sponsors

Artelo BIOSCIENCES

CANOPY GROWTH CORPORATION

GREEN Sponsors

Fundación CANNA

B BRAUN SHARING EXPERTISE

Government Sponsors

National Institute on Drug Abuse

Non-Profit Organization Sponsors

Kang Tsou Memorial Fund

Monique C. and George L. Braude Memorial Foundation

Fundación CANNA

Cannabis science and education.

www.fundacion-canna.es

2022 Symposium on the Cannabinoids

Conference Coordinators

David Finn, Ph.D.
Melanie Kelly, Ph.D.
Jason Schechter, Ph.D.
Sara Whynot
Shlomi Ashkenazi - VERUS
Rami Laor – VERUS
Michelle Roche, Ph.D. – NUI Galway
Patricia Walsh – NUI Galway

2022 Programme Committee

Steve Alexander, Ph.D.
Shanna Babalonis, Ph.D.
Heather Bradshaw, Ph.D.
Stevie Britch, Ph.D.
Vicky Chapman, Ph.D.
Damien Cohall, Ph.D.
Ziva Cooper, Ph.D.
Vincenzo Di Marzo, Ph.D.
Eilís Dowd, Ph.D.
Eric Downer, Ph.D.
David Finn, Ph.D. (President and Chair)
Josee Guindon, Ph.D.
Caroline Herron, Ph.D.
Matt Hill, Ph.D.
Cecilia Hillard, Ph.D.
Andrea Hohmann, Ph.D.
Yasmin Hurd, Ph.D.
Steve Kinsey, Ph.D.
Linda Klumpers, Ph.D.
Magdalena Kostrzewa, Ph.D.
Aron Lichtman, Ph.D.
Mary Lynch, Ph.D.
Mauro Maccarrone, Ph.D.
Ken Mackie, M.D.
Giovanni Marsicano, Ph.D.
Iain McGregor, Ph.D.
John McPartland, D.O.
Spyros Nikas, Ph.D.
Saoirse O'Sullivan, Ph.D.
Pal Pacher, M.D., Ph.D.
Roger Pertwee, MA, D.Phil., D.Sc.
Daniele Piomelli, Ph.D.
Michelle Roche, Ph.D.
Ruth Ross, Ph.D.
Ethan Russo, M.D.
Katarzyna Starowicz, Ph.D.
Yossi Tam, D.M.D., Ph.D.

Lifetime Achievement Award Committee

Cecilia Hillard, Ph.D.
Allyn Howlett, Ph.D.
Steve Kinsey, Ph.D (Chair)
Aron Lichtman, Ph.D.

Young Investigator Award Committee

Lumír Hanuš, PhD, D.Sc., Dr.h.c.mult.
Allyn Howlett, Ph.D. (Chair)
Michelle Glass, Ph.D.

Student Prizes and Awards Committee Chair

Steve Alexander, Ph.D.

Equality, Diversity and Inclusion Committee

Damian Cohall, Ph.D.
Rebecca Craft, Ph.D.
W.S. Vanessa Ho, Ph.D. (Chair)
Haley Vecchiarelli, Ph.D.

Mechoulam Award Committee

Mary Abood, Ph.D.
Francis Barth, Ph.D.
Benjamin Cravatt, Ph.D.
Vincenzo Di Marzo, Ph.D.
Javier Fernández-Ruiz, Ph.D.
Cecilia Hillard, Ph.D.
Allyn Howlett, Ph.D.
John Huffman, Ph.D.
George Kunos, M.D., Ph.D.
Gerard Le Fur, Ph.D.
Aron Lichtman, Ph.D. (Chair)
Beat Lutz, Ph.D.
Ken Mackie, M.D.
Mauro Maccarrone, Ph.D.
Alex Makriyannis, Ph.D.
Roger Pertwee, M.A., D.Phil, D.Sc.
Daniele Piomelli, Ph.D.
Raj Razdan, Ph.D.
Patti Reggio, Ph.D.
Murielle Rinaldi-Carmona, Ph.D.
Natsuo Ueda, M.D., Ph.D.
Andreas Zimmer, Ph.D.

2022 ICRS Board of Directors

Executive Director
Melanie Kelly, Ph.D.

President
David Finn, Ph.D.

President-Elect
Ruth Ross, Ph.D.

Past President
Yossi Tam, Ph.D., D.M.D.

Secretary
Saoirse O'Sullivan, Ph.D.

Treasurer
Steve Kinsey, Ph.D.

International Secretary
Roger Pertwee, M.A., D.Phil, D.Sc.

Student Representative
Magdalena Kostrzewa, Ph.D.

Equality, Diversity and Inclusion Representative
W.S. Vanessa Ho, Ph.D.

Newsletter Editor
Stephen P.H. Alexander, Ph.D.

Mentorship Programme Coordinator
Stevie Britch, Ph.D.

Grant co-PIs
Heather Bradshaw, Ph.D.
Steve Kinsey, Ph.D.

Managing Director
Jason B. Schechter, Ph.D.

Registration: June 25th, 2022 (16.00 – 18.00)
Bailey Allen Hall, NUI Galway, Ireland

Welcome Reception: 18.00 – 20.00
(Includes a talk by Dr. Ethan Russo on the history of cannabinoid research in Ireland and the contribution of Irish physicians and scientists to cannabinoid research, followed by drinks and BBQ)

Day 1
Sunday, June 26th

8.30	Welcome and Opening Remarks		
Oral Session 1. New tools for cannabinoid research and drug development Chairs: Colm Collins and Ruth Ross			
8.45	Ming Jiang, Mirjam Huizenga*, Jonah Wirt, Avand Amedi, Richard van der Berg, Joerg Benz, Ludovic Collin, Hui Deng, Bobby Florea, Uwe Grether, Anthe Janssen, Laura Heitman, Tsang-Wai Lam, Florian Mohr, Anto Pavlovic, Iris Ruf, Helma Rutjes, Floor Stevens, Daan van der Vliet, Tom van der Wel, Matthias Wittwer, Stan van Boeckel, Andrea Hohmann and Mario van der Stelt	DISCOVERY OF LEI-515: AN *IN VIVO* ACTIVE, PERIPHERALLY RESTRICTED, REVERSIBLE MAGL INHIBITOR	1
9.00	Charlie Bell, Jörg Benz, Ludovic Collin, Martin Edelmann, Thais Gazzi, Maude Giroud, Luca Gobbi, Monica Guberman, Yingfang He, Dominik Heer, Axel Hentsch, Manuel Hilbert, Michael Honer, Benoit Hornsperger, Sylwia Huber, Melanie Hug, Claudia Keller, Stefanie Krämer, Carsten Kroll, Bernd Kuhn, Marius Lutz, Rainer Martin, Carla Meier, Yelena Mostinski, Linjing Mu, Adrienne Müller Herde, Marc Nazare, Fionn O'Hara, Annemarieke Postmus, Hans Richter, Martin Ritter, Didier Rombach, Roger Schibli, Mario van der Stelt, Floor Steven, Marco Taddio, Haiyan Wang, Matthias Wittwer and Uwe Grether*	DEVELOPMENT OF NOVEL REVERSIBLE MONOACYLGLYCEROL LIPASE PET AND FLUORESCENT PROBES	2

9.15	Partha Mukhopadhyay, Janos Paloczi, Csaba Matyas, Eszter Trojnar, Resat Cinar, Szabolcs Dvoracsko, Yuri Persidsky, Laura H. Heitman, Mario van der Stelt, Jürgen Fingerle, Sabine Grüner, Jürgen Funk, Christian Apfel, Matthias Wittwer, Pawel Dzygiel, Stefanie Bendels, Christoph Ullmer, Wolfgang Guba, Uwe Grether and Pal Pacher*	CHARACTERIZATION OF NOVEL CANNABINOID RECEPTOR AGONISTS FOR THE TREATMENT OF KIDNEY DISEASES	3
9.30	Saoirse O'Sullivan*, Anna Pereira, Linette Ruston, Paul Duffy, Martin Kaczocha, Iwao Ojima and Andrew Yates	DISCOVERY AND PRECLINICAL EVALUATION OF A NOVEL INHIBITOR OF FABP5, ART26.12, EFFECTIVE IN CHEMOTHERAPY-INDUCED PAIN	4
9.45	Anthony English, Fleur Uittenbogaard, Anna Slaven, Michael Bruchas, Benjamin Land* and Nephi Stella	DEVELOPMENT OF A PALATABLE GELATIN THAT PROMOTES VOLUNTARY ORAL CONSUMPTION OF THC IN MICE	5
10.00	Lucas Laudermilk, Jennifer Lucitti, George Amato, Alan Fulp, Vineetha Vasukuttan, Joel Schlosburg, Erica Golden, Rosamond Goodson, Herbert Seltzman, Scott Runyon and Rangan Maitra*	A NOVEL CB1 RECEPTOR NEUTRAL ANTAGONIST REVERSES NAFLD/NASH IN MICE	6
10.15	COFFEE BREAK		
ORAL SESSION 2. CANNABINOID EXPOSURE IN EARLY LIFE CHAIRS: ÁLVARO LLORENTE-BERZAL AND MICHELLE ROCHE			
10.45	Ryan McLaughlin*, Halle Weimar, Darren Ginder, Alexandra Malena, Amanda Brown and James Peters	MULTIGENERATIONAL EFFECTS OF CANNABIS EXPOSURE ON EMOTIONALITY, VAPOR SELF-ADMINISTRATION, AND CORTICOSTRIATAL INPUTS IN A NOVEL RAT MODEL OF MATERNAL CANNABIS USE	7

11.00	Lin Lin*, Kwang-Mook Jung, Steve Mahler, Faizy Ahmed, Erica Squire, Shiqi Su, Alexa Torrens, Yannick Fotio, Jade Ramirez, Hyelim Lee, Francesca Palese, Georgia Colleluori, Courtney Wood, Saverio Cinti, Nicholas DiPatrizio and Daniele Piomelli	ADOLESCENCE THC EXPOSURE DISRUPTS WHITE ADIPOSE TISSUE HOMEOSTASIS IN ADULTHOOD	8
11.15	Julia Evanski*, Shreya Desai, Samantha Baglot, Matthew Hill and Hilary Marusak	IMPACT OF PRENATAL CANNABIS EXPOSURE ON LIMBIC PATHWAYS AND NEUROBEHAVIORAL OUTCOMES IN CHILDREN	9
11.30	Jeffrey Edwards*, Isaac Ostlund and Calvin Smith	DIFFERENTIAL ACTIVATION AND Δ^9-TETRAHYDROCANNABINOL EFFECT ON CB1-DEPENDENT LONG-TERM DEPRESSION IN VENTRAL TEGMENTAL AREA GABA NEURONS IN ADULT VERSUS ADOLESCENT MICE	10
11.45	**PRESIDENTIAL PLENARY LECTURE** **THE MICROBIOTA-GUT-BRAIN AXIS: A GUT FEELING ABOUT STRESS, PAIN & MENTAL HEALTH** **JOHN CRYAN, BSC (HONS), PHD** Professor and Chair, Department of Anatomy and Neuroscience University College Cork Ireland CHAIR: DAVID FINN		
12.45	**LUNCH**		
ORAL SESSION 3. CANNABINOIDS IN IMMUNOMODULATION, INFLAMMATION AND METABOLISM CHAIRS: MEL KELLY AND YOSSI TAM			
14.15	Abhishek Basu*, Muhammad Arif, Kaelin Wolf, Charles N. Zawatsky and Resat Cinar	CANNABINOID RECEPTOR 1 (CB1R) IN ALVEOLAR MACROPHAGES REGULATES THE DEVELOPMENT OF PULMONARY FIBROSIS	11

14.30	Mauro Maccarrone*, Alessandro Leuti, Lucia Scipioni, Marina Fava, Niccolò Pellegrini, Francesca Ciaramellano and Sergio Oddi	CROSSTALK BETWEEN ENDOCANNABINOID SYSTEM AND RESOLUTION OF INFLAMMATION IN INNATE IMMUNITY AND COGNITIVE DECLINE	12
14.45	Malliga Iyer*, Resat Cinar, Szabolcs Dvorascko and George Kunos	STUDIES ON THE PERIPHERALLY RESTRICTED DUAL-TARGET 3,4-DIARYLPYRAZOLINES AS POTENT ANTAGONISTS OF CANNABINOID-1 (CB1R) RECEPTOR	13
15.00	Sam Johnson* and James Burston	12/15 LIPOXYGENASE IS A KEY REGULATOR OF THE RESOLUTION OF INFLAMMATION IN WOUND HEALING	14
15.15	John-Mark Fitzpatrick, Becky Hackett, Lisa Costelloe, William Hind and Eric Downer*	BOTANICALLY-DERIVED HIGHLY PURIFIED CANNABIDIOL AND DELTA-9-TETRAHYDROCANNABINOL, AND THEIR 1:1 COMBINATION, MODULATE TOLL-LIKE RECEPTOR 3 AND 4 SIGNALLING IN IMMUNE CELLS FROM PEOPLE WITH MULTIPLE SCLEROSIS	15
15.30	Hemanth Cherukury*, Donovan Argueta, Natalie Garcia, Raghda Fouda, Stacy Kiven, Jianxun Lei, Varun Sagi, Graham Velasco and Kalpna Gupta	CANNABIDIOL AMELIORATES CHRONIC HYPERALGESIA IN A MOUSE MODEL OF SICKLE CELL DISEASE	16
15.45	POSTER DATABLITZ SESSION 1 CHAIR: ERIC DOWNER		DB1
16.15 – 18.15	POSTER SESSION 1 RECEPTION		P1

Notes:

Presenting Author*

Day 2
Monday, June 27th

8.25	Opening Remarks		
8.30 - 9.30	**Kang Tsou Memorial Speaker** Adventures understanding pain, analgesia and anaesthesia-induced altered states of consciousness through human neuroimaging **Irene Tracey,** MA (Oxon), DPhil., FRCA, FMedSci Nuffield Chair of Anaesthetic Science University of Oxford United Kingdom Chair: David Finn		
Oral Session 4. Cannabinoids, The Endocannabinoid System, and Pain Chairs: Stevie Britch and Steve Kinsey			
9.30	Donovan Argueta*, Bryant Avalos, Stacy Kiven, Francisco Aguirre, Reina Lomeli, Yannick Fotio, Daniele Piomelli, Nicholas DiPatrizio and Kalpna Gupta	PALMITOYLETHANOLAMIDE ATTENUATES NEURAL INJURY AND PAIN VIA NOGO-A PATHWAY IN SICKLE CELL DISEASE	17
9.45	Hai Tran*, Yin Fang, Dongman Chao, Quinn Hogan and Bin Pan	DESCENDING MECHANISM BY WHICH MEDIAL PREFRONTAL CORTEX ENDOCANNABINOID SIGNALING CONTROLS NEUROPATHIC PAIN	18
10.00	Juliet Chung*, Vengadeshprabhu Karuppagounder, Ahmed Abdeen, Amy Thompson, Andreas Bouboukas, William Pinamont, Natalie Yoshioka, Wesley Raup-Konsavage, Nicholas M. Graziane, Kent E. Vrana, Reyed Elbarbary and Fadia Kamal	THE EFFECT OF CBD AND CBG OILS ON OSTEOARTHRITIS DISEASE PROGRESSION, INFLAMMATION, AND PAIN	19

10.15	Christopher Norris*, Dana Selley and Bradley Taylor	ENDOGENOUS ANALGESIC SYNERGY BETWEEN MU OPIOID AND CB1 RECEPTOR CONSTITUTIVE ACTIVITY	20
10.30	Stephanie Bourke*, Barira Islam, Maurizio Manca, John Stephenson, David P. Finn and Patrick C. McHugh	ELEVATED LEVELS OF 2-ARACHIDONOYLGLYCEROL (2-AG) IN PLASMA OF PATIENTS WITH NEUROPATHIC PAIN	21
10.45	Kennedy Goldsborough*, Karan Muchhala, Atuahene Adu-Gyamfi, Donald Jessup, Kalpna Gupta, Benjamin F. Cravatt, Micah Niphakis, M. Imad Damaj, Joyce Lloyd, Hamid Akbarali, Wally Smith and Aron Lichtman	MONOACYLGLYCEROL LIPASE INHIBITION: A MULTIMODAL APPROACH TO TREAT CHRONIC PAIN IN THE BERKELEY SICKLE CELL MOUSE MODEL	22
11.00	COFFEE BREAK		
ORAL SESSION 5. CANNABINOIDS AND GASTROINTESTINAL TRACT FUNCTION CHAIRS: HEATHER BRADSHAW AND NEPHI STELLA			
11.30	Mariam S. Gh. Alketbi*, Megan R. Sanctuary, Care L. Wilkinson, Alexandre J. Jones, Edward J. Hoffenberg, Cecilia J. Hillard, J Romero and Colm Collins	THE IMPACT OF CANNABIS IN THE INTESTINAL IMMUNE SYSTEM TO IDENTIFY NOVEL THERAPEUTIC TARGETS AND BETTER INFORMING THE PATIENTS	23
11.45	Grzegorz Godlewski*, Alexa Herrerias, Anna Oliverio, Resat Cinar, Jie Liu, Malligha Iyer and George Kunos	CANNABINOID CB1 AND GHRELIN GHSR1A RECEPTORS ON SENSORY AFFERENTS OF THE GASTROINTESTINAL TRACT CONTRIBUTE TO THE CONTROL OF VOLUNTARY ALCOHOL DRINKING IN MICE	24
12.00	Kelsey Andreis, Amanda Thayer, Jenna Billingsley, Kian Naimi Shirazi, Jim Wager-Miller, Heather Bradshaw and Alex Straiker*	CANNABINOID CB1 RECEPTOR REGULATION OF SALIVATION	25

12.15	Isabel González Mariscal*, Valery Gmyr, Francisco Javier Bermudez Silva, Julie Kerr-Conte, François Pattou, Josephine M Egan and Marisol Ruiz de Adana	BLOCKADE OF CANNABINOID 1 RECEPTOR ARRESTS INSULITIS AND PRESERVES HUMAN ISLET FUNCTION IN A NOVEL *EX VIVO* MODEL OF ISLET INFLAMMATION	26
12.30	Courtney P. Wood* and Nicholas V. DiPatrizio	PARASYMPATHETIC SIGNALING CONTROLS BIOSYNTHESIS OF OREXIGENIC ENDOCANNABINOIDS IN THE SMALL-INTESTINE OF WESTERN DIET-INDUCED OBESE MICE	27
12.45	**LUNCH**		
12.45	NIDA TRAINEE LUNCH AND LEARN CHAIRS: MAGDALENA KOSTRZEWA AND DAVID FINN 12.45 - 13.15: COMMUNICATION OF CANNABINOID SCIENCE TO DIVERSE AUDIENCES (LINDA KLUMPERS AND MATT HILL) 13.13 - 14.15: MENTORSHIP NETWORKING LUNCH		
ORAL SESSION 6. ENDOCANNABINOID SYSTEM REGULATION OF BEHAVIOUR CHAIRS: REBECCA CRAFT AND MATT HILL			
14.15	Anthony English, Simar Singh, Dennis Sarroza, David J. Marcus, Ao Dong, Yulong Li, Larry Zweifel, Benjamin Land, Michael R. Bruchas and Nephi Stella*	MEASURING CHANGES IN 2-AG SIGNALING WITH THE GRABECB2.0 SENSOR: CONTROL BY ABHD6 AND ROLE IN THC-MEDIATED INHIBITION OF LOCOMOTION	28
14.30	David Marcus, Emma Seth, Sabrina Hwang, Christian Pedersen, Avery Hunker, Selena Schrieber, Yulong Li, Benjamin Land, Larry Zweifel, Nephi Stella and Michael Bruchas*	ENDOCANNABINOID CONTROL OF BEHAVIORAL ENGAGEMENT VIA A NEUROPETIDERGIC PVT-NAC CIRCUIT	29
14.45	Taylor Woodward*, Sarah Stockman, Fezaan Kazi, Hasaan Kazi, Romario Pacheco, Wouter Driever, Mario van der Stelt, Ken Mackie and Andrea Hohmann	NAPE-PLD REGULATES AFFECTIVE BEHAVIORS, UNCONDITIONED FEAR RESPONSES, AND THE REINFORCING PROPERTIES OF ORAL OXYCODONE CONSUMPTION IN MICE	30
15.00	Matthew Jones*, Taygun Uzuneser, Hanna Szkudlarek and Steven Laviolette	A NOVEL FABP5 INHIBITOR REGULATES ANXIETY AND FEAR EXPRESSION WHEN ADMINISTERED LOCALLY TO THE BASOLATERAL AMYGDALA	31

15.15	Catharine Mielnik*, Claudia Lutelmowski, Clare Johnson, Julia Zebarth, Marija Milenkovic, Wendy Horsfall, Walter Swardsfager, Heather Bradshaw, Ali Salahpour and Ruth Ross	THE UNIQUE EFFECT OF MJN110 (MAGL INHIBITOR) IN HYPERDOPAMINERGIC STATES: IMPLICATIONS FOR 2-AG MODULATION IN PSYCHOSIS	32
15.30	POSTER DATABLITZ SESSION 2 Chair: Katarzyna Starowicz		DB2
16.00 – 18.00	Poster Session 2 Reception		P2
18:00	Business meeting		

Notes:

Presenting Author*

Day 3
Tuesday, June 28th

8.25	Opening Remarks		
Oral Session 7. CB_2 Receptors Chairs: Steve Alexander and Josee Guindon			
8.30	Jara Bouma*, Xiaoting Li, Hao Chang, Laura V. de Paus, Cas van der Horst, Sanjay S. Kumar, Lijie Wu, Antonius P.A. Janssen, Zhi-Jie Liu, Mario van der Stelt, Laura H. Heitman and Tian Hua	MOLECULAR BASIS FOR SELECTIVE ACTIVATION AND TARGET ENGAGEMENT OF CANNABINOID RECEPTOR 2 AGONISTS	33
8.45	Christina A. Behlke*, Leslie Krause, Jennifer Sterrett, Alison Moe and Cecilia J. Hillard	CELL SPECIFIC ROLES OF THE CB2R IN THE ACUTE LOCOMOTOR RESPONSE TO COCAINE	34
9.00	Ana M. Martínez Relimpio*, Samuel Ruiz de Martín Esteban, Irene Benito-Cuesta, M Teresa Grande, Diego Herráez-Aguilar, Ricardo Mostany, Cecilia J. Hillard and Julián Romero	EVALUATION OF THE IMPACT OF MICROGLIAL CANNABINOID CB2 RECEPTOR DELETION IN THE CONTEXT OF ALZHEIMER'S DISEASE	35
9.15	Idan Carmon*, Sai Rama Krishna Meka, Jinan Elayyan, Lital Zecharyahu, George Batshon, Eli Reich, Leonid Kandel, Andras Bilkei-Gorzo, Andreas Zimmer, Raphael Mechoulam and Mona Dvir-Ginzberg	THE CANNABINOID RECEPTOR 2 AGONIST, HU308, MITIGATES OSTEOARTHRITIS VIA CREB-MEDIATED SOX9 ACTIVATION	36
9.30	Jennifer Ana Iden*, Bitya Raphael-Mizrahi, Zamzam Awida, Aaron Naim, Dan Zyc, Tamar Liron, Marilena Vered and Yankel Gabet	THE ANTI-TUMORIGENIC ROLE OF THE CANNABINOID RECEPTOR 2 IN COLON CANCER	37

9.45	IN MEMORIAM		IM
10.00	COFFEE BREAK		
ORAL SESSION 8. PHYTOCANNABINOIDS - PHARMACOKINETICS AND THERAPEUTIC POTENTIAL 1 CHAIRS: DAMIAN COHALL AND SAOIRSE O'SULLIVAN			
10.30	Ryan Vandrey*, Tory Spindle, George Bigelow, C. Austin Zamarripa and Ethan Russo	A HUMAN LABORATORY STUDY ON THE INTERACTION BETWEEN INHALED D-9-THC AND LIMONENE	38
10.45	Austin Zamarripa*, Elise Weerts, Catherine Moore, Sumit Bansal, Tory Spindle, Jashvant Unadkat, Mary Paines and Ryan Vandrey	EVALUATION OF CYTOCHROME P450-MEDIATED CANNABINOID-DRUG INTERACTIONS IN HEALTHY ADULT PARTICIPANTS	39
11.00	Amir Englund*, Dominic Oliver, Edward Chesney, Lucy Chester, Jack Wilson, Simina Sovi, Andrea De Micheli, John Hodsoll, Paolo Fusar-Poli, Robin Murray, John Strang, Tom Freeman and Philip McGuire	CAN WE MAKE CANNABIS SAFER? AN EXPERIMENTAL STUDY OF FOUR CBD:THC RATIOS IN HEALTHY VOLUNTEERS	40
11.15	Jacqueline-Marie N. Ferland*, Randall J. Ellis, Joseph A. Landry, James E. Callen, Alexandra M. Chisholm, Micah D. Frier, Heather B. Bradshaw and Yasmin L. Hurd	CANNABIDIOL ALLEVIATES CUE-INDUCED ANXIETY LINKED TO ALTERATIONS IN CNR1 AND LIPID LEVELS IN THE NUCLEUS ACCUMBENS SHELL	41
11.30	Deepak Kumar Khajuria*, Vengadeshprabhu Karuppagounder, Muhammad Daniyal, Wesley Raup-Konsavage, Kent Vrana, Fadia Kamal and Reyad Elbarbary	CANNABIDIOL AND CANNABIGEROL PROMOTE BOTH EARLY AND LATE PHASES OF FRACTURE HEALING	42

11.45	ICRS Mechoulam Award Speaker TOWARDS A CANNABINOID-BASED NEUROPROTECTIVE THERAPY FOR NEURODEGENERATIVE DISORDERS: THE RECENT EXAMPLE OF TDP-43 DEPENDENT FRONTOTEMPORAL DEMENTIA **Javier Fernandez-Ruiz, Ph.D.** Professor UCM Department of Biochemistry and Molecular Biology Complutense University of Madrid SPAIN Chair: Cecilia Hillard
12.30	**Box Lunch** A quick box lunch (≈30 mins) and then onto buses for outings. Box lunch for all, regardless of whether going on outing or not.
13.15 -	**Free Time and Outings**

Notes:

Presenting Author*

Day 4
Wednesday, June 29th

8.25	Opening Remarks		
Oral Session 9. Phytocannabinoids - Therapeutic potential 2 Chairs: Cecilia Hillard and Daniele Piomelli			
8.30	Catherine Hume*, Samantha Baglot, Savannah Lightfoot and Matthew Hill	EFFECTS OF TETRAHYDROCANNABINOL (THC) VAPOUR EXPOSURE ON RAT FEEDING BEHAVIOURS AND HOMEOSTATIC APPETITE-REGULATING PATHWAYS	43
8.45	Ivori Zvorsky*, L. Riley Gournay, Jordan Petry, Sarah Bilsky, Morgan Hill, Matthew Feldner, Erica Peters, Marcel Bonn-Miller and Ellen Leen-Feldner	CANNABIDIOL REDUCES SYMPTOMS OF ANXIETY AND WITHDRAWAL DURING ACUTE E-CIGARETTE ABSTINENCE	44
9.00	Morgan Ferretti*, Taylor Stanley, Erica Peters, Marcel Bonn-Miller and Jessica Irons	THE EFFECTS OF CBD ISOLATE ON MENSTRUAL-RELATED SYMPTOMS	45
9.15	Carrie Cuttler* and Marcella Oreta	DOES CANNABIS PROTECT AGAINST AGE-RELATED DECLINES IN COGNITIVE FUNCTIONING?	46
9.30	Margaret Haney* and Diana Martinez	ORAL CANNABIS FOR TAXANE-INDUCED NEUROPATHY: A RANDOMIZED PLACEBO-CONTROLLED PILOT STUDY	47
9.45	Anastasia Suraev*, Iain S. McGregor, Nathaniel S. Marshall, Ron R. Grunstein and Camilla M. Hoyos	EFFECTS OF CANNABIDIOL (CBD) AND Δ9-TETRAHYDROCANNABINOL (THC) ON SLEEP AND DAYTIME FUNCTION IN INSOMNIA DISORDER: A RANDOMISED, DOUBLE-BLINDED, PLACEBO-CONTROLLED, PROOF-OF-CONCEPT TRIAL	48
10.00	Coffee Break		

Oral Session 10. Sex differences in cannabinoid effects and the endocannabinoid system

Chairs: Iain McGregor and Magdalena Kostrzewa

10.30	Aoife Thornton, David Finn and Michelle Roche*	INCREASING ENDOCANNABINOID TONE ELICITS SEX-SPECIFIC EFFECTS ON BEHAVIOURAL RESPONDING IN A PRENATAL MODEL OF AUTISM	49
10.45	Elise Weerts*, Catherine Moore and Catherine Davis	SEX AND HISTORY OF PASSIVE VAPOR EXPOSURE INFLUENCE Δ9-TETRAHYDROCANNABINOL (THC) VAPOR SELF ADMINISTRATION IN RATS	50
11.00	Melissa McHann*, Isabel Castro-Piedras, Daniel J. Morgan and Josee Guindon	SEX AND DOSE-DEPENDENT DELAY IN ANTINOCICEPTIVE TOLERANCE TO CP55,940 IN CB1 DESENSITIZATION-RESISTANT MUTANT MICE DUE TO JNK INHIBITION	51
11.15	Erin Martin* and Aimee McRae-Clark	SEX DIFFERENCES IN CANNABIS WITHDRAWAL AND THE ROLE OF THE ENDOCANNABINOID SYSTEM	52
11.30	Laura Boullon*, David P Finn and Alvaro Llorente-Berzal	EVALUATION OF SEX DIFFERENCES IN ENDOCANNABINOID-MEDIATED MODULATION OF NOCICEPTIVE BEHAVIOUR IN PERIPHERAL NEUROPATHY	53
11.45	Andrew Kesner*, Stephanie Ramos-Maciel, Yolanda Mateo, Alexa Gracias and David Lovinger	SEX-DEPENDENT CHANGES IN MURINE STRIATAL DOPAMINE RELEASE, SLEEP, AND BEHAVIOR DURING SPONTANEOUS Δ-9-TETRAHYDROCANNABINOL ABSTINENCE	54
12.00	**Presidential Plenary Lecture** Endocannabinoids Sculpt Sex Differences in the Developing Social Behavior Network **Margaret McCarthy, B.S., M.A., Ph.D.** James and Carolyn Frenkil Dean's Professor and Chair, Department of Pharmacology University of Maryland School of Medicine USA Chair: David Finn		

13.00	LUNCH		
ORAL SESSION 11. CLINICAL TRIALS, USE AND ORIGINS OF CANNABINOIDS CHAIRS: MARGARET HANEY AND ETHAN RUSSO			
14.00	Thomas Dowling III, Nicholas Frane*, Erik Stapleton, Larry Good, Timothy Garrett and Thomas Dowling	TOPICAL CANNABIDIOL IN PATIENTS WITH LOW BACK PAIN SECONDARY TO SPONDYLOLISTHESIS: A RANDOMIZED, DOUBLE-BLIND, 3-ARM, PARALLEL-GROUP PILOT STUDY	55
14.15	Stephanie Lake*, Margaret Haney and Ziva Cooper	SEX DIFFERENCES IN SUBJECTIVE AND REINFORCING EFFECTS OF SMOKED CANNABIS: A SECONDARY ANALYSIS OF TWO RANDOMIZED CONTROLLED TRIALS	56
14.30	Lucy Troup*, Simon Erridge, Beata Ciesluk and Mikael Hans Sodergren	AN ASSESSMENT OF CURRENT AND PREVIOUS EXPOSURE TO ILLICITLY OBTAINED CANNABIS IN UK MEDICAL CANNABIS PATIENTS	57
14.45	John McPartland* and Saoirse O'Sullivan	ORIGINS OF CANNABIS IN IRELAND: AN INTERDISCIPLINARY STUDY	58
15.00	ICRS WILLIAM A. DEVANE YOUNG INVESTIGATOR AWARDEE ALLOSTERIC PHARMACOLOGY AT THE CANNABINOID RECEPTORS: COMPLEX PATTERNS AND NOVEL QUESTIONS ROBERT LAPRAIRIE, PH.D. Associate Professor GSK-CIHR Research Chair in Drug Discovery and Development University of Saskatchewan CANADA CHAIR: ALLYN HOWLETT		
15.30	POSTER DATABLITZ SESSION 3 CHAIR: LINDA KLUMPERS		DB3

16.00 – 18.00	Poster Session 3 Reception	P3
19.00	AWARDS CEREMONY AND ICRS BANQUET	

Notes:

Presenting Author*

Departure: Thursday, June 30th

ICRS2022 DATABLITZ SESSION 1

DAY 1, SUNDAY, JUNE 26TH: 15:45 - 16:15

Andras Bilkei-Gorzo*, Britta Schürmann, Marion Schneider, Michael Krämer, Prakash Nidadavolu, Christa Müller and Andreas Zimmer	CHANGES IN THE METABOLOME MAY CONTRIBUTE TO THE POSITIVE EFFECTS OF CHRONIC LOW-DOSE Δ^9-TETRAHYDROCANNABINOL ON BRAIN AGEING IN MICE	DB1-1 [P1-9]
Erin Hughes*, Sandra Leone-Kabler, Andrew England, Jill Clodfelter, W. Todd Lowther and Allyn Howlett	CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A): FUNCTION AS A GAI CARRIER	DB1-2 [P1-32]
Barkha J. Yadav-Samudrala*, Elizabeth Kim, Shreya Ramineni, Benjamin Gorman, Dai Lu, Justin Poklis, Aron Lichtman, Bogna Ignatowska-Jankowska and Sylvia Fitting	POSITIVE ALLOSTERIC MODULATOR OF CB1 RECEPTOR: A POTENTIAL THERAPEUTIC TARGET FOR HAND	DB1-3 [P1-81]
Zheng-Xiong Xi*, Briana Hempel and Chloe Jordan	CANNABINOID CB1 RECEPTORS ARE EXPRESSED IN A SUBSET OF DOPAMINE NEURONS AND FUNCTIONALLY INVOLVED IN CANNABINOID ACTION IN MICE	DB1-4 [P1-80]
Ruyi Cai*, Ao Dong, Kaikai He, Barna Dudok, Jordan Farrell, Jiali Duan, Lei Wang, Shangkun Wang, Ivan Soletesz, Chen Song and Yulong Li	GENETICALLY ENCODED FLUORESCENT SENSORS FOR MONITORING ENDOCANNABINOID DYNAMICS *IN VITRO* AND *IN VIVO*	DB1-5 [P1-11]
Cesar Martinez Ramirez*, Garrett Sauber, Ashley Doherty, Leslie Krause, Gonzalo Ruiz-Pérez and Cecilia Hillard	MATERNAL CBD EXPOSURE IN MICE ALTERS MOTOR COORDINATION IN THE OFFSPRING AT ADULTHOOD	DB1-6 [P1-48]

Anna Permyakova*, Alina Nemirovski and Joseph Tam	THE CROSSTALK BETWEEN CANNABINOID 1 RECEPTOR (CB1R) AND ADENINE NUCLEOTIDE TRANSLOCASE 2 (ANT2) IN THE REGULATION OF MITOCHONDRIAL FUNCTION IN THE KIDNEY	DB1-7 [P1-55]

DATABLITZ presenters present BOTH a 4-minute presentation AND a POSTER at ICRS2022.

DATABLITZ abstract page numbers are indicated [IN BRACKETS].

Notes:

Presenting Author*

ICRS2022 DATABLITZ SESSION 2

DAY 2, MONDAY, JUNE 27TH: 15:30 - 16:00

Rebecca Craft*, Hannah Gogulski, Timothy Freels, Nicholas Glodosky and Ryan McLaughlin	VAPORIZED CANNABIS EXTRACT-INDUCED ANTINOCICEPTION IN MALE VS. FEMALE RATS	DB2-1 [P2-8]
Robert Leddy*, Carol Aherne, Mariam Alketbi and Colm Collins	INVESTIGATING THE ROLE OF THE ENDOCANNABINOID SYSTEM IN INTESTINAL INFLAMMATION	DB2-2 [P2-30]
Stefan Hall*, Sufyan Faridi, Irene Euodia, Juan Zhou, Melanie Kelly and Christian Lehmann	MODULATION OF ACUTE LUNG INJURY-INDUCED SYSTEMIC INFLAMMATION VIA CANNABINOID TYPE 2 RECEPTOR ACTIVATION	DB2-3 [P2-21]
Antonio Farina, Martha Lopez-Canul*, Anahita Oveisi, Alexandra Teggin, Justine Enns, Maria Luisa Vigano, Luca Posa, Danilo DeGregorio, Elena He and Gabriella Gobbi	THC AND CBD IN INSOMNIA ASSOCIATED TO NEUROPATHIC PAIN: EFFECT ON SLEEP ARCHITECTURE AND FIRING ACTIVITY OF THE RVM NEURONS	DB2-4 [P2-36]
Mary A. Hopkins*, Maria C. Redmond, Mehnaz I. Ferdousi, Stephanie Bourke, Catherine Healy, Álvaro Llorente-Berzal and David P. Finn	SEXUALLY DIMORPHIC ENDOCANNABINOID CHANGES IN A RAT MODEL OF CHRONIC LOW BACK PAIN ASSOCIATED WITH INTERVERTEBRAL DISC INJURY	DB2-5 [P2-22]
Hayden Wright*, Zachary Fisher, Rafael Urrutia-Camargo, Darren Ginder, Amanda Brown, Hannah Arey, Jobe Ritchie, Rita Fuchs and Ryan McLaughlin	2-ARACHIDONOYLGLYCEROL SIGNALING IN THE LATERAL HABENULA: IMPACTS ON STRESS COPING BEHAVIOR AND DOWNSTREAM IMMEDIATE EARLY GENE EXPRESSION	DB2-6 [P2-73]

Kelsey Guenther*, Julian Romero, Cecelia Hilliard, Ken Mackie, Zhili Xu and Andrea Hohmann	CB2 RECEPTORS IN PRIMARY SENSORY NEURONS MEDIATE ANTI-ALLODYNIC EFFICACY OF THE CB2 AGONIST LY2828360 IN A MOUSE MODEL OF INFLAMMATORY PAIN	DB2-7 [P2-19]
DATABLITZ presenters present BOTH a 4-minute presentation AND a POSTER at ICRS2022. DATABLITZ abstract page numbers are indicated [IN BRACKETS].		

Notes:

Presenting Author*

DATABLITZ SESSION 3

DAY 4, WEDNESDAY, JUNE 29TH: 15:30 - 16:00

Kayle Dickson*, Cassidy Scott, Hannah White and Christian Lehmann	BETA-CARYOPHYLLENE AS A NOVEL ADJUNCT THERAPY FOR THE TREATMENT OF URINARY TRACT INFECTIONS	DB3-1 [P3-17]
Tory Spindle*, Hayleigh Tilton, Spencer Lin, Ed Cone, Ruth Winecker, Eric Welsh, Lynn Wagner, Ron Flegel and Ryan Vandrey	PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS OF HEMP-DERIVED CANNABIDIOL (CBD) TOPICAL PRODUCTS	DB3-2 [P3-73]
Taryn Bosquez*, Jessie Gudorf, Michael VanNieuwenhze and Alex Straiker	CANNABIDIOL-DERIVED VARIANTS AS POTENTIAL NEGATIVE ALLOSTERIC MODULATORS AT THE MU OPIOID RECEPTOR	DB3-3 [P3-6]
Ali Mokhtar Mahmoud*, Magdalena Kostrzewa, Marianna Cerasuolo, Roberto Ronca and Alessia Ligresti	CANNABIDIOL TRIGGERS MITOCHONDRIAL DYSFUNCTION AND CELL DEATH IN HORMONE-REFRACTORY PROSTATE CANCER BY TARGETING VOLTAGE-DEPENDENT ANION-SELECTIVE CHANNEL (VDAC1) AND HEXOKINASE II (HK-II)	DB3-4 [P3-52]
Nicola Forte*, Serena Boccella, Lea Tunisi, Alba Clara Fernández-Rilo, Maria De Risi, Monica Iannotta, Fabiana Piscitelli, Elvira De Leonibus, Sabatino Maione, Vincenzo Di Marzo and Luigia Cristino	OREXIN-A, 2-AG AND 2-AG-DERIVED 2-AGP ARE INVOLVED IN OBESITY-ASSOCIATED ALTERATIONS OF HIPPOCAMPAL MEMORY IN MICE	DB3-5 [P3-33]
Conor Murray* and Ziva Cooper	ASSOCIATIONS BETWEEN CHRONIC CANNABIS USE AND RESTING EEG	DB3-6 [P3-60]

Justin Strickland*, Rhiannon Mayhugh, Renuka Surujnarain and Ryan Vandrey	ECOLOGICAL MOMENTARY ASSESSMENT OF ANXIETY AND DEPRESSION IN NEWLY INITIATED MEDICINAL CANNABIS PATIENTS: MOMENTARY AND LONG-TERM CLINICAL EFFECTS	DB3-7 [P3-75]
DATABLITZ presenters present BOTH a 4-minute presentation AND a POSTER at ICRS2022. DATABLITZ abstract page numbers are indicated [IN BRACKETS].		

Notes:

Presenting Author*

POSTER SESSION 1
DAY 1, SUNDAY, JUNE 26TH: 16:15 - 18:15

WITHDRAWN	WITHDRAWN	P1-1
Adam Ametovski*, Elizabeth Cairns, Rochelle Boyd, Jonathon Arnold, Iain McGregor and Samuel Banister	A NEW SYNTHESIS OF 11-HYDROXY-CANNABINOL AND PHARMACOLOGICAL CHARACTERISATION AT HUMAN CANNABINOID RECEPTORS	P1-2
Dongchen An*, Steve Peigneur and Jan Tytgat	OLEOYL SEROTONIN, AN ACTIVE SMALL MOLECULE IN VENOMS OF STEPHANOCONUS SNAILS, IS AN ANTAGONIST OF THE CANNABINOID RECEPTOR TYPE 1	P1-3
M. Andrea Arnanz*, Kevin Troule, Gonzalo Ruiz-Pérez, Irene Benito-Cuesta, Fernando Puente-Sánchez, Ana M. Martínez Relimpio, María Posada-Ayala, Benjamin F. Cravatt, Fátima Al-Shahrour, M. Teresa Grande, Rocío Palenzuela and Julián Romero	FAAH GENETIC INACTIVATION LEADS TO A PRO-INFLAMMATORY, YET NEUROPROTECTIVE, PHENOTYPE IN 5XFAD MICE	P1-4
Bryant Avalos*, Courtney P. Wood and Nicholas V. DiPatrizio	CHRONIC EXPOSURE TO CANNABIS SATIVA EXTRACTS IMPROVES METABOLIC DYSFUNCTION AND DYSREGULATION OF THE ADIPOINSULAR AXIS IN DIET-INDUCED OBESE MICE	P1-5
Samantha L. Baglot*, Catherine Hume, Lucy Javorcikova, Laine M. Grace, Robert J. Aukema, Gavin N. Petrie and Matthew N. Hill	THE EFFECTS OF INHALED CANNABIS EXPOSURE DURING PREGNANCY ON ENDOCANNABINOID AND IMMUNE SYSTEM DEVELOPMENT IN MALE AND FEMALE RATS	P1-6
Daniel G. Barrus*, Jenny L. Wiley and Thomas F. Gamage	PARADOXICAL PHARMACOLOGICAL PARAMETERS OF CANNABIDIOL IN CB1 RECEPTOR BINDING AND FUNCTIONAL ASSAYS	P1-7
Patrycja Bielawiec*, Karolina Konstantynowicz-Nowicka, Adrian Chabowski and Ewa Harasim-Symbor	CANNABIDIOL INHIBITS MUSCLE DE NOVO LIPOGENESIS, POSITIVELY INFLUENCING FATTY ACIDS METABOLISM IN A RAT MODEL OF OBESITY INDUCED BY A HIGH-FAT DIET	P1-8

Andras Bilkei-Gorzo*, Britta Schürmann, Marion Schneider, Michael Krämer, Prakash Nidadavolu, Christa Müller and Andreas Zimmer	CHANGES IN THE METABOLOME MAY CONTRIBUTE TO THE POSITIVE EFFECTS OF CHRONIC LOW-DOSE Δ^9-TETRAHYDROCANNABINOL ON BRAIN AGEING IN MICE	P1-9
Angela D. Bryan*, Laurel P. Gibson, Gregory Giordano, Carillon Skrzynski, Chasmine Malabanan, Jinqiu Yang, Madeline Stanger, L. Cinnamon Bidwell, Kent E. Hutchison and Leigh Perreault	ASSOCIATIONS OF CANNABIS USE TO INSULIN SENSITIVITY, PHYSICAL ACTIVITY AND DIET: IMPLICATIONS FOR OBESITY	P1-10
Ruyi Cai*, Ao Dong, Kaikai He, Barna Dudok, Jordan S Farrell, Jiali Duan, Lei Wang, Shangkun Wang, Ivan Soltesz, Chen Song and Yulong Li	GENETICALLY ENCODED FLUORESCENT SENSORS FOR MONITORING ENDOCANNABINOID DYNAMICS *IN VITRO* AND *IN VIVO*	P1-11
Isabel Castro-Piedras*, Melissa C. McHann, Henry L. Blanton, Haley De Selle, Canice Lei Dancel, Jose-Luis Redondo, Deborah Molehin, Nadia German, Scott Trasti, Kevin Pruitt and Josée Guindon	CANNABINOID RECEPTOR 2 AGONIST (JWH-133) INCREASES ECTOPIC OVARIAN TUMOR GROWTH AND ENDOCANNABINOIDS (ANANDAMIDE AND 2-ARACHIDONOYL GLYCEROL) LEVELS FOLLOWING CHRONIC ADMINISTRATION IN FEMALE SCID MICE	P1-12
Costanza Ceni*, Robert B. Laprairie, Kawthar A. Mohamed, Massimo Valoti, Giulio Poli, Tiziano Tuccinardi, Maria Digiacomo, Marco Macchia and Simone Bertini	ADVANCES TOWARD THE DISCOVERY OF NOVEL 3-BENZYLQUINOLIN-2(1H)-ONES AS POTENT AGONISTS OF THE GPR55 RECEPTOR	P1-13
Amy F. Walsh, Hannah MacAulay, Antonia Dunbar, Helen Sheridan and Mark O. Cunningham*	AN ELECTROPHYSIOLOGICAL ASSESSMENT OF THE IMPACT OF THE CANNABIS-DERIVED TERPENES ON ACUTE SEIZURE ACTIVITY *IN VITRO*	P1-14
Shreya Desai*, Julia M. Evanski, Christine A. Rabinak and Hilary A. Marusak	GENETIC VARIATION IN ENDOCANNABINOID SIGNALING AND THREAT- AND REWARD-RELATED BRAIN FUNCTIONING IN CHILDREN	P1-15
Wesley Raup-Konsavage, Diana Sepulveda, Daniel Morris, Shantu Amin, Kent Vrana, Nicolas Graziane and Dhimant Desai*	A NOVEL SYNTHESIS METHOD FOR ALTERING SIDE CHAIN LENGTH ON CANNABIGEROL MOLECULES AND THE IMPACT OF SIDE-CHAIN LENGTH ON PAIN AND CYTOTOXICITY	P1-16
Marieka V. DeVuono*, Mohammed H. Sarikahya and Steven R. Laviolette	LONG-TERM SEX AND DOSE-DEPENDENT NEURODEVELOPMENTAL CONSEQUENCES OF ADOLESCENT EDIBLE THC CONSUMPTION IN RATS	P1-17

Marie-Eve Di Raddo*, Marija Milenkovic, Yalin Sun, Bertha K. Madras and Susan R. George	ESCALATING Δ^9-TETRAHYDROCANNABINOL (THC) ADMINISTRATION IN ADOLESCENCE LEADS TO PERSISTENT SEX-SPECIFIC DYSREGULATION OF DOPAMINE D1-D2 RECEPTOR HETEROMER	P1-18
Wouter Driever*, Berend Gagestein, Dirk Minnee, Tessa Gote, Brenda Pijper, Elliot Mock, Anthe Janssen, Stan van Boeckel and Mario van der Stelt	LEI-401 IS AN ALLOSTERIC INHIBITOR OF NAPE-PLD	P1-19
Megan Drupals*, Janice Hicks, Benjamin Steinberg and Michael Salter	THE EFFECT OF PRENATAL THC AND CBD EXPOSURE ON BEHAVIOUR IN THE ADULT RODENT	P1-20
Michaela Dvorakova*, Wenwen Du, Catherine S. Wright and Ken Mackie	ADOLESCENT THC TREATMENT PROMOTES AN INFLAMMATORY PHENOTYPE IN MALE MEDIAL PREFRONTAL CORTEX	P1-21
Sylwia Dziemitko*, Patrycja Bielawiec, Klaudia Sztolsztener, Adrian Chabowski and Ewa Harasim-Symbor	EFFECTS OF CHRONIC CANNABIDIOL ADMINISTRATION ON THE DE NOVO LIPOGENESIS AND DESATURATION RATIO IN THE WHITE SKELETAL MUSCLE IN HIGH-FAT DIET FED RATS	P1-22
Alysha L. Ellison*, José-Javier Rosado Franco, Cory J. White, Lisa B. Fridman and Dionna W. Williams	ENDOCANNABINOID SYSTEM RECEPTOR EXPRESSION IN THE CONTEXT OF SIV AND cART	P1-23
Rebecca Ferrisi*, Francesca Gado, Kawthar A. Mohamed, Caterina Ricardi, Vittoria Carnicelli, Lorenzo Niccoli, Robert B. Laprairie, Clementina Manera and Grazia Chiellini	WHAT'S NEW ABOUT THE FIRST CB2R HETEROBIVALENT DUALSTERIC/BITOPIC LIGAND? LATEST RESEARCH ON ITS NEUROPROTECTIVE BENEFITS	P1-24
Tyson Follack*, Ayat Zagzoog, Vancy To, Adam Hupka, Deborah Michael, Anna-Maria Smolyakova, Sumanta Garai, Ganesh A Thakur and Robert B Laprairie	PHARMACOKINETIC AND *IN VIVO* BEHAVIORAL CHARACTERIZATION OF A NOVEL CANNABINOID RECEPTOR MODULATOR: GAT1102	P1-25
Christa M. Frodella*, Stephen B. Pruett and Barbara L.F. Kaplan	EXPLORING THE IMPACT OF CANNABIDIOL ON THE BRAIN TRANSCRIPTOME IN A MILD DISEASE MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS	P1-26

WITHDRAWN	WITHDRAWN	P1-27
Briana Hempel*, Guo-Hua Bi, Madeline Crissman, Sruti Pari, Ben Klein and Zheng-Xiong Xi	A FUNCTIONAL AND ANATOMICAL ASSESSMENT OF ASTROCYTIC CB1RS IN CANNABINOID ACTION	P1-28
Axel Hentsch*, Monica Guberman, Yelena Mostinski, Thais Gazzi, Jerome Paul, Jörg Benz, Bernd Kuhn, Uwe Grether and Marc Nazare	DEVELOPMENT OF HIGHLY POTENT AND SELECTIVE FLUORESCENT MAGL PROBES WITH DRUG-LIKE PROPERTIES	P1-29
Beatriz Gil and Caroline Herron*	CANNABIDIOLIC ACID TREATMENT REVERSES DEFICITS IN SYNAPTIC PLASTICITY IN HIPPOCAMPAL SLICES FROM THE APP/PS1 MOUSE MODEL OF ALZHEIMER'S DISEASE	P1-30
Liad Hinden*, Rami Ludyansky, Yoav Harris, Sary Leidershnaider, Joseph Tam and Guy Hidas	PERIPHERAL CB1 RECEPTOR BLOCKADE REDUCES THE SEVERITY OF HEMORRHAGIC CYSTITIS	P1-31
Erin K. Hughes*, Sandra Leone-Kabler, Andrew C. England, Jill C. Clodfelter, W. Todd Lowther and Allyn C. Howlett	CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A): FUNCTION AS A Gαi CARRIER	P1-32
Andrew J Irving*, Erica Millet O'Beirne, Jenni Harvey and Mark Cunningham	CHARACTERISATION OF A NOVEL, POTENT PEPTIDE LIGAND FOR GPR55 THAT MODULATES HIPPOCAMPAL SYNAPTIC PLASTICITY	P1-33
Christiana J. Smith, Daniela Vergara, Brian Keegan and Nick Jikomes*	THE PHYTOCHEMICAL DIVERSITY OF COMMERCIAL CANNABIS IN THE UNITED STATES	P1-34
Cameron Jordan* and Luis E. Rodriguez-Saona	USE OF HANDHELD FT-NIR SENSORS TO RAPIDLY QUANTIFY CANNABINOIDS OF HEMP *IN SITU*	P1-35

Célia Roger-Villeboeuf, Laetitia Jacquot, Patricia Passilly-Degrace, Chloé Buch, Julia Leemput, Laurent Demizieux, Bruno Vergès, Pascal Degrace, Glenn Crater and Tony Jourdan*	THE CB1R INVERSE-AGONIST INV-202 REDUCES RENAL INJURY IN A MURINE MODEL OF STREPTOZOTOCIN (STZ)-INDUCED TYPE-1 DIABETES	P1-36
Kwang-Mook Jung*, Hye-Lim Lee, Yannick Fotio, Francesca Palese, Lin Lin, Erica Squire, Alexa Torrens, Faizy Ahmed, Jade Ramirez and Daniele Piomelli	THC EXPOSURE IN ADOLESCENCE DISRUPTS MICROGLIA HOMEOSTASIS AND DISABLES RESPONSES TO INFECTION AND SOCIAL STRESS IN ADULTHOOD	P1-37
Michael Tagen and Linda Klumpers*	THE CB1 ANTAGONIST ANEB-001, A CANDIDATE FOR ACUTE CANNABINOID INTOXICATION TREATMENT, BLOCKS CANNABINOID ACTIVITIES *IN VITRO* AND *IN VIVO*	P1-38
Anastasia Kolousek*, Ezra Pak-Harvey, Oliver Lam, Mia White and Joshua M Levy	EFFECTS OF ENDOCANNABINOIDS IN MAMMALIAN RESPIRATORY SYSTEM THROUGH COX-DEPENDENT PATHWAYS: A SCOPING REVIEW	P1-39
Mohammad Ebrahimzadeh, Ashwini Joshi, Joanne Speed, Nidhi Kanwar, Sandra Lottes, Ram Saralaya, Joga Gobburu and Lakshmi Kotra*	PRECLINICAL SAFETY PHARMACOLOGY AND TOXICOLOGY OF FSD201, A PALMITOYLETHANOLAMIDE COMPOSITION	P1-40
Magdalena Kostrzewa, Poulami Kumar*, Maria Pina Mollica, Rosa Purgatorio, Modesto de Candia, Claudia Mugnaini and Alessia Ligresti	SELECTIVE CB2 RECEPTOR LIGANDS WITH NOVEL HYBRID MOLECULAR SCAFFOLD AS POTENTIAL TARGET FOR NEURODEGENERATIVE DISEASES OF MULTIFACTORIAL ORIGIN: A MULTI-TARGET-DIRECTED LIGANDS (MTDL) APPROACH	P1-41
Savannah H. Lightfoot*, Andrei S. Nastase, Samantha L. Baglot, Catherine Hume, Ryan J. McLaughlin and Matthew N. Hill	INVESTIGATING THE EFFECTS OF ACUTE THC VAPOUR EXPOSURE ON STRESS REACTIVITY AND FEAR CONDITIONING	P1-42
James N. Lowder*, Patrick Tso, Min Liu, Vassili Kotlov, Chad Pope, Nobumitsu Sakai and Olapeju Bolarinwa	FORMULATION OF CANNABINOIDS PROMOTING TRAFFICKING THROUGH CHYLOMICRONS INCREASES BIOAVAILABILITY IN RATS	P1-43
Camilla Di Meo, Daniel Tortolani, Sara Standoli, Clotilde Beatrice Angelucci, Salam Kadhim, Eric Hsu, Cinzia Rapino and Mauro Maccarrone*	MODULATION OF ENDOCANNABINOID SIGNALING IN HUMAN KERATINOCYTES BY “MINOR” PHYTOCANNABINOIDS	P1-44

Leonard Mach*, Malgorzata Wasinska-Kalwa, Anahid Omran, David Sykes, Yelena Mostinski, Thais Gazzi, Wolfgang Guba, Arne Rufer, Johannes Broichhagen, Dimitry Veprintsev, Uwe Grether and Marc Nazare	DESIGN, SYNTHESIS AND EVALUATION OF NOVEL IMAGING PROBES FOR THE VISUALIZATION OF THE CANNABINOID RECEPTORS TYPE 1 AND 2	P1-45
Nagina Mangal*, Vikash Reebye, Barbara Pacchetti, Anguraj Sadanandam and Mikael Sodergren	CYTOTOXICITY OF CANNABIDIOL IN PANCREATIC DUCTAL ADENOCARCINOMA OCCURS VIA UPREGULATION OF CERAMIDE SYNTHASE 1 AND INDUCTION OF ER STRESS	P1-46
Jack Markham*, Lewis Martin, Adam Ametovski, Samuel Banister and David Hibbs	THE APPLICATION AND COMPARISON OF VARIOUS COMPUTER-ASSISTED MEDICINAL CHEMISTRY METHODS: INDAZOLE-BASED CB1 AGONISTS	P1-47
César E. Martinez Ramirez*, Garrett Sauber, Ashley Doherty, Leslie Krause, Gonzalo Ruiz-Pérez and Cecilia J. Hillard	MATERNAL CBD EXPOSURE IN MICE ALTERS MOTOR COORDINATION IN THE OFFSPRING AT ADULTHOOD	P1-48
María Martinez Vega*, María Villa Cruz, Laura Silva Colmenar, María de Hoz Rivera, Ángela Romero Sánchez, Juan Miguel Godoy Corchuelo and José Martínez Orgado	NEUROPROTECTIVE EFFICACY OF VCE-004.8 IN A NEWBORN RAT MODEL OF PERINATAL ARTERIAL ISCHEMIC STROKE	P1-49
Kawthar Mohamed, Francesca Gado, Rebecca Ferrisi*, Beatrice Polini, Elena Lucarini, Sara Carpi, Federica Domenichini, Lesley Stevenson, Paola Nieri, Roger Pertwee, Carla Ghelardini, Lorenzo Di Cesare Mannelli, Grazia Chiellini, Robert Laprairie and Clementina Manera	EVALUATION OF NOVEL BITOPIC LIGANDS AT THE TYPE 2 CANNABINOID RECEPTOR	P1-50
Erica O'Beirne* and Jenni Harvey	CANNABIDIOL INCREASES EXCITATORY SYNAPTIC TRANSMISSION AT TEMPOROAMMONIC-CA1 SYNAPSES IN RAT HIPPOCAMPUS	P1-51
Placid Orji*, Asher Brandt, Ayat Zagzoog, John Howland, Robert Laprairie and Christopher Phenix	SYNTHESIS OF FLUORINATED CANNABINOID DERIVATIVES; TOWARDS PET RADIOTRACERS FOR IMAGING THE CANNABINOID RECEPTOR	P1-52
Sang-Hyuck Park*, Sam Koch and Brian D. Vanden Heuvel	DEFENSIVE ROLE OF CANNABIDIOL (CBD) AGAINST PEST INSECT TOBACCO HORNWORM *MANDUCA SEXTA* THROUGH DISRUPTING EXOSKELETON DEVELOPMENT	P1-53

Marzia Pendino*, Sandra Garcia Mulero, Rebeca Sanz Pamplona, Simone Marcone, Kayleigh Slater, Josep Piulats and Breandan N. Kennedy	EVALUATING CANNABINOID RECEPTORS AS A THERAPEUTIC TARGET FOR UVEAL MELANOMA	P1-54
Anna Permyakova*, Alina Nemirovski and Joseph Tam	THE CROSSTALK BETWEEN CANNABINOID 1 RECEPTOR (CB1R) AND ADENINE NUCLEOTIDE TRANSLOCASE 2 (ANT2) IN THE REGULATION OF MITOCHONDRIAL FUNCTION IN THE KIDNEY	P1-55
Pavel Powlowski*, David Bodenstein, Kassandra Zachos, Catherine Mielnik, Ana Andreazza and Ruth Ross	THE EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON MITOCHONDRIAL FUNCTION IN HUMAN EMBRYONIC KIDNEY 293 (HEK 293) CELLS	P1-56
Itay Lipka, Eliran Malki, Nurit Tweezer Zaks, Yossi Ben Amram, Talma Gotteiner and Neta Rimmerman*	BUILDING A GLOBAL CANNABIS SOCIAL NETWORK: DATA FROM THE NEW EcoCaNN MOBILE APPLICATION	P1-57
Angela Romero Sanchez*, Aaron Del Pozo Sanz, María De Hoz Rivera, Laura Silva Comenar, María Villa Cruz, María Martínez Vega and José Martínez Orgado	CANNABIDIOL NEUROPROTECTION IN INTRAVENTRICULAR HEMORRHAGE-INDUCED BRAIN DAMAGE IN IMMATURA RATS IS RELATED TO ANTI-INFLAMMATORY EFFECTS THROUGH MICROGLIAL MODULATION	P1-58
Sarah H. Shrader*, Nicholas Mellen, Gregory Barnes and Zhao-Hui Song	CANNABIDIOL ALTERS ABERRANT IMMUNE CELL POPULATIONS IN A MODEL OF IDIOPATHIC AUTISM SPECTRUM DISORDER	P1-59
Laura Silva Colmenar, María Villa Cruz, María Martínez Vega, Ángela Romero Sánchez, María de Hoz Rivera* and José Martínez Orgado	EFFECTS OF CANNABIDIOL ON MICROGLIA TO MODULATE NEUROINFLAMMATION AFTER ACUTE ISCHEMIC STROKE IN NEWBORN RATS	P1-60
Simar Singh*, Dennis Sarroza, Anthony English, Dale Whittington, Ao Dong, Mario van der Stelt, Yulong Li, Larry Zweifel, Michael Bruchas, Benjamin Land and Nephi Stella	ABHD6 DIFFERENTIALLY CONTROLS STIMULI-DEPENDENT INCREASES IN 2-AG LEVELS	P1-61
Lucy Sloan*, Shigeo Tamiya and Zhao-Hui Song	EFFECTS OF N-OLEOYL DOPAMINE ON PORCINE RETINAL PIGMENT EPITHELIAL CELLS	P1-62

Eric Sparkes*, Rochelle Boyd, Shuli Chen, Tahira Foyzun, Humayra Zaman, Jia Lin Luo, Ross Ellison, Iain McGregor, Roy Gerona, Marina Santiago, Mark Connor, Michelle Glass, Adam Ametovski, Samuel Banister and Elizabeth Cairns	CHEMISTRY AND PHARMACOLOGY OF SYNTHETIC CANNABINOID RECEPTOR AGONISTS AB-4CN-BUTICA, MMB-4CN-BUTINACA, MDMB-4F-BUTICA, MDMB-4F-BUTINACA AND THEIR ANALOGUES	P1-63
William W. Stoops* and Joshua A. Lile	INFLUENCE OF CO-MORBID CANNABIS AND COCAINE USE ON IMMUNE BIOMARKERS	P1-64
Kim Sugamori*, Catharine Mielnik, David Finlay, Mohammed Mustafa, Daniel Liput, Mostafa Abdelrahman, Laurent Trembleau, Ali Salahpour, Amy Ramsey, David Lovinger, Aron Lichtman, Michelle Glass, Iain Greig and Ruth Ross	NOT ALL CB1 ALLOSTERIC MOLECULES ARE CREATED EQUAL: EVIDENCE FOR SELECTIVE EFFECTIVENESS IN DOPAMINE-DYSREGULATED SYMPTOMS	P1-65
WITHDRAWN	WITHDRAWN	P1-66
Yalin Sun*, Meenalochani Sivasubramanian, Marija Milenkovic, Marie-Ève Di Raddo, Sarah L. Withey, Bertha K. Madras and Susan R. George	DAILY EXPOSURE TO Δ^9-TETRAHYDROCANNABINOL (THC) INDUCES REACTIVE GLIOSIS IN ADOLESCENT, BUT NOT ADULT NONHUMAN PRIMATE AND RAT AMYGDALA: ANTAGONISM BY CANNABIDIOL (CBD)	P1-67
Klaudia Sztolsztener*, Katarzyna Hodun, Adrian Chabowski, Sylwia Dziemitko and Karolina Konstantynowicz-Nowicka	THE EFFECT OF CANNABIGEROL ON THE EXPRESSION OF PROTEINS INVOLVED IN EXTRACELLULAR MATRIX FORMATION IN PRIMARY RAT HEPATOCYTES EXPOSED TO PALMITATE AND FRUCTOSE CONDITIONS	P1-68
Elad Ben-Cnaan, Liad Hinden and Joseph Tam*	TREATING AND PREVENTING HYPERPHAGIA AND OBESITY IN PRADER-WILLI SYNDROME WITH A CANNABIDIOLIC ACID DERIVATIVE	P1-69
Mark A. Tripson*, Alexis Papariello, Karen Litwa and Ken Soderstrom	CANNABIDIOL (CBD) INHIBITS NEUROINFLAMMATORY RESPONSES AND SYNAPTIC LOSS FOLLOWING DAMAGE TO SONGBIRD VOCAL MOTOR CORTEX	P1-70

Kazuhito Tsuboi*, Tatsuya Tai, Ryouhei Yamashita, Hanif Ali, Takashi Watanabe, Toru Uyama, Yoko Okamoto, Keisuke Kitakaze, Yasuhiro Takenouchi, Iffat Ara Sonia Rahman, Hitoshi Houchi, Tamotsu Tanaka, Akira Tokumura, Junko Matsuda, Yasuo Okamoto and Natsuo Ueda	ACID CERAMIDASE AS A THIRD N-ACYLETHANOLAMINE HYDROLASE IN CELLS AND TISSUES	P1-71
Toru Uyama*, Zahir Hussain, Katsuya Morito, S. M. Khaledur Rahman, Mohammad Mamun Sikder, Tamotsu Tanaka, Kenichi Ota, Masaki Ueno, Hiroo Takahashi, Tohru Yamamoto, Makoto Murakami and Natsuo Ueda	ACCUMULATION OF N-ACYL-PHOSPHATIDYLETHANOLAMINES IN BRAIN ISCHEMIA BY CYTOSOLIC PHOSPHOLIPASE A2ε	P1-72
Noëlle van Egmond*, Floor Stevens, Bobby Florea, Berend Gagestein, Tom van der Wel, Daan van der Vliet, Sander van Kasteren and Mario van der Stelt	A MULTI-DIMENSIONAL PROFILE OF THE ENDOCANNABINOID SYSTEM IN POLARIZED MICROGLIA	P1-73
Haley A. Vecchiarelli*, Hayley Thorpe, Sophia Loewen, Jibran Y. Khokhar and Marie-Ève Tremblay	MICROGLIA DENSITY, DISTRIBUTION AND MORPHOLOGY FOLLOWING ACUTE VAPOURIZED CANNABINOID EXPOSURE	P1-74
María Villa*, Ianire Gallego, Aarón Del Pozo, María Martínez, Laura Silva, María De Hoz-Rivera, Ángela Romero, Ana Gómez-Soria, María José Casarejos and José Martínez-Orgado	CANNABIDIOL AS A TREATMENT FOR POST STROKE MOOD DISORDERS IN NEWBORN RATS	P1-75
Amey S. Dhopeshwarkar, Jim Wager-Miller*, Madison Walenga and Ken Mackie	Δ^9THC AND ITS MAJOR METABOLITES ACTIVATE GPR119 *IN VITRO* AND INDUCE WEIGHT LOSS IN DIET-INDUCED OBESE MICE	P1-76
Emily T. Wilson*, David H. Eidelman and Carolyn J. Baglole	AHR REGULATES PULMONARY INFLAMMATION FROM CANNABIS SMOKE	P1-77
María E. Prados, Matthias Winkler*, Juan D. Unciti Unciti, Francisco J, Ponce, Juan A. Collado, Isabel Lastres, Marcus R. Götz and Eduardo Muñoz	CHARACTERIZATION OF A JUNIPER PHYTOEXTRACT AS A POSITIVE ALLOSTERIC MODULATOR FOR CB1R. IMPLICATIONS FOR PSORIASIS	P1-78

Christopher Gaffney, Jack Cunnington, Naomi Greathead, Evie Rigby, Dan Scott, Michelle Montemayor, Hannah Garvey and Karen Wright*	CANNABIDIOL (CBD) MAY INFLUENCE SUBSTRATE UTILISATION DURING AND MUSCLE RECOVERY FOLLOWING DAMAGING EXERCISE	P1-79
Zheng-Xiong Xi*, Briana Hempel and Chloe Jordan	CANNABINOID CB1 RECEPTORS ARE EXPRESSED IN A SUBSET OF DOPAMINE NEURONS AND FUNCTIONALLY INVOLVED IN CANNABINOID ACTION IN MICE	P1-80
Barkha J. Yadav-Samudrala*, Elizabeth Kim, Shreya Ramineni, Benjamin Gorman, Dai Lu, Justin Poklis, Aron H. Lichtman, Bogna M. Ignatowska-Jankowska and Sylvia Fitting	POSITIVE ALLOSTERIC MODULATOR OF CB1 RECEPTOR: A POTENTIAL THERAPEUTIC TARGET FOR HAND	P1-81
Yi Yang* and Lakshmi P. Kotra	TOTAL SYNTHESIS AND CHARACTERIZATION OF NOVEL *CIS*-CBD STEREOISOMERS	P1-82
WITHDRAWN	WITHDRAWN	P1-83
Alexander P. Young* and Eileen M. Denovan-Wright	CANNABINOIDS SUPPRESS THE NEUROTOXIC PROPERTIES OF PRO-INFLAMMATORY MICROGLIA *IN VITRO*	P1-84
Ayat Zagzoog*, Ashley Cabecinha, Hanan Abramovici and Robert B Laprairie	THE ACTIVITY OF CANNABINOID BY PRODUCTS AND TERPENES ON TYPE 1 CANNABINOID RECEPTOR	P1-85

Notes: Presenting Author*

POSTER SESSION 2
DAY 2, MONDAY, JUNE 27TH: 16:00 - 18:00

Cassandra Occelli Hanbury Brown, Anand Gururajan, Lyndsey Anderson, Joel Raymond, Adam Ametovski, Samuel Banister, Peter Doohan, Miguel Bedoya-Perez, Iain McGregor and Jonathon Arnold*	THE PHYTOCANNABINOID CANNABINOL (CBN) ENHANCES SLEEP AND ALTERS SLEEP ARCHITECTURE IN RATS	P2-1
Nicole M Ashpole*, Fakhri Mahdi, Mohammed Salahuddin, Emaya Moss, Alaa Qrareya, Miguel A De Leon, Matthew J Foster, Mohamed O Marzouk, Jessica P Marshall, Amira Wanas, Mohamed M Radwan, Mahmoud A ElSohly and Jason J Paris	MINOR CANNABINOIDS AND TERPENES AMELIORATE HIV REPLICATION AND HIV PROTEIN-RELATED NEUROINFLAMMATORY PAIN	P2-2
Samantha M. Ayoub*, Erin M. Rock, Reem Smoum, Cheryl L. Limebeer, Marieka DeVuono, Raphael Mechoulam and Linda A. Parker	ORALLY ADMINISTERED N-OLEOYL ALANINE BLOCKS ACUTE MORPHINE WITHDRAWAL-INDUCED CONDITIONED PLACE AVERSION AND ATTENUATES SOMATIC WITHDRAWAL FOLLOWING CHRONIC OPIATE EXPOSURE IN RATS	P2-3
WITHDRAWN	WITHDRAWN	P2-4
Zeeta Bawa*, Daniel Lewis, Paul Gavin, Roksan Libinaki, Lida Joubran, Mahmoud El-Tamimy, Greg Taylor, Ryan Meltzer and Iain McGregor	THE CANNABIDIOL TOPICAL FOR HAND OSTEOARTHRITIS PILOT (CATCH-OP): A SINGLE CENTRE OPEN LABEL PILOT TRIAL	P2-5
Henry Blanton*, Linda Yin, Joseph Duong and Khalid Benamar	COMBINATION OF CANNABIDIOL AND BETA-CARYOPHYLLENE SYNERGISTICALLY MITIGATE INFLAMMATORY PAIN	P2-6
Chris Breivogel*, Samantha Harrell, Linda Nguyen and Hunnain Siddiqui	CBD INTERACTIONS WITH PROPOFOL IN MICE	P2-7

Rebecca Craft*, Hannah Gogulski, Timothy Freels, Nicholas Glodosky and Ryan McLaughlin	VAPORIZED CANNABIS EXTRACT-INDUCED ANTINOCICEPTION IN MALE VS. FEMALE RATS	P2-8
María de Hoz Rivera*, Aarón del Pozo Sanz, Ángela Romero Sanchez, Laura Silva Colmenar, María Martínez Vega, María Villa Cruz and José Martínez Orgado	INVOLVEMENT OF CB2 RECEPTORS IN CANNABIDIOL NEUROPROTECTION AFTER INTRAVENTRICULAR HEMORRHAGE IN IMMATURE RATS	P2-9
Miguel De Leon*, Waseem Gul, Vishvesh Raje, Mahmoud ElSohly, Nicole Ashpole and Hannah Harris	CANNABICHROMENE EFFECTIVELY REDUCES CISPLATIN-INDUCED NEUROPATHIC PAIN	P2-10
Oleh Durydivka*, Agnieszka Kubik-Zahorodna, Michaela Dvorakova, Matej Gazdarica, Ken Mackie and Jaroslav Blahos	SGIP1 MODULATES ACUTE AND CHRONIC NOCICEPTION IN A SEX-SPECIFIC FASHION	P2-11
Anthony English*, David Marcus, Fleur Uittenbogaard, Yulong Li, Larry Zweifel, Benjamin Land, Nephi Stella and Michael Bruchas	THC ENHANCES 2-AG SIGNALING IN MOUSE PFC AT LOCOMOTION INITIATION	P2-12
Lara Bapir*, Simon Erridge, Carl Holvey, Ross Coomber, Mohammed Sajad, Azfer Usmani, Mona Mubarak, Wendy Holden, Jonathan Hoare, Shaheen Khan, Mark Weatherall, Michael Platt, James Rucker and Mikael Sodergren	COMPARISON OF CLINICAL OUTCOMES OF CHRONIC PAIN PATIENTS WITH CO-MORBID ANXIETY SYMPTOMS TREATED WITH MEDICAL CANNABIS: A COHORT STUDY	P2-13
Joaquin Bello, Simon Erridge*, Carl Holvey, Ross Coomber, Michael Platt, James Rucker, Shaheen Khan and Mikael Sodergren	AN UPDATED ANALYSIS OF CLINICAL OUTCOME MEASURES FOR PALLIATIVE CARE FROM THE UK MEDICAL CANNABIS REGISTRY	P2-14
Nish Dalavaye*, Simon Erridge, Carl Holvey, Ross Coomber, James Rucker, Jonathan Hoare and Mikael Sodergren	ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P2-15
Claire Wang, Simon Erridge*, Carl Holvey, Ross Coomber, Azfer Usmani, Mohammed Sajad, Mona Mubarak, Wendy Holden, James Rucker, Michael Platt and Mikael Sodergren	ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH FIBROMYALGIA: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P2-16

Joel Gagnier*, Daniel Nechi and Kevin Boehnke	A SYSTEMATIC REVIEW AND META-ANALYSIS OF CANNABIDIOL FOR PAIN AND RELATED OUTCOMES	P2-17
Eliot Gardner* and Zheng-Xiong Xi	ADVERSE PSYCHIATRIC SIDE EFFECTS OF THE CANNABINOID CB1 INVERSE AGONIST SR141716 (RIMONABANT) WERE LIKELY PREDICTABLE FROM PRECLINICAL ANIMAL MODELS	P2-18
Kelsey Guenther*, Julian Romero, Cecilia Hilliard, Ken Mackie, Zhili Xu and Andrea Hohmann	CB2 RECEPTORS IN PRIMARY SENSORY NEURONS MEDIATE ANTI-ALLODYNIC EFFICACY OF THE CB2 AGONIST LY2828360 IN A MOUSE MODEL OF INFLAMMATORY PAIN	P2-19
Stefan Hall*, Sufyan Faridi, Irene Euodia, Juan Zhou, Melanie Kelly and Christian Lehmann	MODULATION OF ACUTE LUNG INJURY-INDUCED SYSTEMIC INFLAMMATION VIA CANNABINOID TYPE 2 RECEPTOR ACTIVATION	P2-20
Robin Heider*, Izzabella Green, Han Lee, Randall Krug II and Karl J. Clark	MODELING FATTY ACID AMIDE HYDROLASE FUNCTION WITH ZEBRAFISH	P2-21
Mary A. Hopkins*, Maria C. Redmond, Mehnaz I. Ferdousi, Stephanie Bourke, Catherine Healy, Álvaro Llorente-Berzal and David P. Finn	SEXUALLY DIMORPHIC ENDOCANNABINOID CHANGES IN A RAT MODEL OF CHRONIC LOW BACK PAIN ASSOCIATED WITH INTERVERTEBRAL DISC INJURY	P2-22
Valerie Joers*, Sean Kelly, Fredric P Manfredsson, Bob M Moore II and Malú G Tansey	MODULATION OF CANNABINOID RECEPTOR 2 TO AMELIORATE NEUROINFLAMMATION AND ALTER FORMATION OF ALPHA-SYNUCLEIN AGGREGATES IN A RAT MODEL OF PARKINSON'S DISEASE	P2-23
Kimberly Karin*, Erica Golden, Raphael Mechoulam, Linda Parker, M. Imad Damaj, Aron Lichtman and Joel Schlosburg	OLEOYL GLYCINE REDUCES NICOTINE SEEKING AND REINSTATEMENT IN RATS	P2-24
Daman Kaur*, Stephanie Bourke, Magda Bucholc, Stephen Todd, KongFatt Wong-Lin, Paula McClean and David Finn	PLASMA ENDOCANNABINOID LEVELS AS NOVEL BLOOD-BASED MARKERS FOR MILD COGNITIVE IMPAIRMENT AND DEMENTIA	P2-25

Marta Kedziora*, Serena Boccella, Jakub Mlost, Sabatino Maione and Katarzyna Starowicz	THERAPEUTIC POTENTIAL OF CANNABIDIOL FOR THE TREATMENT OF NEUROPATHIC PAIN AND ASSOCIATED COGNITIVE IMPAIRMENT	P2-26
Hye Ji Kim*, Ayat Zagzoog, Tallan Black, Sarah Baccetto, Udoka Ezeaka and Robert Laprairie	BRAIN REGION-SPECIFIC MOLECULAR AND BEHAVIOURAL EFFECTS OF CP55,940 THROUGHOUT THE MOUSE ESTRUS CYCLE	P2-27
Jace B. King*, Molly B. D. Prigge, Lubdha M. Shah and Jeffrey S. Anderson	THC PROLONGS SYNCHRONOUS BRAIN ACTIVITY	P2-28
Alex Kuklish*, Ali Sualeh, Ken Mackie and Anna Kalinovsky	ENDOCANNABINOID SIGNALING REGULATES MOSSY FIBER AXON GROWTH	P2-29
Robert Leddy*, Mariam Alketbi, Carol Aherne and Colm Collins	INVESTIGATING THE ROLE OF THE ENDOCANNABINOID SYSTEM IN INTESTINAL INFLAMMATION	P2-30
Nadia Leen*, Antoin de Weijer, Sanne van Rooij, Mitzy Kennis, Johanna Baas and Elbert Geuz	ENDOCANNABINOIDS 2-AG AND ANANDAMIDE INDICATE CLINICAL SYMPTOMS AND DO NOT PREDICT TREATMENT OUTCOME IN VETERANS WITH PTSD	P2-31
Tim Lefever*, Kristen Trexler, Rayetta Henderson and Marcel Bonn-Miller	PRE-CLINICAL EVALUATION OF CANNABIDIOL TOXICITY IN RATS	P2-32
Kristen Trexler, Daniela Schwotzer, Marcel Bonn-Miller, Jacob McDonald and Timothy Lefever*	DELTA-8-TETRAHYDROCANNABIVARIN (THCv), CANNABICHROMENE (CBC), AND CANNABINOL (CBN) SHOW NO TOXICITY FOLLOWING 14 DAY ORAL EXPOSURE	P2-33
Alan Morris, Michelle Adkins, Jacci Bainbridge, Rachael Rzasa Lynn and Emily Lindley*	DESIGN OF A CLINICAL TRIAL PROTOCOL TO STUDY DAILY ORAL CANNABIS FOR CHRONIC SPINE PAIN	P2-34
Nicole Lookfong*, Mariam Melkumyan, Wesley Raup-Konsavage, Kent Vrana and Yuval Silberman	EFFECTS OF CANNABIDIOL WITH AND WITHOUT OTHER CANNABINOIDS AND TERPENES ON SHORT-TERM AND LONG-TERM STRESS-RELATED BEHAVIORS	P2-35

Martha López-Canul*, Anahita Oveisi, Antonio Farina, Alexandra Teggin, Justine Enns, Maria-Luisa Vigano, Luca Posa, Danilo De Gregorio, Elena He, Shelly Yin and Gabriella Gobbi	THC AND CBD IN INSOMNIA ASSOCIATED TO NEUROPATHIC PAIN: EFFECT ON SLEEP ARCHITECTURE AND FIRING ACTIVITY OF RVM NEURONS	P2-36
Sarah Lunardi Baccetto*, Tallan Black, Ilne Barnard, Dan McElroy, John Howland and Robert Laprairie	ASSESSING SUSTAINED TETRAD RESPONSIVENESS IN FEMALE RATS FOLLOWING CHRONIC CANNABIS OR CANNABINOID EXPOSURE DURING PREGNANCY	P2-37
Claudia Lutelmowski*, Catharine Mielnik, Marija Milenkovic, Wendy Horsfall, Amy Ramsey, Heather Bradshaw and Ruth Ross	THE ROLE OF ANANDAMIDE IN A HYPOGLUTAMATERGIC MODEL OF PSYCHOSIS-LIKE BEHAVIOURS	P2-38
Elena Martin-Garcia*, Maria Cajiao, Veronica Casado-Anguera, Alejandra García-Blanco and Rafael Maldonado	NEUROBIOLOGICAL MECHANISMS UNDERLYING VULNERABILITY AND RESILIENCE TO CANNABIS ADDICTION	P2-39
WITHDRAWN	WITHDRAWN	P2-40
Jakub Mlost*, Magdalena Białoń, Marta Kędziora and Katarzyna Starowicz	ANANDAMIDE IN OSTEOARTHRITIS AND DEPRESSION: BEHAVIOURAL, BIOCHEMICAL AND NETWORK ANALYSIS	P2-41
WITHDRAWN	WITHDRAWN	P2-42
Catherine Moore*, Catherine Davis and Elise Weerts	BEHAVIORAL AND NEUROCOGNITIVE EFFECTS OF THE CANNABINOID CANNABIGEROL (CBG)	P2-43
Daniel Kaoru Mori-Fegan*, Che-Yuan Wu, Yuen Yan Wong, Ruth Ross and Walter Swardfager	THE EFFECTS OF G-PROTEIN COUPLED RECEPTOR 55 (GPR55) SINGLE NUCLEOTIDE POLYMORPHISMS ON BRAIN VOLUME AND ATROPHY IN LATER LIFE	P2-44

Alan W. J. Morris*, Rahwa Netsanet, Jacci Bainbridge, Vikas V. Patel, Rachael Rzasa Lynn and Emily M. Lindley	PRELIMINARY DATA COMPARING THE EFFICACY OF ACUTE VAPORIZED CANNABIS TO ORAL OXYCODONE AND PLACEBO FOR CHRONIC SPINE PAIN	P2-45
Mohammed Mustafa*, Joseph Porter, Joel Schlosburg, Jayden Elmer, Justin Poklis and Aron Lichtman	INVESTIGATION OF CANNABIDIOL IN THE DRUG DISCRIMINATION PARADIGM	P2-46
Nathalie Nadales*, R. Michael Little and Nancy E. Buckley	EFFECT OF THC AND THE ROLE OF PERIPHERAL CANNABINOID RECEPTOR (CB2R) ON RESISTANCE TO SYSTEMIC *CANDIDA ALBICANS* INFECTION IN IMMUNE SUPPRESSED AND IMMUNE COMPETENT MALE MICE	P2-47
John Patrick Neary*, Jane Alcorn, Robert B. Laprairie, Payam Dehghani, Bruce H. Bjornson, Thomas Hadjistavropoulos, Kim D. Dorsch, Cameron S. Mang, Holly A. Bardutz, Lanishen Bhagaloo, Zachary Walsh, Philip N. Ainslie, Michael Szafron, Jyotpal Singh and Elizabeth S. Thompson	NATURALLY PRODUCED CANNABINOIDS FOR PAIN MANAGEMENT AND NEUROPROTECTION FROM CONCUSSION DURING PARTICIPATION IN CONTACT SPORTS: NFL FUNDED STUDY PROTOCOL	P2-48
Lesley O'Brien*, Delaney Place, Jones Aidan and Aron Lichtman	MAGL INHIBITION PREVENTS MEMORY DEFICIT CHEMOTOXICITY BY AROMATASE INHIBITION IN MICE	P2-49
WITHDRAWN	WITHDRAWN	P2-50
Bolanle F. Olabiyi*, Eike Geissmar, Andreas Zimmer and Anne-Caroline Schmöle	CANNABINOID RECEPTOR 2 DELETION ENHANCES RESPIRATORY CAPACITY IN NAÏVE AND TLR4-STIMULATED MICROGLIA	P2-51
Gavin Petrie*, Georgia Balsevich, Hiulan Yau, Tamas Fuzesi, David Rosenegger Robert Aukema, Mario Van Der Stelt, Jaideep Bains and Matthew Hill	ENDOCANNABINOID UPREGULATION INFLUENCES THE BEHAVIORAL AND NEUROENDOCRINE RESPONSE TO STRESS	P2-52

Diana Sepulveda, Daniel Morris, Wesley Raup-Konsavage*, Dongxiao Sun, Kent Vrana and Nicholas Graziane	CANNABIGEROL (CBG) ATTENUATES MECHANICAL HYPERSENSITIVITY ELICITED BY CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY	P2-53
Antonio Reck*, Frances Kim and Steven Kinsey	CANNABINOID AND OPIOID RECEPTOR APPROACHES TO REDUCE HISTAMINE-INDUCED PRURITUS	P2-54
Maria C Redmond*, Catherine R Healy, Mehnaz I Ferdousi, Georgina Gethin, Abhay Pandit and David P Finn	BEHAVIOURAL CHARACTERISATION OF A PRECLINICAL INCISIONAL WOUND MODEL AND INVESTIGATION OF ASSOCIATED ALTERATIONS IN THE ENDOCANNABINOID SYSTEM	P2-55
Rachel Humphrey, Andrés Illanes-Rosales, Daniel Kerr, David Finn and Michelle Roche*	SEX-DEPENDENT SENSORY AND AFFECTIVE INFLAMMATORY PAIN RESPONDING IN A PRECLINICAL RAT MODEL OF AUTISM AND ASSOCIATED CHANGES IN ENDOCANNABINOID GENE EXPRESSION IN THE ANTERIOR CINGULATE CORTEX	P2-56
Carl Erwin B. Rodriguez* and Steven G. Kinsey	MAGL INHIBITION ATTENUATES POST-SURGICAL PAIN IN MICE	P2-57
Alan Morris, Michelle Adkins, Jacci Bainbridge, Rachael Rzasa Lynn* and Emily Lindley	DESIGN OF A CLINICAL TRIAL PROTOCOL OF DAILY ORAL CANNABIS TO REDUCE OPIOID INTAKE IN PATIENTS WITH CHRONIC SPINE PAIN	P2-58
Ayshe Sahinovic*, Peter Doohan, Richard C Kevin, Iain S McGregor and Danielle McCartney	EXPLORING THE EFFECTS OF EXERCISE AND CANNABIDIOL ON THE ENDOCANNABINOID SYSTEM	P2-59
WITHDRAWN	WITHDRAWN	P2-60
Erica Golden, George Amato, Rosamond Goodson, Rangan Maitra and Joel Schlosburg*	SCREENING FOR CENTRALLY-MEDIATED AVERSIVE EFFECTS OF CB1 RECEPTOR ANTAGONISTS USING INTRACRANIAL SELF-STIMULATION	P2-61

WITHDRAWN	WITHDRAWN	P2-62
Gabriella Smith*, Mayil Bhat, Blake Woods, Max Rose, Ken Mackie and Anna Kalinovsky	DEVELOPMENTAL AND BEHAVIORAL CONSEQUENCES OF 2-AG ATTENUATION IN THE CEREBELLUM: IMPLICATIONS FOR AUTISM SPECTRUM	P2-63
Jennifer Spohrs, Michael Prost*, Martin Ulrich, Paul Plener, Laura Bindila and Birgit Abler	ENDOCANNABINOID SYSTEM REACTIVITY DURING STRESS PROCESSING IN HEALTHY HUMANS	P2-64
Chandrani Majumdar*, Mohamed M. Radwan, Donald Stanford, Suman Chandra, Amira Wanas, Mostafa A. Elhendawy, Elsayed A. Ibrahim and Mahmoud A. ElSohly	EFFECT OF GAMMA IRRADIATION ON THE CANNABINOIDS AND TERPENES CONTENT OF CANNABIS BIOMASS	P2-65
James Tait*, Simon Erridge, Carl Holvey, Ross Coomber, Azfer Usmani, Mohammed Sajad, Jonathan Hoare, Shaheen Khan, Mark Weatherall, James Rucker, Michael Platt and Mikael Sodergren	CLINICAL OUTCOME DATA OF CHRONIC PAIN PATIENTS TREATED WITH CANNABIS-BASED OILS AND DRIED FLOWER IN THE UNITED KINGDOM: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P2-66
Marian Twohig*, Andy Baker, Douglas Stevens, Andrew Aubin and Christopher J. Hudalla	CHARACTERISATION OF DELTA-8 THC DISTILLATES USING HIGH RESOLUTION MASS SPECTROMETRY (HRMS) AND CYCLIC ION MOBILITY SPECTROMETRY COUPLED WITH HRMS	P2-67
Michael Udoh*, Adam Ametovski, Charles Marlowe, Jia Luo, Marina Santiago, Jianping Sun, Marika Heblinski, Iain McGregor, Philip Barr, Mark Connor and Jonathon Arnold	CANNABICHROMENE ACTIVITY AT CB2 RECEPTORS IS ENANTIOSELECTIVE	P2-68
Paula Unzueta-Larrinaga*, Alicia Calvo, Aitor Villate, Rocío Barrena-Barbadillo, Néstor Etxebarria, Luis Felipe Callado and Leyre Urigüen	ENDOCANNABINOIDS PLASMA LEVELS IN SUBJECTS WITH SCHIZOPHRENIA WITH AND WITHOUT CANNABIS USE DISORDER	P2-69
Olivia Vanegas*, Matthew Reck, Carl Rodriguez, Julie Marusich, Omer Yassin, Gregory Sotzing, Jenny Wiley and Steven Kinsey	ASSESSMENT OF ABUSE LIABILITY AND DEPENDENCE POTENTIAL OF HEMP-DERIVED Δ^8-TETRAHYDROCANNABINOL IN MICE	P2-70

Beth Wiese*, Erika Liktor-Busa, Sarah Couture, Spyros Nikas, Lipin Ji, Yingpeng Liu, Alexandros Makriyannis, Todd Vanderah and Tally Largent-Milnes	OPIOID AND CANNABINOID INTERACTIONS: A STRATEGIC APPROACH TO REDUCE THE RISK OF OVERDOSE	P2-71
Jonah Wirt*, Ming Jiang, Mirjam Huizenga, Alex Makriyannis, Mario van der Stelt and Andrea Hohmann	LEI-515, A PERIPHERALLY RESTRICTED INHIBITOR OF MONOACYLGLYCEROL LIPASE, SUPPRESSES NEUROPATHIC NOCICEPTION IN A MOUSE MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY	P2-72
Hayden Wright*, Zachary Fisher, Rafael Urrutia-Camargo, Darren Ginder, Amanda Brown, Hannah Arey, Jobe Ritchie, Rita Fuchs and Ryan McLaughlin	2-ARACHIDONOYLGLYCEROL SIGNALING IN THE LATERAL HABENULA: IMPACTS ON STRESS COPING BEHAVIOR AND DOWNSTREAM IMMEDIATE EARLY GENE EXPRESSION	P2-73

Notes:

Presenting Author*

POSTER SESSION 3
DAY 4, WEDNESDAY, JUNE 29TH: 16:00 - 18:00

WITHDRAWN	WITHDRAWN	P3-1
Jacquelyn Bainbridge*, Sarah Rajkovic, Maureen Leehey, Ying Liu, Mahmoud ElSohly and Michelle Adkins	RESEARCH PHARMACIST EXPERIENCE COMPOUNDING THE NIDA CANNABIS EXTRACT FOR PRACTICAL USE IN A CLINICAL TRIAL	P3-2
L. Cinnamon Bidwell, Renée Martin-Willett* and Sharon R Sznitman	DAILY ASSOCIATION WITH CANNABIS USE AND SLEEP QUALITY IN ANXIOUS CANNABIS USERS	P3-3
Tallan Black*, Sarah L. Bacetto, Ilne L. Barnard, Dan L. McElroy, Emma Finch, Faith V. Austin-Scott, Quentin Greba, Deborah Michel, Ayat Zagzoog, John G. Howland and Robert B. Laprairie	PHARMACOKINETIC AND MOLECULAR CHARACTERIZATION IN A RAT MODEL OF PRENATAL CANNABIS SMOKE EXPOSURE	P3-4
Kevin Boehnke*, Owen Dean and Avinash Hosanagar	US MEDICAL CANNABIS PATIENT REGISTRATION AND REASONS FOR USE: 2016-2020	P3-5
Taryn Bosquez*, Jessie Gudorf, Michael VanNieuwenhze and Alex Straiker	CANNABIDIOL-DERIVED VARIANTS AS POTENTIAL NEGATIVE ALLOSTERIC MODULATORS AT THE MU OPIOID RECEPTOR	P3-6
Stephanie Bourke*, Therese O' Connor, Mary Hopkins, Nikkita N. Burke, Massieh Moayedi, Brian E. McGuire and David P. Finn	SEX DIFFERENCES IN SOMATOSENSORY SENSITIVITY, ACUTE PSYCHOLOGICAL STRESS RESPONSE AND ENDOCANNABINOIDS	P3-7
Heather B Bradshaw*, Hannah R Bentz, Clare T Johnson and Alex Straiker	SEX DIFFERENCES IN CANNABINOID ACTIVITY IN THE SALIVARY GLAND DRIVES CHANGES IN LIPIDOMIC PROFILES: IMPLICATIONS FOR DRY MOUTH MECHANISMS	P3-8

Stevie Britch*, Sharon Walsh, Rachel Vickers-Smith, Shanna Babalonis and Svelta Slavova	CANNABINOID TOXICITY-RELATED EMERGENCY DEPARTMENT VISITS AND INPATIENT HOSPITALIZATIONS IN KENTUCKY, 2017 TO 2019	P3-9
Douglas Bruce*, Gertrude Palillo, Suzanne McLone, Meha Singh and Nikhil Prachand	PROFILES OF MEDICAL AND RECREATIONAL CANNABIS USERS IN A POPULATION-BASED SAMPLE DURING COVID-19	P3-10
Teah-Marie Bynion*, L. Riley Gournay, Graham M. L. Eglit, Marcel O. Bonn-Miller, Matthew T. Feldner and Ellen W. Leen-Feldner	A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TEST OF THE EFFECTS OF CANNABIDIOL ON FEAR ELICITED BY A 10% CARBON-DIOXIDE-ENRICHED AIR BREATHING CHALLENGE	P3-11
Teah-Marie Bynion*, L. Riley Gournay, Jordan Petry, Matthew T. Feldner, Marcel Bonn-Miller and Ellen W. Leen-Feldner	A MULTIPLE BASELINE STUDY OF THE EFFECTS OF CANNABIDIOL ON DAILY PERCEIVED STRESS AMONG PEOPLE EXPERIENCING HIGH LEVELS OF COVID DISTRESS	P3-12
Sara MacPhail, Miguel Bedoya-Perez, Rhys Cohen, Vicki Kotsirilos, Iain McGregor and Elizabeth Cairns*	FIVE YEARS OF MEDICINAL CANNABIS PRESCRIBING IN AUSTRALIA: WHERE ARE WE HEADING?	P3-13
Luis Felipe Callado*, Inés Ibarra-Lecue, Paula Unzueta-Larrinaga, Rocío Barrena-Barbadillo, Begoña Mendivil, Miguel Ángel Landabaso and Leyre Urigüen	5-HT2AR AND AKT PROTEIN EXPRESSION IS DIFFERENTLY REGULATED BY CANNABIS ABUSE IN PLATELETS OF SCHIZOPHRENIA SUBJECTS	P3-14
Carrie Cuttler*, Amanda Stueber, and Aria Petrucci	ATTENTION PLEASE! CHRONIC AND ACUTE EFFECTS OF CANNABIS ON COGNITION IN PEOPLE WITH ADHD	P3-15
Mary Kathryn Dahlgren*, Kelly Sagar, Ashley Lambros, Rosemary Smith, Celine El-Abboud, Deniz Kosereisoglu and Staci Gruber	REGRESSION MODELING TO IDENTIFY VARIABLES ASSOCIATED WITH CLINICAL IMPROVEMENT FOLLOWING 3 MONTHS OF TREATMENT WITH MEDICAL CANNABIS	P3-16
Kayle Dickson, Cassidy Scott, Hannah White and Christian Lehmann	BETA-CARYOPHYLLENE AS A NOVEL ADJUNCT THERAPY FOR THE TREATMENT OF URINARY TRACT INFECTIONS	P3-17

Denise Vidot, Bria-Necole Diggs*, Brandie Bentley, Johis Ortega, Juan Hernandez, Eulalia Kahwa, Jermaine Whyte and Marvin Reid	BRIEF REPORT: PREVALENCE AND PATTERNS OF CANNABIS USE IN RURAL JAMAICA	P3-18
Simon Erridge*, Beata Ciesluk, Lucy Troup and Mikael Hans Sodergren	PERCEIVED EFFECTIVENESS OF CANNABIS-BASED MEDICINE PRODUCTS: A QUESTIONNAIRE STUDY	P3-19
Simon Erridge*, Fabian Olsson and Mikael Sodergren	PATIENT PRIORITIES FOR RESEARCH: A FOCUS GROUP STUDY OF UK MEDICAL CANNABIS PATIENTS	P3-20
Simon Erridge*, Carl Holvey, Ross Coomber, Sushil Beri, Jonathan Hoare, Shaheen Khan, Mark Weatherall, Michael Platt and Mikael Sodergren	AN ANALYSIS OF CLINICAL OUTCOME MEASURES FOR SCOTTISH PATIENTS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-21
Simon Erridge*, Carl Holvey, Ross Coomber, Sushil Beri, Jonathan Hoare, Shaheen Khan, Mark Weatherall, Michael Platt, James Rucker and Mikael Sodergren	AN ANALYSIS OF CLINICAL OUTCOME MEASURES FOR ENGLISH PATIENTS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-22
Simon Erridge*, Jess Kerr-Gaffney, Carl Holvey, Ross Coomber, Daniela Barros, Urmila Bhoskar, Gracia Mwimba, Kavita Praveen, Chris Symeon, Simmi Sachdeva-Mohan, Mikael Sodergren and James Rucker	CLINICAL OUTCOME ANALYSIS OF PATIENTS WITH AUTISM SPECTRUM DISORDER: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-23
Simon Erridge*, Carl Holvey, Ross Coomber, Jonathan Hoare, Shaheen Khan, Michael Platt, James Rucker, Mark Weatherall, Sushil Beri and Mikael Hans Sodergren	CLINICAL OUTCOME DATA OF CHILDREN TREATED WITH CANNABIS BASED MEDICINAL PRODUCTS FOR TREATMENT RESISTANT EPILEPSY – ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-24
Sajed Mangoo, Simon Erridge*, Carl Holvey, Ross Coomber, Kavita Praveen, Chris Symeon, Urmila Bhoskar, Simmi Sachdeva-Mohan, James Rucker and Mikael Sodergren	ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH DEPRESSION: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-25

Martha Nicholas*, Simon Erridge, Carl Holvey, Ross Coomber, Ezzat Awad, James Rucker, Mark Weatherall and Mikael Sodergren	ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH HEADACHE DISORDERS: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-26
Fabian Olsson*, Simon Erridge, James Tait, Carl Holvey, Ross Coomber, Sushil Beri, Jonathan Hoare, Shaheen Khan, Michael Platt, James Rucker and Mikael Sodergren	AN UPDATED ANALYSIS OF CLINICAL OUTCOME MEASURES ACROSS PATIENT GROUPS IN THE UK MEDICAL CANNABIS REGISTRY	P3-27
Manaswini Pillai*, Simon Erridge, Carl Holvey, Ross Coomber, Kavita Praveen, Chris Symeon, Urmila Bhoskar, Simmi Sachdeva-Mohan, James Rucker and Mikael Sodergren	ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH POST-TRAUMATIC STRESS DISORDER: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-28
Raphael Rifkin-Zybutz*, Simon Erridge, Carl Holvey, Ross Coomber, Daniela Barros, Urmila Bhoskar, Gracia Mwimba, Kavita Praveen, Chris Symeon, Simmi Sachdeva-Mohan, James Rucker and Mikael Sodergren	CLINICAL OUTCOME DATA OF ANXIETY PATIENTS TREATED WITH CANNABIS-BASED OILS AND DRIED FLOWER IN THE UNITED KINGDOM: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-29
Lucy Troup*, Simon Erridge, Beata Ciesluk and Mikael Hans Sodergren	PERCEIVED STIGMA OF PATIENTS UNDERGOING TREATMENT WITH CANNABIS-BASED MEDICINAL PRODUCTS	P3-30
Kavyesh Vivek, Zekiye Karagozolu, Simon Erridge*, Carl Holvey, Ross Coomber, Ezzat Awad, James Rucker, Mark Weatherall and Mikael Sodergren	CLINICAL OUTCOME DATA OF PATIENTS WITH PRIMARY INSOMNIA DISORDER TREATED WITH CANNABIS-BASED MEDICINAL PRODUCTS: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY	P3-31
Cheryl Fitzer-Attas*, Shrey Joshi and Connie Pascal	EVIDENCE-BASED CUSTOMER DISCOVERY CONFIRMS PERCEIVED CUSTOMER VALUE FOR A DECENTRALIZED RESEARCH PLATFORM FOR MEDICAL CANNABIS	P3-32
Nicola Forte*, Serena Boccella, Lea Tunisi, Alba Clara Fernández-Rilo, Maria De Risi, Monica Iannotta, Fabiana Piscitelli, Elvira De Leonibus, Sabatino Maione, Vincenzo Di Marzo and Luigia Cristino	OREXIN-A, 2-AG AND 2-AG-DERIVED 2-AGP ARE INVOLVED IN OBESITY-ASSOCIATED ALTERATIONS OF HIPPOCAMPAL MEMORY IN MICE	P3-33

Albert Garcia-Romeu*, Sandeep Nayak, David Mathai, Erin Wang and Natalie Gukasyan	HEALTHCARE PROVIDERS' KNOWLEDGE AND ATTITUDES ON CANNABIS-BASED TREATMENTS	P3-34
Durga Ghosh*, Alexander Upfill-Brown and Elizabeth Lord	PREVALENCE AND IMPACT OF CANNABINOID USE IN PATIENTS WITH SPINAL DISORDERS	P3-35
Laurel Gibson, Raeghan Mueller, Angela Bryan*, L. Cinnamon Bidwell and Kent Hutchison	CANNABINOID EXPOSURE AND SUBJECTIVE EFFECTS AFTER ACUTE *AD LIBITUM* ADMINISTRATION OF ORAL CANNABIS IN A NATURALISTIC SETTING	P3-36
Rina Goldstein*, Sean Madden and Nehal P. Vadhan	PRELIMINARY DATA ON THE IMPACT OF RECREATIONAL CANNABIS LEGALIZATION ON CANNABIS USE PATTERNS IN THE NY METROPOLITAN AREA	P3-37
Gregory Burnett, David Gorelick* and Kevin Hill	MEDICAL CANNABIS LEGALIZATION IN THE UNITED STATES: EVIDENCE-BASED OR POLITICALLY DRIVEN?	P3-38
Deniz Kosereisoglu, Rosie Smith, Kelly Sagar, Ashley Lambros, Mary Kathryn Dahlgren, Celine El-Abboud and Staci Gruber*	A MAJOR QUESTION: ACCURACY OF MINOR CANNABINOID CONTENT ON CANNABIS-CONTAINING PRODUCT LABELS	P3-39
Charleen Gust*, Laurel Gibson, Jarrod Ellingson, Sophie YorkWilliams, Cristina Sempio, Jost Klawitter, Angela Bryan, Cinnamon Bidwell and Kent Hutchison	SEX-DEPENDENT DIFFERENCES IN ACUTE OBJECTIVE AND SUBJECTIVE INTOXICATION AMONG HIGH POTENCY CANNABIS FLOWER USERS	P3-40
WITHDRAWN	WITHDRAWN	P3-41
Bryan Jenkins*, Hayley Wilson, Kate Nicholson, Amy Newman and Jibran Khokhar	CHRONIC CANNABIDIOL TREATMENT SEX-DEPENDENTLY ALTERS HPA AXIS RESPONSIVITY WITHOUT MODULATING FEAR MEMORY OR ANXIETY-LIKE BEHAVIOUR	P3-42

Erin Johnson*, Michael Kilgore and Shanna Babalonis	QUANTITATION OF 17 PHYTOCANNABINOIDS IN HEMP-DERIVED OILS: LC-MS/MS METHOD VALIDATION AND PRODUCT DATA	P3-43
Clare T. Johnson*, Hannah R. Bentz, Gabriel H.D. Abreu, Ken Mackie, Hui-Chen Lu and Heather B. Bradshaw	Δ^9-TETRAHYDROCANNABINOL, CANNABIDIOL, AND THEIR COMBINATION RESULT IN SEXUALLY DIMORPHIC MODULATIONS OF PLASMA LIPIDS	P3-44
Faiz Kassim*, Sophie Tod, Matthew Albrecht, Sean Hood, Joseph Lee, Jennifer Rodger and Mathew Martin-Iverson	NABILONE IMPAIRS SPATIAL AND VERBAL WORKING MEMORY IN HEALTHY SUBJECTS	P3-45
Richard Kevin*, Rebecca Gordon, Thomas Arkell, Frederick Vinckenbosch, Eef Theunissen, Johannes Ramaekers and Iain McGregor	DO CANNABIS "BREATHALYSERS" WORK? RELATIONSHIP BETWEEN BREATH CANNABINOIDS, PLASMA CANNABINOIDS, AND DRIVING PERFORMANCE	P3-46
Paul Kocis and Kent Vrana*	CANNabinoid DRUG INTERACTION REVIEW (CANN-DIR™) WEB APP	P3-47
Justyna Kulpa*, Graham Eglit, Laura MacNair, Helena Yardley, Mark Ware, Marcel O. Bonn-Miller and Erica N. Peters	SERUM MARKERS OF BONE TURNOVER FOLLOWING ADMINISTRATION OF ORAL MEDICAL CANNABIS PRODUCTS IN HEALTHY ADULTS	P3-48
Caroline MB Kwee*, Johanna MP Baas, Febe E Van der Flier, Lucianne Groenink, Puck Duits, Merijn Eikelenboom, Date C Van der Veen, Mirjam Moerbeek, Neeltje M Batelaan, Anton JLM Van Balkom and Danielle C Cath	CANNABIDIOL ENHANCEMENT OF EXPOSURE THERAPY IN TREATMENT REFRACTORY PATIENTS WITH ANXIETY DISORDERS: A RANDOMISED CONTROLLED TRIAL	P3-49
Caroline M.B. Kwee*, Joop M.A. van Gerven, Fleur L.P. Bongaerts, Danielle C. Cath, Gabriël Jacobs, Johanna M.P. Baas and Lucianne Groenink	CANNABIDIOL IN CLINICAL AND PRECLINICAL ANXIETY RESEARCH. A SYSTEMATIC REVIEW INTO CONCENTRATION-EFFECT RELATIONS USING THE IB-DE-RISK TOOL	P3-50

Lindsay Lo*, April Christiansen, Lauren Eadie, David Kim, Alasdair Barr and Caroline MacCallum	CANNABIDIOL (CBD)-ASSOCIATED LIVER ENZYME ELEVATION: A SYSTEMATIC REVIEW OF CLINICAL TRIALS	P3-51
Ali Mokhtar Mahmoud*, Magdalena Kostrzewa, Marianna Cerasuolo, Roberto Ronca and Alessia Ligresti	CANNABIDIOL TRIGGERS MITOCHONDRIAL DYSFUNCTION AND CELL DEATH IN HORMONE-REFRACTORY PROSTATE CANCER BY TARGETING VOLTAGE-DEPENDENT ANION-SELECTIVE CHANNEL (VDAC1) AND HEXOKINASE II (HK-II)	P3-52
Jahan Marcu*, Teresa Simon, Andreas Gomez and Matt Wabwire	COMPARISON OF CANNABIS AND PSILOCYBIN ADVERSE EVENTS IN A NATIONAL REPORTING SYSTEM	P3-53
Jahan Marcu*, Teresa Simon, Mathew Wabwire, Sara Jane Ward and Tim Smale	GET YOUR THINKING CAP ON: IMPLEMENTATION OF THE CANNABIS AWARENESS PROGRAM (CAP) FOR HIGH SCHOOL STUDENTS	P3-54
Renée Martin-Willett*, Elizabeth Zambrano Garza and L. Cinnamon Bidwell	CANNABIS USE PATTERNS, PERCEPTIONS, AND RELATED HEALTH OUTCOMES AMONG SPANISH SPEAKERS IN THE UNITED STATES AND INTERNATIONALLY	P3-55
Danielle McCartney*, Richard Kevin, Anastasia Suraev, Peter Doohan and Iain McGregor	HOW LONG DOES A SINGLE ORAL DOSE OF CANNABIDIOL PERSIST IN PLASMA? FINDINGS FROM THREE RECENT CLINICAL TRIALS	P3-56
Jake McDonald	PHARMACOKINETICS AND TOXICITY EVALUATION AFTER INHALATION OF DELTA-9-TETRA-HYDROCANNABINOL AEROSOLS GENERATED FROM A PRESSURIZED METERED DOSE INHALER (PMDI)	P3-57
Danielle McCartney, Anastasia Suraev and Iain McGregor*	'NEXT DAY' EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON COGNITIVE FUNCTION AND SAFETY-SENSITIVE TASKS: A SYSTEMATIC REVIEW	P3-58
John McPartland	DOUBLE YOUR FUN WITH EDIBLES? THE RELATIVE POTENCY OF 11-OH-THC COMPARED TO THC – A META-ANALYSIS	P3-59

Conor H. Murray* and Ziva D. Cooper	ASSOCIATIONS BETWEEN CHRONIC CANNABIS USE AND RESTING EEG	P3-60
John Patrick Neary*, Jyotpal Singh, Jane Alcorn, Stephanie Vuong and Lanishan Bhagaloo	EFFECTS OF PHYTOCANNABINOIDS ON BLOOD PRESSURE AND PREFRONTAL CORTEX OXYGENATION IN FEMALE POST-CONCUSSION SYNDROME PATIENTS: CASE SERIES	P3-61
Karen Oldfield*, Harry Delany, Allie Eathorne, Giles Newton-Howes and Irene Braithwaite	MEDICINAL CANNABIS USE IN NEW ZEALAND FOLLOWING THE INTRODUCTION OF THE MEDICINAL CANNABIS SCHEME	P3-62
Laura MacNair, Maja Kalaba, Erica Peters*, Matthew Feldner, Graham Eglit, Lucile Rapin, Erin Prosk and Mark Ware	MEDICAL CANNABIS AUTHORIZATION PATTERNS, SAFETY, AND ASSOCIATED EFFECTS IN OLDER ADULTS	P3-63
Aria Petrucci*, Daniela Palombo and Carrie Cuttler	AUTOBIOGRAPHICAL MEMORY FOR EMOTIONAL AND NEUTRAL EVENTS IN CHRONIC CANNABIS USERS	P3-64
WITHDRAWN	WITHDRAWN	P3-65
Hudson Reddon*, Zach Walsh, Stephanie Lake, Cameron Grant, M. Eugenia Socias, Dan Werb, Kanna Hayashi and M-J Milloy	CANNABIS USE AND THE INCIDENCE OF MENTAL HEALTH DIAGNOSES: A 12-YEAR STUDY OF PEOPLE WHO USE UNREGULATED DRUGS	P3-66
Antonia Sánchez de Medina, Juan Manuel Serrano-Rodríguez, Elisa Díez de Castro, María Teresa García-Valverde, Aritz Saitua, Mireia Becero, Ana Muñoz, Carlos Ferreiro-Vera and Verónica Sánchez de Medina*	PHARMACOKINETICS AND ORAL BIOAVAILABILITY OF CANNABIDIOL IN HORSES AFTER INTRAVENOUS AND ORAL ADMINISTRATION WITH OIL AND MICELLAR FORMULATIONS	P3-67

Daniela Schwotzer*, Wendy Dye, Jacob Jantzi, Hammad Irshad, Kristen Trexler, Timothy Lefever, Marcel Bonn-Miller, Mark Ware and Jacob McDonald	PHARMACOKINETICS OF CANNABIDIOL IN SPRAGUE-DAWLEY RATS AFTER PULMONARY AND ORAL ADMINISTRATION	P3-68
Kassidy Seol* and Xueyu Zhu	AN INVESTIGATION OF COVID-19'S IMPACT ON DEPRESSIVE SYMPTOMS AMONG CANNABIS USERS USING MACHINE LEARNING	P3-69
Teresa Simon*, Andres Gomez, John Simon and Jahan Marcu	AN UPDATE TO SAFETY DATA ON CANNABIS-LIKE PRODUCTS IN THE FDA FAERS DATABASE	P3-70
Teresa Simon*, Anthony Silvestrone, Yomei Shaw and Jahan Marcu	"FREE WEED THAT GETS YOU HIGH": DID THE FDA AND CDC HEALTH ALERTS ON DELTA-8 THC REACH MAINSTREAM SOCIAL MEDIA?'	P3-71
Gregory L. Smith	HAIR REGROWTH WITH NOVEL HEMP EXTRACT – A CASE SERIES	P3-72
Tory Spindle*, Hayleigh Tilton, Spencer Lin, Ed Cone, Ruth Winecker, Eric Welsh, Lynn Wagner, Ron Flegel and Ryan Vandrey	PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS OF HEMP-DERIVED CANNABIDIOL (CBD) TOPICAL PRODUCTS	P3-73
Jenna Billingsley, Kelsey Andreis, Amanda Thayer, Jim Wager-Miller, Heather Bradshaw and Alex Straiker*	FATTY AMIDE HYDROLASE REGULATES TEARING IN A SEX-DEPENDENT MANNER IN MICE	P3-74
Justin Strickland*, Rhiannon Mayhugh, Renuka Surujnarain and Ryan Vandrey	ECOLOGICAL MOMENTARY ASSESSMENT OF ANXIETY AND DEPRESSION IN NEWLY INITIATED MEDICINAL CANNABIS PATIENTS: MOMENTARY AND LONG-TERM CLINICAL EFFECTS	P3-75
Amanda Stueber*, Aria Petrucci and Carrie Cuttler	CHRONIC AND ACUTE EFFECTS OF CANNABIS ON SYMPTOMS OF ATTENTION-DEFICIT/HYPERACTIVITY DISORDER	P3-76

James Tait*, Simon Erridge and Mikael Sodergren	UK MEDICAL CANNABIS REGISTRY: A PATIENT EVALUATION	P3-77
Alexa Torrens*, Christina Ruiz, Faizy Ahmed, Stephen Mahler and Daniele Piomelli	EVIDENCE FOR SUBSTANTIAL NASAL ABSORPTION OF Δ^9-TETRAHYDROCANNABINOL AND ITS ACTIVE METABOLITE FOLLOWING AEROSOL ADMINISTRATION	P3-78
Nir Treves*, Noa Yakirevich-Amir, Wiessam Abu Ahmad, Omer Bonne, Elyad Davidson, Tyler Dautrich and Ilan Matok	THE CHARACTERIZATION OF CANNABIS USERS AND PRODUCTS AND THE EXPERIENCE OF NEGATIVE MENTAL EMOTIONS AFTER CANNABIS USE	P3-79
Erin M. Rock, Cheryl L. Limebeer and Linda A Parker*	EVALUATION OF SEX DIFFERENCES IN THE POTENTIAL OF Δ^9-TETRAHYDROCANNABINOL, CANNABIDIOL AND CANNABIDIOLIC ACID TO REDUCE NAUSEA-INDUCED CONDITIONED GAPING REACTIONS IN SPRAGUE-DAWLEY RATS	P3-80
Sara Jane Ward*, Saadet Inan and Ajay Nayak	TOPICAL CHRONIC EXPOSURE TO BETA-CARYOPHYLLENE, BUT NOT TO CANNABIDIOL, INDUCES DERMATITIS IN MICE	P3-81
Maja Kalaba*, Adela Leiva Centeno, Jessica Hart and Mark A. Ware	ADVERSE EVENTS OF CANNABIS SINCE LEGALIZATION: A THREE-YEAR REVIEW	P3-82

Notes:

Presenting Author*

ICRS2022 - Presidential Plenary Speaker
11.45 - 12.45 Sunday, June 26th

The Microbiota-Gut-Brain Axis: A Gut Feeling About Stress, Pain & Mental Health

John Cryan, BSc (Hons), PhD

Professor and Chair
Department of Anatomy and Neuroscience
University College Cork
Ireland

The microbiota-gut-brain axis is emerging as a research area of increasing interest for those investigating the biological and physiological basis of neurodevelopmental, age-related and neurodegenerative disorders. The routes of communication between the gut and brain include the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or via microbial metabolites such as short chain fatty acids. These mechanisms also impinge on neuroendocrine function at multiple levels. Studies in animal models have been key in delineating that neurodevelopment and the programming of an appropriate stress response is dependent on the microbiota. Developmentally, a variety of factors can impact the microbiota in early life including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. Stress can significantly impact the microbiota-gut-brain axis at all stages across the lifespan. Moreover, animal models have been key in linking the regulation of fundamental brain processes ranging from adult hippocampal neurogenesis to myelination to microglia activation by the microbiome. Finally, studies examining the translation of these effects from animals to humans are currently ongoing. Further studies will focus on understanding the mechanisms underlying such brain effects and developing nutritional and microbial-based intervention strategies and how these interact with various systems in the body including the cannabinoid system.

ICRS2022 – Kang Tsou Memorial Lecturer
8.30 – 9.30 Monday, June 27th

Adventures understanding pain, analgesia and anaesthesia-induced altered states of consciousness through human neuroimaging

Irene Tracey, M A (O x o n), D P h i l . , F R C A , F M e d Sci

Nuffield Chair of Anaesthetic Science
University of Oxford
United Kingdom

The brain is key to the experience of pain and pain relief. Relating specific neurophysiological mechanisms from the brain, brainstem and spinal cord, identified using neuroimaging, that are relevant in acute and chronic pain experiences has tremendous value. Chronic pain is one of the largest medical health problems in the developed world with one in five adults suffering. Relating these neural mechanisms to individual pain experiences, measures of pain relief, persistence of pain states, degree of injury and genetics as well as determining neural network vulnerabilities towards chronic pain development has neuroscientific and potential diagnostic relevance. Objective evidence of target engagement and pharmacodynamic efficacy of potential new analgesics is desperately needed if we are to improve analgesic drug development - hampered by over-reliance on subjective pain ratings. Advanced neuroimaging can powerfully aid explanation of a subject's multidimensional pain experience, analgesia and even what makes them vulnerable or resilient to developing chronic pain. There are many scenarios, in and out of the clinic, whereby having robust composite pain biomarkers would be helpful. Neuroimaging can powerfully contribute to their generation.

Less work has been directed at understanding what brain changes occur during altered states of consciousness induced either endogenously (e.g. sleep) or exogenously (e.g. anaesthesia). However, that situation is changing rapidly. Our multimodal neuroimaging work explores how anaesthetic agents produce altered states of consciousness such that perceptual experiences of pain and awareness are degraded. This is bringing us fascinating insights into the complex phenomenon of anaesthesia and the concept of self-hood.

ICRS2022 – Mechoulam Award Speaker
11.45 – 12.30 Tuesday, June 28th

Towards a cannabinoid-based neuroprotective therapy for neurodegenerative disorders: the recent example of TDP-43 dependent frontotemporal dementia

Javier Fernandez-Ruiz, PH.D.

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

(1) Worldwide life span has significantly elevated over the past 50 years and, despite the recent COVID-19 pandemy that affected particularly aged people, it is expected that this elevation continues in the forthcoming years. Such elevation has been paralleled by an increased incidence of neurodegenerative disorders, as they are strongly dependent on aging as the major risk factor, which gives these disorders more opportunities to become visible. This situates the need of a better therapeutic management for these disorders as an important biomedical challenge in future years.

(2) Cannabinoids form a singular family of signaling lipids, plant-derived compounds and synthetic derivatives capable to preserve, rescue, repair and/or replace neurons and other neural cells against a myriad of insults that deteriorate their homeostasis and integrity in neurodegenerative disorders. It is important to remark that these insults cooperate each other to kill neural cells, which require a multi-target strategy capable to limit all these events in a coordinated manner, something that cannabinoids may provide (they may cooperate to attenuate excitotoxicity, oxidative stress, glia-driven inflammation, mitochondrial failure, and protein aggregation). Such pleiotropic potential is possible through their action at their multiple canonical targets (e.g. cannabinoid receptors and endocannabinoid enzymes) and also via non-canonical elements, which are located at CNS structures (e.g. blood-brain barrier) and cellular substrates (e.g. neurons, astrocytes, resting and reactive microglia, perivascular microglial cells, oligodendrocytes and oligodendrocyte precursor cells, and neural progenitor cells) that are critical in cell degeneration, protection, and/or repair.

(3) The neuroprotective potential of cannabinoids has been extensively investigated at the preclinical level in the most frequent neurodegenerative disorders (e.g. Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's chorea), whereas similar studies have been recently initiated in experimental models of frontotemporal dementia (FTD). FTD is a heterogeneous group of early onset and progressive neurodegenerative disorders, characterized by degeneration in the frontal and temporal lobes, which causes deterioration in cognition, personality, social behavior and language. Around 65% of the cases are characterized for the presence of aggregates of the RNA-binding protein TDP-43. Mice overexpressing TDP-43 exclusively in forebrain neurons recapitulate cardinal signs of FTD as cognitive deficits, emotional impairment, disinhibition in social behaviour and higher mortality, which were associated with important neuronal losses and elevated glial reactivity in the medial prefrontal cortex and the hippocampus, as well as with a dysregulation in the endocannabinoid system in both structures. The treatment of these mice with several endocannabinoid-acting compounds (e.g., pharmacological inactivation of FAAH enzyme using URB597, selective activation of CB_1 and CB_2 receptors with ACEA and HU-308, respectively) delayed the progression of the pathological phenotype in these mice, improving cognitive, emotional and social behaviour deterioration, preserving pyramidal neurons of the medial prefrontal cortex and the hippocampus, and reducing glial reactivity in both structures, which situates the elevation of the endocannabinoid tone as a promising therapy against TDP-43-induced neuropathology in FTD.

ICRS2022 – Presidential Plenary Speaker
12.00 – 13.00 Wednesday, June 29th

Endocannabinoids Sculpt Sex Differences in the Developing Social Behavior Network

Margaret McCarthy, B.S., M.A., Ph.D.

James and Carolyn Frenkil Dean's Professor and Chair
Department of Pharmacology and Program in Neuroscience
University of Maryland School of Medicine
USA

The social behavior network consists of essential nodes and critical connections that drive social behaviors, including courting, mating, parenting and aggression, all of which are components of reproductive success. Less appreciated is the control of adolescent play behavior which is also embedded within the same neural network controlling adult behaviors. Major progress has been made in elucidating specific cellular populations and connections that control discrete adult reproductive behaviors, often differently in males and females. Where knowledge has lagged is in characterizing how the social network develops, how it develops differently as a function of sex and how it goes awry in response to challenge.

In this talk we will explore the role of endocannabinoids in establishing a sex difference in rough-and-tumble play using the laboratory rat as our model species. As membrane-derived fast acting small molecules, endocannabinoids are at first pass a surprising purveyor of social network formation. Derived from lipid membrane precursors, endocannabinoids are among the most evolutionarily ancient and earliest expressed signaling molecules in the brain, detectable as early as 5-weeks of gestation in humans. When placed in the context of ancient evolutionary origins, pan species importance and early appearance in brain development, the centrality of endocannabinoids seems self-evident and is probably underestimated.

We have found an unexpected role for endocannabinoid tone in the developing amygdala which is higher in neonatal males than females and promotes phagocytosis by the innate immune cells of the brain, microglia. The phagocytosis is not random but instead targeted towards proliferative precursors of future astrocytes. The higher rates of phagocytosis by microglia in the male amygdala reduces the population of astrocytes and thereby increases the excitation of neurons within this region that promote playfulness[1]. Manipulating the endocannabinoid tone early in life with either THC or CB1 and CB2 receptor agonists alters playfulness during the adolescent period[2]. The importance of play to males is reflected in the enduring consequences on adult behavior of being denied of play as an adolescent. Understanding how this happens and why it appears specific to males are the targets of ongoing studies.

1. VanRyzin JW, Marquardt AE, Argue KJ, et al. Microglial Phagocytosis of Newborn Cells Is Induced by Endocannabinoids and Sculpts Sex Differences in Juvenile Rat Social Play. *Neuron* 2019.
2. Argue KJ, VanRyzin JW, Falvo DJ, Whitaker AR, Yu SJ, McCarthy MM. Activation of Both CB1 and CB2 Endocannabinoid Receptors Is Critical for Masculinization of the Developing Medial Amygdala and Juvenile Social Play Behavior. *eNeuro* 2017; **4**(1).

ICRS2022 – William A Devane Young Investigator Awardee Lecture
15.00 – 15.30 Wednesday, June 29th

Allosteric Pharmacology at the Cannabinoid Receptors: Complex Patterns and Novel Questions

Robert Laprairie, Ph.D.

Associate Professor, CIHR-GSK Research Chair in Drug Discovery and Development
College of Pharmacy and Nutrition
University of Saskatchewan, Saskatoon, Saskatchewan, Canada

The type 1 and type 2 cannabinoid receptors (CB1R and CB2R) participate in the regulation of numerous physiology functions and have been a major focus of pharmaceutical drug research since their identification and cloning. Over the past decade work from our lab and others has begun to explore allosteric modulation of the cannabinoid receptors. Allosteric modulators act at a distinct receptor site from that of the endogenous ligand to augment (positive allosteric modulators [PAMs]) or diminish (negative allosteric modulators [NAMs]) endogenous ligand signaling. Allosteric modulation offers several potential advantages over 'traditional' orthosteric compounds that directly activate or inactivate a receptor such as an effect ceiling, greater receptor subtype selectivity, and reduced tolerance, dependence, or desensitization.

Our lab group has been characterizing and developing a group of PAMs based on the scaffold of the compound GAT211. GAT211 possesses both allosteric agonist and PAM properties attributable to the *R*- and *S*-enantiomers of GAT211, respectively. We have been working closely with several other groups to improve the structure-activity relationship (SAR) and pharmacokinetics of these compounds through iterative testing processes. Thus far, our group and others have found that this family of PAMs shows pre-clinical potential in rodent models of pain, glaucoma, Huntington's disease, and absence epilepsy. Meanwhile others have shown efficacy for NAMs of CB1R signaling in rodent models of hyperdopaminergia.

Considering the impressive strides made in allosteric pharmacology, many questions have arisen. Many NAMs of CB1R signaling display complex activity in vitro: increasing ligand binding but inhibiting signal transduction. CB1R PAMs and allosteric agonists may bind multiple receptor sites with unique effects on receptor conformation and signaling. Moreover, allosteric ligands participate in probe dependence and ligand bias, deepening the complexity of the field. Clear pre-clinical evidence exists to warrant the advancement of cannabinoid allosteric modulators as future medicines. We are becoming increasingly aware of how intricate cannabinoid receptor regulation is as these investigations continue.

DISCOVERY OF LEI-515: AN *IN VIVO* ACTIVE, PERIPHERALLY RESTRICTED, REVERSIBLE MAGL INHIBITOR

Ming Jiang[1], Mirjam Huizenga[1], Jonah Wirt[2], Avand Amedi[1], Richard van der Berg[3], Joerg Benz[4], Ludovic Collin[4], Hui Deng[1], Bobby Florea[3], Uwe Grether[4], Anthe Janssen[1], Laura H. Heitman[5], Tsang-Wai Lam[6], Florian Mohr[1], Anto Pavlovic[4], Iris Ruf[4], Helma Rutjes[6], Floor Stevens[1], Daan van der Vliet[1], Tom van der Wel[1], Matthias Wittwer[4], Stan van Boeckel[1], Andrea G. Hohmann[2] and Mario van der Stelt[1]

[1]Department of Molecular Physiology, Leiden University & Oncode Institute, Netherlands
[2]Department of Psychological and Brain Sciences, Program in Neuroscience, Gill Center for Biomolecular Science, Indiana University, Bloomington, IN, United States
[3]Department of Bio-organic Synthesis, Leiden University, Netherlands
[4]Roche Innovation Center Basel, F. Hoffman-La Roche Ltd., Basel, Switzerland
[5]Division of Drug Discovery and Safety, Leiden University & Oncode Institute, Netherlands
[6]Pivot Park Screening Centre, Oss, Netherlands

Introduction: Monoacylglycerol lipase (MAGL) is the principle enzyme responsible for hydrolysis of the endocannabinoid 2-arachidonoylglycerol (2-AG). MAGL inhibition provides several potential therapeutic opportunities, including anti-nociceptive, anti-inflammatory and anti-cancer activity. ABX-1413, a covalent, irreversible MAGL inhibitor, is tested in phase 2 clinical trials for neurological disorders. Our aim was to develop a new class of reversible MAGL inhibitors with reduced potential for CNS-side effects and off-target toxicity.

Methods: A library of 233.820 compounds was screened at the Pivot Park Screening Center. Several confirmed hits were identified and a medicinal chemistry program guided by activity-based protein profiling was started. The hits were optimized on potency, selectivity and metabolic stability. Co-crystallization studies elucidated the binding mode of the lead compound. Pharmacokinetic experiments were performed to determine the oral bioavailability and target modulation. A mouse model of chemotherapy-induced neuropathy was used to assess *in vivo* efficacy.

Results: The high throughput screen yielded 7 confirmed hits. Over 100 analogues of the most promising hit were designed, synthesized and evaluated in a natural substrate assay and activity-based protein profiling. This resulted in the identification of LEI-515, which has subnanomolar inhibitory potency, high selectivity and good metabolic stability. LEI-515 is a reversible inhibitor that forms a hemiketal with catalytic Ser122, stabilized by hydrogen bonds with Ala53 and Met123. LEI-515 is > 100-fold selective over a panel of 44 ion channels, receptors and enzymes, including the cannabinoid CB1 and CB2 receptor, hERG and cyclooxygenases. Targeted lipidomics revealed that LEI-515 increased cellular 2-AG levels in a concentration and time-dependent manner. Pharmacokinetic studies indicated that LEI-515 has excellent oral bioavailability, but does not penetrate the brain. It dose-dependently increased 2-AG levels in peripheral tissue, but not in brain. LEI-515 was able to suppress dose-dependently paclitaxel-induced mechanical allodynia in mice after i.p. and oral administration. Moreover, antinociceptive efficacy of LEI-515 was preserved following chronic dosing.

Conclusion: LEI-515 is a peripherally restricted, reversible MAGL inhibitor with oral bioavailability that exhibits anti-nociceptive properties in mice.

DEVELOPMENT OF NOVEL REVERSIBLE MONOACYLGLYCEROL LIPASE PET AND FLUORESCENT PROBES

Charlie Bell[1], Jörg Benz[1], Ludovic Collin[1], Martin R. Edelmann[1], Thais Gazzi[2], Maude Giroud[1], Luca Gobbi[1], Monica Guberman[2], Yingfang He[3], Dominik Heer[1], Axel Hentsch[2], Manuel Hilbert[1], Michael Honer[1], Benoit Hornsperger[1], Sylwia Huber[1], Melanie N. Hug[1], Claudia Keller[3], Carsten Kroll[1], Bernd Kuhn[1], Marius Lutz[1], Rainer E. Martin[1], Carla Meier[3], Yelena Mostinski[2], Adrienne Müller Herde[3], Fionn O'Hara[1], Annemarieke Postmus[4], Hans Richter[1], Martin Ritter[1], Didier Rombach[1], Floor Steven[4], Marco F. Taddio[3], Haiyan Wang[1], Matthias Wittwer[1], Stefanie D. Krämer[3], Linjing Mu[3], Marc Nazare[2], Roger Schibli[3], Mario van der Stelt[4] and Uwe Grether[1]

[1]Roche Pharma Research & Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., 4070 Basel (Switzerland); [2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie FMP, Campus Berlin-Buch, 13125 Berlin (Germany); [3]Department of Nuclear Medicine, University Hospital Zurich, CH-8091 Zurich (Switzerland); [4]Department of Molecular Physiology, Leiden Institute of Chemistry, Leiden University, 2333 CC, Leiden (The Netherlands)

Introduction: The serine hydrolase monoacylglycerol lipase (MAGL) is the key regulator of 2-arachidonoylglycerol (2-AG) brain concentrations. Its inhibition increases 2-AG while concomitantly reducing arachidonic acid (AA) and proinflammatory eicosanoids levels in the central nervous system. Thereby neuroinflammation is reduced and hence MAGL inhibition holds great therapeutic potential for treating neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis (MS) or Amyotrophic Lateral Sclerosis (ALS) for which chronic neuroinflammatory processes are characteristic features.

Methods: In this study, we report the discovery of morpholine-3-ones as a novel series of highly potent and selective reversible MAGL inhibitors.

Results: A focused Roche library screen provided highly attractive benzoxazinone-derived starting points which were successfully developed into a lead series. Due to the high relevance of chemical probes for supporting drug discovery programs throughout the whole value chain, e.g. by enabling the set-up of cellular assays as well as the generation of tools for studying target occupancy in humans, the morpholine-3-one scaffold was also exploited for the generation of fluorescently and radioisotope-labelled MAGL ligands. Structure-guided optimization work toward the generation of these subnanomolar MAGL probes which can be applied across species will be reported. Key absorption, distribution, metabolism, and excretion (ADME) properties such as lipophilicity, protein binding, and rodent pharmacokinetic profiles relevant for the individual probe types will be highlighted. Successful utilizations such as the development of a cellular assay based on a fluorescently labelled ligand will be shown. Furthermore, novel ^{3}H and ^{11}C labelled MAGL ligands and respective applications such as autoradiography and *in vivo* positron emission tomography (PET) studies will be disclosed.

Conclusions: Novel reversible MAGL probes have been developed which are promising tools to deepen the understanding of target biology and to support clinical studies.

CHARACTERIZATION OF NOVEL CANNABINOID RECEPTOR AGONISTS FOR THE TREATMENT OF KIDNEY DISEASES

Partha Mukhopadhyay[1], Janos Paloczi[1], Csaba Matyas[1], Eszter Trojnar[1], Resat Cinar[1], Szabolcs Dvoracsko[1], Yuri Persidsky[2], Laura H. Heitman[3], Mario van der Stelt[3], Jürgen Fingerle[4], Sabine Grüner[4], Jürgen Funk[4], Christian Apfel[4], Matthias Wittwer[4], Pawel Dzygiel[4], Stefanie Bendels[4], Christoph Ullmer[4], Wolfgang Guba[4], Uwe Grether[4] and Pal Pacher[1]

[1]National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, USA
[2]Department of Pathology, Temple University, PA, USA
[3]Department of Molecular Physiology, Leiden University & Oncode Institute, Netherlands
[4]Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland

Introduction: Previously the most widely used cannabinoid 2 receptor (CB2R) agonists were independently tested in multiple academic and industry laboratories and were found to have numerous of off-targets. Except for a few most were not advocated for *in vivo* use (Soethoudt et al., 2017, Nat. Commun.). The consortium study concluded that HU910 was the best CB2R agonist recommended for *in vivo* use based on its specificity, selectivity, and pharmacokinetics. We aimed to develop novel CB2R agonists with superior off-target profile, selectivity, and improved pharmacokinetics *in vivo*.

Methods: We describe two novel structurally distinct CB2R agonists RO6871304 ((3*S*)-1-{5-*tert*-butyl-3-[(1-cyclopropyl-1*H*-tetrazol-5-yl)methyl]-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-7-yl}pyrrolidin-3-ol) and RO6839828 (*N*-[(1*S*)-2-Amino-1-(cyclopropylmethyl)-2-oxoethyl]-6-(cyclopropylmethoxy)-5-(3,3-difluoro-1-azetidinyl)-2-pyrazinecarboxamide). These ligands were characterized in detail on human and mouse CB1/CB2R binding and signaling in 3 independent labs. All ligands were also tested against 50 representative off-targets, toxicology *in vitro* and detailed *in vivo* pharmacokinetics following oral, intraperitoneal, and intravenous administration. For target validation the effects of HU910, RO6871304, and RO6839828 were evaluated in acute and chronic models of kidney inflammation and fibrosis in mice.

Results: We demonstrate that RO6871304, and RO6839828 are potent, selective, and specific CB2R agonists with superior off-target profile and pharmacokinetics compared to previously published CB2R agonists. In contrast to previous studies based on the use of nonspecific CB2R antibodies, utilizing multiple approaches (RNA sequencing, RT-PCR, digital droplet PCR and RNA scope) we demonstrate negligible CB2R expression in human, mouse kidneys and isolated kidney proximal tubular cells. We show that during kidney injury induced by ureter occlusion there is massive inflammatory cell infiltration, which is positively correlating with fibrosis and CB2R expression. There is also increase in CB2R expression in activated fibroblasts. In contrast, inflammation and fibrosis is negatively correlating with tubular markers, because of the destruction of the kidney parenchyma. HU910, RO6871304, and RO6839828 dose-dependently improved kidney fibrosis by attenuating influx of inflammatory cells and fibroblast activation.

Conclusions: RO6871304, and RO6839828 are selective and specific CB2R agonists with excellent oral bioavailability. CB2R agonists may represent promising treatment in kidney inflammation and fibrosis.

DISCOVERY AND PRECLINICAL EVALUATION OF A NOVEL INHIBITOR OF FABP5, ART26.12, EFFECTIVE IN CHEMOTHERAPY-INDUCED PAIN

SE O'Sullivan[1], A Pereira[2], P Duffy[3], L Ruston[4], M Kaczocha[5], I Ojima[5] and A Yates[1]

[1]Artelo Biosciences, Solana Beach, USA, [2]Transpharmation Ltd., London, UK, [3]Apconix Ltd., UK, [4]Seda Pharma Development Services Ltd, UK, [5]Stony Brook University, New York, USA.

Stony Brook University (SBU) developed a series of inhibitors of fatty acid binding protein 5 (FABP5) effective in pain[1,2], cancer[3] and inflammatory models[4], which were licensed by Artelo Biosciences (ART). The analgesic effects of these compounds involve reduced degradation of endocannabinoids (FABP5 delivers endocannabinoids to fatty acid amide hydrolase (FAAH) for metabolism) and can inhibited by antagonists of CB_1, TRPV1 and PPARα[1,2]. The SBU/ART partnership undertook a medicinal chemistry optimisation programme to develop a more potent and selective lead compound, from which ART2612 was identified, with a greater than ten-fold selectivity over FABP3 and FABP7.

ART26.12 showed selectivity against a broad panel of enzymes and receptors. ART26.12 showed no in vitro toxicological effects, and no clinical signs, gross pathology or histopathology up to 300 mg/kg/day for 5 days (No-Observable-Effect-Level, NOEL) in rodents in vivo. ART26.12 displayed drug-like properties, and dose-dependent oral exposure in rodent pharmacokinetic studies with an early T_{max} (~1 hr) and drug exposure for 5-6 hours from an oral solution.

Seven days oral dosing with ART26.12 resulted in dose-dependent reduction in mechanical sensitivity in an oxaliplatin (10 mg/kg i.p., single dose)-induced model of chemotherapy-induced pain (CIPN) in Sprague Dawley rats. ART26.12 treatment began 7 days after the administration of oxaliplatin. Greater efficacy was observed when ART26.12 was dosed orally at 25 mg/kg twice per day than 50 mg/kg once per day. Mean plasma levels corresponding to efficacy were in the 4.4-6.0 µM range. The effects of ART26.12 were not different to the effect of pregabalin, but without the sedating effect on animals. Cytokines analysis is ongoing.

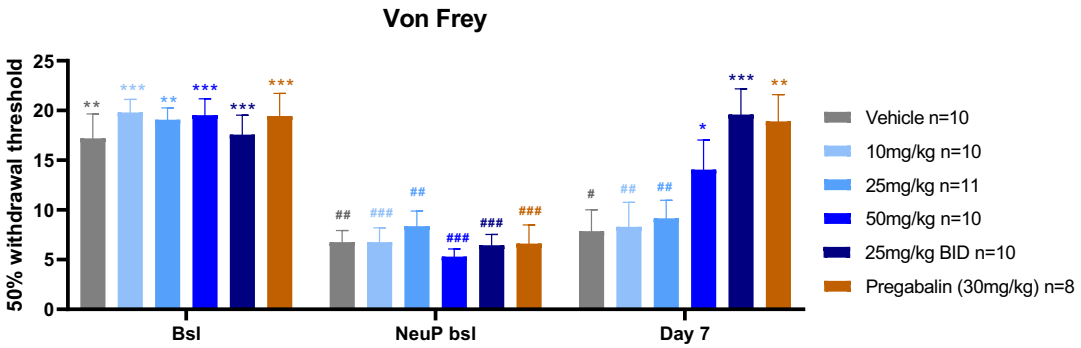


Figure: Von Frey thresholds for vehicle and test compound treated groups. #p<0.05 ##p<0.01 ###p<0.001 indicate significant decrease in von Frey threshold when compared to the respective group's baseline values. *<0.05, **<0.01, ***<0.001 indicate significant reversal of mechanical allodynia by treatment when compared to the respective group's neuropathic baseline values. Values are presented as mean ± s.e.m. (n=8-11).

In summary, we have identified a novel, selective FABP5 inhibitor with an acceptable safety profile and efficacy in chemotherapy-induced pain. These data support the further development of ART26.12.

Peng X, Studholme K, Kanjiya MP, et al. Fatty-acid-binding protein inhibition produces analgesic effects through peripheral and central mechanisms. *Molecular Pain*; 2017 Jan;13:1744806917697007.
Kaczocha M, Rebecchi MJ, Ralph BP, et al. Inhibition of fatty acid binding proteins elevates brain anandamide levels and produces analgesia. *PLoS ONE*; 2014 Apr 4;9(4):e94200.
Carbonetti G, Converso C, Clement T, et al. Docetaxel/cabazitaxel and fatty acid binding protein 5 inhibitors produce synergistic inhibition of prostate cancer growth. *Prostate* 2020; 80: 88–98.
Bogdan D, Falcone J, Kanjiya MP, et al. Fatty acid-binding protein 5 controls microsomal prostaglandin E synthase 1 (mPGES-1) induction during inflammation. *Journal of Biological Chemistry* 2018; 293: 5295–5306.

DEVELOPMENT OF A PALATABLE GELATIN THAT PROMOTES VOLUNTARY ORAL CONSUMPTION OF THC IN MICE

Anthony English[1,4], Fleur Uittenbogaard[1,4], Anna Slaven[1,4], Michael R. Bruchas[1,2,4], Benjamin B. Land[1,4] and Nephi Stella[1,3,4]

[1]Departments of Pharmacology, [2]Anesthesiology, [3]Psychiatry and Behavioral Sciences, [4]Center of Excellence in the Neurobiology of Addiction, Pain and Emotion, University of Washington, Seattle, USA

Introduction: There are a limited number of available experimental models for studying the health impact of acute, voluntary consumption of edibles containing the psychoactive phytocannabinoid, Δ^9-tetrahydrocannabinol (THC). Previous work by our group has demonstrated that mice will consume THC via gelatin, albeit at relatively low levels. Further, as the concentration of THC increases in the gelatin mixture, the relative consumption decreases. Thus, here we developed and validated a new, highly palatable gelatin formulation and assessed consumption, pharmacokinetics, cannabimimetic responses, and THC-induced startle responsivity.

Methods: We allowed C57/Bl6 mice 2 hr, ad libitum access to a novel gelatin mixture of dark chocolate 100% Ensure (E-gelatin). We measured consumption and mg/kg THC of this E-gelatin compared to our previous gelatin preparation containing 1% sucrose. Further, we measured cannabimimetic responses (analgesia, locomotor activity, body temperature), blood/brain levels of THC and its metabolites (11-OH-THC and 11-COOH-THC) at various time points during/post consumption, and startle responses compared to i.p. injection of THC.

Results: The THC-E-gelatin resulted in significantly higher THC consumption compared to our previous formulation. Consumption of 10 mg/15 ml THC-E-gelatin lead to a 4.58-fold increase in THC consumption (29.2 ± 1.8 mg of THC/kg/2h). Animals consuming the 10 mg/15 ml gelatin showed significant cannabimimetic responses that were equivalent to a 5 mg/kg i.p. dose of THC. Further, cannabimimetic responses correlated to individual mg/kg/2 hr consumption regardless of THC gelatin concentration. Analysis of plasma demonstrated elevated THC and its metabolites at all time points post-consumption. Finally, orally consumed THC affected acoustic startle responses.

Conclusions: These data suggest we have established a simple approach that promotes significant oral consumption of THC in mice. This paradigm provides the opportunity to determine how individual intake impacts THC-related behaviors, providing a useful comparison to traditional, investigator-administered (non-contingent) THC routes of administration. This model may also be broadly applicable across a wide range of animal THC behavioral research, as diverse mixtures of cannabinoid compounds may also be included in the gelatin.

Acknowledgements: Funded by NIH (DA051558 to NS and BL) and (R37DA033396 to MRB)

A NOVEL CB1 RECEPTOR NEUTRAL ANTAGONIST REVERSES NAFLD/NASH IN MICE

Lucas Laudermilk, Jennifer Lucitti, George Amato, Alan Fulp[#], Vineetha Vasukuttan, Schlosburg[*], Erica Golden[*], Rosamond Goodson[*], Herbert Seltzman, Scott Runyon and Rangan Maitra

RTI International, Research Triangle Park, NC, USA;
[#]Liberty University, Lynchburg, VA, USA;
[*]Virginia Commonwealth University, Richmond, VA, USA

Introduction: Obesity associated non-alcoholic fatty-liver disease (NAFLD) can progress to non-alcoholic steatohepatitis (NASH), which represents a significant healthcare burden without an FDA approved medication. Cannabinoid receptor 1 (CB1) antagonism is a proven therapeutic strategy for NAFLD. However, clinical development of centrally penetrant inverse agonists ceased following reports of adverse psychiatric effects. Basal activity of CB1 is necessary for emotional welfare and first-generation inverse agonists like rimonabant interfered with this process. Neutral antagonists offer a safer approach to targeting CB1 as these compounds selectively inhibit dysregulated endocannabinoid signaling reported in NAFLD/NASH but do not suppress necessary basal CB1 activity. One such novel compound was characterized and tested.

Methods: The purine RTI-348 is a neutral antagonist of CB1 with reduced brain exposure. This compound was tested in a mouse diet-induced model of NAFLD/NASH for efficacy. This model is relevant to and recapitulates many features of human NASH. Various i*n vitro*, ADMET and pharmacokinetic (PK) studies were used to characterize RTI-348. These studies indicated that RTI-348 has good drug-like properties including oral bioavailability. Mice (male, B6) maintained on a modified Amylin (AMLN) liver NASH diet for 26 weeks received vehicle or increasing doses of RTI-348. Various disease associated biomarkers were evaluated. In addition, behavioral profiling of RTI-348 was performed using intracranial electrical self-stimulation (ICSS) in rats.

Results: Administration of RTI-348 reversed diet-induced total body and liver weight gain. Treatment with RTI-348 reduced hepatic triglycerides and steatosis. Further, these animals had lower circulating levels of plasma AST, ALT, ALP, and LDH -- biomarkers associated with liver damage. Mice maintained on the NASH diet had elevated expression of genes associated with fibrosis, immune response, and extracellular matrix remodeling. Treatment with RTI-348 reversed these changes in gene expression. In ICSS studies, acute treatment with rimonabant but not RTI-348 produced dysphoria in rats, confirming a more benign behavioral profile of this neutral antagonist compound.

Conclusions: RTI-348 administered therapeutically in mice with established NAFLD/NASH reversed phenotypes and biomarkers associated with disease progression and pathogenesis. Unlike rimonabant, RTI-348 did not produce adverse behavioral effects. These encouraging studies support further preclinical development of RTI-348 for NAFLD/NASH and other important indications. (Support from NIDDK/NIH: DK100414, DK124615, DK130765)

MULTGENERATIONAL EFFECTS OF CANNABIS EXPOSURE ON EMOTIONALITY, VAPOR SELF-ADMINISTRATION, AND CORTICOSTRIATAL INPUTS IN A NOVEL RAT MODEL OF MATERNAL CANNABIS USE

Ryan J. McLaughlin[1,2], Halle V. Weimar[1], Darren E. Ginder[2], Alexandra N. Malena[1], Amanda M. Brown[1] and James H. Peters[1]

[1]Department of Integrative Physiology and Neuroscience
[2]Department of Psychology, Washington State University, Pullman, WA, USA

Introduction: Cannabis is the most commonly used illicit drug during pregnancy, yet little is known regarding its impacts on developing offspring. This is partly due to a lack of animal studies that use volitional drug delivery models in pregnant dams. To address this gap, we developed a novel model of maternal cannabis use that employs response-contingent delivery of vaporized cannabis extracts in pregnant rat dams. We used this model in the current study to determine whether prenatal cannabis exposure alters emotionality, vapor self-administration, and basal properties of corticostriatal inputs in adulthood.

Methods: Sprague Dawley rat dams self-administered cannabis (69.9% Δ^9-tetrahydrocannabinol; 150 mg/mL) or vehicle (4:1 propylene glycol:vegetable glycerol) vapor on a fixed-ratio-1 schedule twice daily in hour-long sessions throughout mating and gestation. Another group not exposed to vapor was included for emotional behavior experiments to rule out unintended effects of vapor delivery per se. Isolation-induced ultrasonic vocalizations (USVs) were measured in early life and anxiety-like behaviors were measured in the elevated plus maze and novelty-suppressed feeding tests in adulthood. Other offspring from the same litters were trained to self-administer cannabis vapor or vehicle vapor according to an escalating schedule of reinforcement over 21 days during adulthood. Remaining offspring received injections of fluorescent retrobeads (200 nl/side) into the nucleus accumbens core (NAc) during adulthood and brain slices containing the medial prefrontal cortex (mPFC) were harvested for recordings of spontaneous excitatory postsynaptic currents (sEPSCs) in NAc-projecting mPFC neurons.

Results: Cannabis self-administering dams received significantly more vapor reinforcers than vehicle-administering rats and showed greater discrimination for the cannabis-paired operandum. Importantly, cannabis-exposed pups weighed significantly less than both vehicle- and air-exposed pups on postnatal day (PND) 1, which parallels human data. Cannabis-exposed offspring emitted significantly more USVs on PND 6 but no differences in anxiety-like behavior were observed in adulthood. Female offspring self-administered cannabis vapor significantly more than vehicle vapor, irrespective of prenatal exposure condition. Conversely, male cannabis-exposed offspring showed reduced responding for both cannabis vapor and vehicle vapor in adulthood. Lastly, male (but not female) cannabis-exposed offspring had significantly higher sEPSC frequencies in NAc-projecting mPFC neurons, which suggests enhanced excitability within the corticostriatal pathway.

Conclusions: These results support the use of the cannabis vapor self-administration approach to investigate long-term effects of maternal cannabis use on developing offspring. Moreover, our data indicate transient effects of prenatal cannabis exposure on emotional behavior and long-lasting sex-specific effects on vapor self-administration and corticostriatal activity in male offspring.

ADOLESCENCE THC EXPOSURE DISRUPTS WHITE ADIPOSE TISSUE HOMEOSTASIS IN ADULTHOOD

Lin Lin[1], Kwang-Mook Jung[1], Steve Mahler[2], Faizy Ahmed[1], Erica Squire[1], Shiqi Su[1], Alexa Torrens[1], Yannick Fotio[1], Jade Ramirez[1], Hyelim Lee[1], Francesca Palese[1], Georgia Colleluori[3], Courtney Wood[4], Saverio Cinti[3], Nicholas DiPatrizio[4] and Daniele Piomelli[1]

[1]Department of Anatomy and Neurobiology, University of California-Irvine, CA, USA, 92617
[2]Department of Neurobiology and Behavior, University of California-Irvine, CA, USA, 92617
[3]Department of Experimental and Clinical Medicine, Marche Polytechnic University, Ancona, Italy, 60020
[4]Department of Biomedical Sciences, University of California-Riverside, CA, USA, 92521

Introduction: Epidemiological studies have shown that frequent cannabis use is associated with lower body-mass index (BMI), improved fasting insulin and HDL-C levels and smaller waist circumference. This finding is paradoxical, because activation of CB_1 cannabinoid receptors by Δ^9-tetrahydrocannabinol (THC), the psychoactive constituent of cannabis, stimulates food intake as well as lipogenesis in white adipose tissue (WAT). As frequent cannabis use often starts during the teenage years, we evaluated the immediate and long-term effects of daily exposure to THC during adolescence (ado-THC) on WAT, the primary site of energy storage and a major source of metabolic fuel to maintain glucose and lipid homeostasis.

Methods: Vehicle (Tween 80/saline) or THC (5 mg/kg) was administered once daily to male and female mice (C57BL/6J) from postnatal day (PND) 30-43 by intraperitoneal (IP) injection. In some experiments, male mice received AM6545 (3 mg/kg) once daily 60 min before THC. In other experiments, mice were placed on a high-fat diet (60 kcal% fat) from PND 57-130.We recorded meal parameters, body weight, motor activity, energy expenditure (metabolic chamber and measured body composition -magnetic resonance spectroscopy-), adipocyte size (hematoxylin-eosin) and microbiome composition. The WAT transcriptome was investigated by bulk RNA sequencing. Levels of THC and endocannabinoids were measured by LC-MS/MS. Fasting plasma was used for comprehensive blood panel analysis.

Results: Residual effects of ado-THC, assessed on PND44-49, included lower body-weight gain, increased energy expenditure and reduced WAT adipocyte area. No change was seen in body length or energy efficiency. The effects of ado-THC were prevented by co-administration of the peripheral CB_1 antagonist AM6545 or by cell-selective deletion on the *Cnr1* gene in adipocytes. Enduring effects of ado-THC, assessed on PND70 (when THC was no longer detectable in the body), included lower fat mass, higher lean mass, and reduced adipocyte area in WAT. Ado-THC mice were partly resistant to high-fat diet, assessed on PND57-130, as shown by reduced body-weight gain, increased energy expenditure, reduced fat mass and WAT adipocyte area, lower fasting glucose, and improved fasting cholesterol and triglyceride levels. No change was noted in adipocyte structure (electron microscopy) or gut microbiome. However, RNAseq analyses revealed substantial transcriptome-wide changes in WAT, which included ectopic expression of a large number of skeletal muscle genes.

Conclusions: Frequent exposure to low-dose THC during adolescence activates CB_1 receptors in WAT, causing profound and persistent alterations in energy metabolism as well as broad changes in gene transcription. This striking effect may contribute to the negative association between cannabis use and metabolic syndrome in humans.

Acknowledgements: This work was supported by the National Institute on Drug Abuse (NIDA) grant (P50DA044118-01 to DP).

IMPACT OF PRENATAL CANNABIS EXPOSURE ON LIMBIC PATHWAYS AND NEUROBEHAVIORAL OUTCOMES IN CHILDREN

Julia M. Evanski[1], Shreya Desai[1], Samantha L. Baglot[2], Matthew N. Hill[2] and Hilary A. Marusak[1]

[1]Department of Psychiatry and Behavioral Neurosciences, Wayne State University
[2]Hotchkiss Brain Institute, University of Calgary

Introduction: A significant portion of Δ-9-tetrahydrocannabinol (THC) and its bioactive metabolite (11-OH-THC) readily crosses and accumulates in the placenta and fetal brain and may therefore disrupt offspring neurodevelopment. Indeed, recent studies link prenatal cannabis exposure (PCE) to smaller total white and gray matter volumes, and greater neurobehavioral problems in children. Here, we tested for differences in neurobehavioral problem, white matter integrity, and gray matter volume of limbic pathways and regions among children with and without PCE using data from the Adolescent Brain Cognitive Development (ABCD) study, which is the largest pediatric neuroimaging study in the U.S.

Methods: This study reports on cross-sectional demographic, neuroimaging, and clinical data collected in 10,579 children (M±SD age=9.92±0.62 years; 48% female). PCE was measured via parent retrospective report. Regression was used to examine the effects of PCE (before and after knowledge of pregnancy) on child neurobehavioral outcomes and fractional anisotropy (FA) of limbic white matter pathways. We also explored associations among PCE, white matter FA, regional gray matter volumes of adjacent brain regions, and neurobehavioral outcomes.

Results: PCE was associated with higher withdrawn depression, social problems, and psychotic-like experiences, and lower FA in several limbic pathways. However, the only tract that remained significant after adjusting for covariates and multiple comparisons was the right fornix, a major efferent pathway from the hippocampus. In particular, relative to children without PCE, children with PCE before (but not after) knowledge of pregnancy demonstrated lower FA of the fornix. There was no difference in hippocampal volumes between children with and without PCE. However, for children *without* PCE, lower FA of the right fornix was associated with larger right hippocampal volumes and lower withdrawn depression, social problems, and psychotic-like experiences. FA of the right fornix was not associated with hippocampal volumes or behavioral outcomes among children with PCE.

Conclusions: These data add to the growing body of evidence that PCE is associated with altered neurodevelopment in offspring. Importantly, PCE — particularly early during pregnancy — was associated with lower FA of the fornix, a white matter pathway implicated in memory and emotional behavior. Microstructural alterations in the fornix were, in turn, associated with volumetric changes in the hippocampus and neurobehavioral outcomes. This suggests that structural changes in hippocampal-based circuitry may explain the previously reported elevated risk of cognitive, behavioral, and social problems following PCE. We are actively engaged in parallel controlled rodent studies of PCE to determine if comparable changes in white matter are observed in preclinical models.

Acknowledgements: Data were obtained from the Adolescent Brain Cognitive Development (ABCD) Study (abcdstudy.org). The ABCD Study is supported by the National Institutes of Health and additional federal partners. This project is also supported by K01MH119241 to HM.

DIFFERENTIAL ACTIVATION AND Δ^9-TETRAHYDROCANNABINOL EFFECT ON CB1-DEPENDENT LONG-TERM DEPRESSION IN VENTRAL TEGMENTAL AREA GABA NEURONS IN ADULT VERSUS ADOLESCENT MICE

Jeffrey G. Edwards*, Isaac Ostlund and Calvin Smith

Department of Cell Biology & Physiology, Neuroscience,
Brigham Young University, Provo, UT, USA

Introduction: The VTA mediates incentive salience and reward prediction error through dopamine (DA), that are regulated by GABA neurons. In adolescent mice, GABA cells exhibit a form of synaptic plasticity known as long-term depression (LTD) that is dependent on cannabinoid 1 (CB1) receptors preceded by metabotropic glutamate receptor 5 (mGluR5) activation to induce endocannabinoid 2-AG production. This LTD was occluded by chronic, but not acute, exposure to the marijuana cannabinoid Δ9 -tetrahydrocannabinol (THC). Our prior findings demonstrated both a novel site of action for THC to induce its rewarding effects, but also illustrated chronic THC induces long-term plasticity changes that outlast the presence of THC in the brain (J Neuroscience, Friend et al., 2017).

Methods: As long-term outcomes of THC use are more dramatic in adolescent versus adult ages, we now examined reasons for this difference in adult/adolescent male and female mice, and whether THC-induced plasticity blockade was reversible (i.e. relevant to withdrawal from cannabis use disorder). We performed whole-cell voltage-clamp electrophysiology experiments in GAD67 (GABA cell marker)-GFP knock-in mice and recorded glutamatergic inputs to VTA GABA cells.

Results: Chronic THC injections occluded CB1 agonist-mediated depression, suggesting chronic THC desensitizes or removes synaptic CB1 receptors and illustrates the mechanism of THC-induced blockade of plasticity. We note that seven days after withdrawal from chronic THC, LTD and CB1 agonist-induced depression were restored, suggesting reversibility of long-term THC-induced changes in the VTA. Interestingly, adult mice do not exhibit LTD, but continue to express functional mGluR5 and CB1. Therefore, to investigate whether plasticity in adults is quantitatively different in nature we doubled the stimulus to induce plasticity, and in this case, it was sufficient to induce LTD in adults. This again suggests a quantitative difference in CB1-dependent plasticity between adolescents and adults. Examining the reason behind this difference, we investigated CB1 functionality in adults as well as differences in AMPA and NMDA glutamate receptors, known to be involved in plasticity induction. Indeed, AMPA/NMDA ratios were increased and NMDA input/output curves decreased in adults compared to adolescents, illustrating adults express fewer NMDA receptors. CB1 receptor-induced depression was observed in adults, illustrating adults continue to express CB1 receptors at this synapse. We also performed quantitative reverse-transcription PCR (qRT-PCR) in the VTA and identified that CB1, DAGLα, and GluA1 levels increased following chronic THC exposure, illustrating the potential THC-induced molecular impact on reward and plasticity.

Conclusions: Collectively, our data demonstrate the first age-dependent GABA neuron plasticity in the brain, which could have implications for decreased THC dependence capacity in adults, as well as the mechanism behind chronic THC-induced synaptic alterations in adolescent VTA GABA cells. This data also illustrates chronic THC-induced changes in VTA plasticity are reversible.

Acknowledgements: This work was funded by the NIH (grants R15DA038092 and R15DA049260).

CANNABINOID RECEPTOR 1 (CB_1R) IN ALVEOLAR MACROPHAGES REGULATES THE DEVELOPMENT OF PULMONARY FIBROSIS

Abhishek Basu, Muhammad Arif, Kaelin Wolf, Charles N. Zawatsky and Resat Cinar

Section on Fibrotic Disorders, National Institute on Alcohol Abuse and Alcoholism (NIAAA), National Institutes of Health (NIH), Maryland, USA

Introduction: Overactivity of endocannabinoids/CB_1R system contributes to development of pulmonary fibrosis (PF) in human, as well as corresponding murine disease model. Furthermore, increased anandamide (AEA) level in BALF was negatively correlated with pulmonary function tests. Therefore, identifying cell type/types responsible for increased levels of AEA in alveolar space, and understanding cell specific roles of CB_1Rs in fibrotic lungs microenvironment may offer new approaches to therapeutically mitigate pulmonary fibrosis development and progression.

Methods: Myeloid cells and alveolar type 2 cells specific CB_1R knockout mice (My-*Cnr1*$^{-/-}$ and AT2-*Cnr1*$^{-/-}$) were generated. Pulmonary fibrosis was induced by oropharyngeal administration of bleomycin (1 U/kg b.w.) in the mice. After 14 days, the intensity of pulmonary fibrosis development was evaluated by pulmonary function tests in conditional CB_1R KO mice. Different biochemical parameters related to fibrosis and endocannabinoid system were also measured. In addition, phenotypic and genotypic characterizations of alveolar macrophages were carried out to explore the CB_1R specific pathways, altered in the alveolar macrophages leading to pulmonary fibrosis.

Results: Myeloid cells specific deletion of CB_1R (My-*Cnr1*$^{-/-}$) showed significant inhibition of pulmonary fibrosis development compared to AT2-*Cnr1*$^{-/-}$ mice and this effect was almost comparable to the global CB_1R KO (*Cnr1*$^{-/-}$) mice. The pulmonary functions and the histologic features were much improved in My-*Cnr1*$^{-/-}$ mice. The level of anandamide was significantly reduced in the My-*Cnr1*$^{-/-}$ mice, indicating suppression of CB_1R-mediated endocannabinoid system in the lungs. The phenotypic profiling of BALF (bronchoalveolar lavage fluid) cells by flow cytometry revealed that the subpopulation of CD11b and CD206 +ve alveolar macrophages expressing CB_1R is one of the major populations in the wildtype fibrotic mice and this population was significantly reduced in the My-*Cnr1*$^{-/-}$ and *Cnr1*$^{-/-}$ mice. Multiplex cytokine analysis in the BALF showed significant reduction in the levels of pro-inflammatory and M2 macrophages specific cytokines and chemokines in My-*Cnr1*$^{-/-}$ and *Cnr1*$^{-/-}$ mice, among which most important were IL-6, CXCL-10, CCL7, LIF, IL-4, GM-CSF and CCL11. The RNA-Seq data from the alveolar macrophages showed downregulation of collagen biosynthesis, cytokine signaling and NIK/NFκβ signaling pathways in the My-*Cnr1*$^{-/-}$ and *Cnr1*$^{-/-}$ mice.

Conclusions: Taken together, the comprehensive analysis indicates CB_1R expression in the alveolar macrophages played a dominant role in the initiation and progression of fibrotic cascade in the lungs and represents a major target population for anti-fibrotic therapy.

Acknowledgements: Funded by the Intramural Research Support of the NIAAA (to R. Cinar)

CROSSTALK BETWEEN ENDOCANNABINOID SYSTEM AND RESOLUTION OF INFLAMMATION IN INNATE IMMUNITY AND COGNITIVE DECLINE

Alessandro Leuti[1,2], Lucia Scipioni[2,3], Marina Fava[2,4], Niccolò Pellegrini[1], Francesca Ciaramellano[2,5], Sergio Oddi[2,5] and Mauro Maccarrone[2,3]

[1]Department of Medicine, Campus Bio-Medico University of Rome, Rome, Italy; [2]Santa Lucia Foundation, Rome, Italy; [3]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy; [4]Faculty of Biosciences and Technology for Food Agriculture and Environment, University of Teramo, Teramo, Italy; [5]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy

Introduction: Specialized pro-resolving mediators (SPMs) are a novel class of ω-3 and ω-6 derived lipids that orchestrate resolution of inflammation, thus avoiding collateral tissue damage due to aberrant immune responses. Although SPMs are considered the main executioners of this process, other bioactive lipids like endocannabinoids (eCBs) can exert pro-resolving-like actions. Recent evidence suggests that SPMs and eCBs communicate and share molecular targets to coordinate immune regulation; however, their functional cross-talk remains largely unknown in both health and disease. In line with this, recent studies have linked neuroinflammatory-related changes in Alzheimer's disease (AD) to an impaired resolution [Leuti et al., *Adv. Drugs. Deliv. Rev*. 159 (2020) 133-169]. Here, we sought to investigate whether eCB administration or FAAH inhibition could modulate metabolic enzymes and target receptors of SPMs during the resolution of neuroinflammation.

Methods: Primary human macrophages were chronically treated with anandamide (AEA) or the FAAH inhibitor URB597, then resolution-related functional readouts (*i.e*., efferocytosis and SPMs production), expression of SPM receptors and metabolic enzymes and immunophenotypical markers (CD80, CD54, CD206, CD163) were measured as compared to vehicle-treated macrophages. In addition, endogenous levels of the main SPMs and expression of SPM receptors and metabolic enzymes were measured in the hippocampus and prefrontal cortex of Tg2576 mice – a murine model of amyloidosis-induced cognitive decline – upon chronic treatment with palmitoylethanolamide (PEA) and URB597.

Results: AEA treatment of human macrophages: i) enhanced efferocytosis in a GPR18-dependent manner, ii) modulated two pivotal enzymes involved in SPM metabolism – namely 5-lipoxigenase (5-LOX) and 15-prostaglandine dehydrogenase (15-PGDH), and iii) induced the synthesis of SPMs such as RvD1, RvD2, MaR1, and LXA4 in these cells. Furthermore, URB597 and PEA treatment resulted, in mice, in a reduced expression of SPM receptors (*e.g*., GPR18, GPR32, ALX, ChemR23, LGR6, and GPR37) and inactivating enzymes (15-PGDH), as well as it enhanced the expression of 5-LOX and promoted pro-resolving-like changes in the production of pro-inflammatory lipids in the hippocampus and prefrontal cortex of WT mice, with respect to Tg2576 littermates.

Conclusions: Pharmacological modulation of the eCB tone – achieved by either direct administration of AEA/PEA or inhibition of the hydrolase FAAH – improves neuroprotection in AD by activating pro-resolving pathways.

Acknowledgements: Funded by the Italian Ministry of University and Research to S.O. (local PI) and M.M. (national PI), under PRIN grants no. 2017BTHJ4R_005 and 2017BTHJ4R_001 respectively.

STUDIES ON THE PERIPHERALLY RESTRICTED DUAL-TARGET 3,4-DIARYLPYRAZOLINES AS POTENT ANTAGONISTS OF CANNABINOID-1 (CB_1R) RECEPTOR

Malliga R. Iyer,[1]* Resat Cinar,[2] Szabolcs Dvoracsko[1,2] and George Kunos[3]

[1]Section on Medicinal Chemistry, [2]Section on Fibrotic Disorders,
[3]Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism,
National Institutes of Health, 5625 Fishers Lane, Rockville, MD 20852, USA.

Introduction: Obesity associated metabolic syndrome (OMS) underlies the pathogenesis of dyslipidemia, organ fibrosis, cardiovascular, metabolic and renal abnormalities. While many signaling pathways are implicated in such chronic conditions, the role of the endocannabinoid (EC) system in modulating the pathophysiology of OMS is well established. Blockade of cannabinoid-1 receptors (CB_1R) is a validated target for alleviating metabolic abnormalities such as adiposity, insulin resistance and related pathologies. With the market withdrawal of first-in-class, brain-penetrant CB_1R blocker rimonabant, attention has turned to harnessing the effects of CB_1R blockade by restricting the drug actions to the periphery. In this paradigm, we report the synthesis and structure-activity relationships of novel, dual-target dihydropyrazoline compounds designed to inhibit CB_1R and simultaneously activate 5-adenosine monophosphate kinase (AMPK). A series of racemic 3,4-diarylpyrazolines were synthesized and evaluated initially in CB_1R binding assays. The compounds were designed to limit brain penetrance and engage CB_1R and a secondary target to induce synergistic improvements in the targeted functions. Evaluated compounds displayed limited brain penetrance attesting to its peripheral restriction in tissue distribution studies. Component enantiomers of potent compounds were evaluated in animal models of metabolic syndrome.

Methods: Design and synthesis of a novel series of 3,4-diarylpyrazolines bearing various N-substituted pendants for peripheral restriction as well as dual-target engagement were carried out. The compounds were synthesized as racemates and evaluated in CB_1R/CB_2R binding and functional assays as well as AMPK assays. The compounds were further evaluated for inverse agonism at the CB_1R. Promising compounds were then subjected to chiral HPLC to yield pure enantiomers which were evaluated for peripheral restriction using tissue distribution studies.

Results: Several compounds showed nanomolar potencies on CB_1R and behaved as inverse agonists with acceptable selectivity over CB_2R along with impaired CNS access. Some of the compounds activated AMPK at low micromolar concentrations.

Conclusion: A new series of peripherally restricted dual-target CB_1R agents were synthesized and pharmacologically evaluated. Structure-Activity Relationship (SAR) studies along with studies showing their role in ameliorating conditions related to metabolic dysfunction will be presented.

Acknowledgements: The Intramural Program of National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health is acknowledged for funding support.

12/15 LIPOXYGENASE IS A KEY REGULATOR OF THE RESOLUTION OF INFLAMMATION IN WOUND HEALING

Sam R C Johnson[1] and James J Burston[1,2]

[1] C21 north Wales medical program, School of medicine, Cardiff University, Wales, U.K
[2] School of Health and Medical Sciences, Bangor University, Bangor, Wales, U.K.

Introduction: 12/15-lipoxygenase (12/15-LOX) is the murine ortholog of the human *15-LOX1*. This enzyme generates multiple free and esterified lipid mediators, which have been shown to have either anti-inflammatory or pro-inflammatory actions.

Methods: In this study, we characterised the role of 12/15-LOX in the co-ordination of wound healing in a mouse model, by inducing full thickness wounds in C57BL/6 wildtype (WT) and 12/15-LOX knockout (KO) mice, followed by ex-vivo analysis of wounds using a combination of immunohistochemistry, LC-MS/MS, RNA-SEQ, and zymography techniques.

Results 1: We found that wounding results in infiltration of 12/15-LOX positive macrophages into the wound bed, which was coupled to a significant increase in multiple lipids generated by 12/15-LOX such as 15-HEPE and 13-HDOHE in Day 1 in wildtype (WT) wounds compared to non-wounded controls: (non-wounded controls 27 ± 4.8 pg/mg vs. WT day 1 post wounding 193 ± 24.1 pg/mg, $p<0.0001$). Wounded 12/15-LOX deficient mice showed a reduction in a variety of lipids relative to wildtype controls and a more pronounced inflammatory phenotype. Specifically, KO wounds showed increased IFN-γ expression, decreased M2 macrophage polarisation ($p=0.03$) and high levels of neutrophil extracellular traps (NETs). In addition to an augmented inflammatory response, KO wounds showed alteration in key regulators of wound proliferation, such as greater TGF-β expression, fibroblast proliferation (WTD7 105 ± 2.7 vs. KOD7 13 ± 5.1 mean grey intensity, $p=0.0006$), coupled with an increase in Ki-67 staining, increased keratinocyte seeding into the wound as well as greater epithelial to mesenchymal transition (EMT).

Results 2: Analysis of RNA-seq data showed that KO wounds (relative to WT wounds at the same timepoint), show a decrease in anti-inflammatory signalling through PPAR gamma and LXR/RXR receptors, with subsequent modulation of 20+ key inflammatory and pro-fibrotic genes such as *Il6*, *Il1 Cxcl2*, *Osm*, *Cd14*, *NLrp3*, *Ptges2* and *Nfkb* inhibitors, which correlated with altered macrophage phenotypes, reduction in MMP activity and an increase in collagen deposition. Furthermore RNA-seq analysis shows modulation of other anti-inflammatory signalling systems, specifically CB2 ligand and receptor expression, which may contribute to a compensatory mechanisms, when PPAR gamma and LXR/RXR signalling is reduced. Lipid profile differences between KO and WT mice lead to the identification of 12 key anti-inflammatory lipids, that were elevated due to wounding in wildtype mice, that were either absent or significantly lower in KO wounds. Addition of these lipids to KO wounds resulted in significant elevation of MMP2 and MMP-9 activity, whilst wound histology reveals that collagen deposition in response to lipid treatment was normalised in the knockout wounds to a level seen in wildtype wounds.

Conclusion: In summary, we demonstrate that 12/15-LOX is a key regulatory enzyme in the temporal orchestration of wound healing by modulation of inflammation, specifically by control of the resolution of the inflammatory phase of wounding, and in the absence of this enzyme we see a fibrotic phenotype, thus 12/15-LOX would be expected to modulate both chronic wounds in addition to fibrotic conditions such as skin scarring.

Acknowledgements: Funded by MRC, grant number: MR/M011445/1.

BOTANICALLY-DERIVED HIGHLY PURIFIED CANNABIDIOL AND Δ⁹-TETRAHYDROCANNABINOL, AND THEIR 1:1 COMBINATION, MODULATE TOLL-LIKE RECEPTOR 3 AND 4 SIGNALLING IN IMMUNE CELLS FROM PEOPLE WITH MULTIPLE SCLEROSIS

John-Mark Fitzpatrick[1], Becky Hackett[1], Lisa Costelloe[2], William Hind[3] and Eric J. Downer[1,*]

[1]Discipline of Physiology, Trinity College Dublin, Ireland, [2]Department of Neurology, Beaumont Hospital, Dublin, Ireland, [3]GW Research Ltd, Sovereign House, Vision Park, Histon, CB24 9BZ, United Kingdom

Introduction: The innate immune response to bacterial and viral molecules involves the production of cytokines, chemokines and type I interferons (IFNs), which is orchestrated by toll-like receptors (TLRs). TLRs, and their signalling intermediates, are associated with multiple sclerosis (MS) pathogenesis. Recent data from our laboratory reported that Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD) regulate viral and bacterial inflammatory signalling pathways controlled by TLR3 and TLR4 in macrophages[1]. The aim of this study was to assess the effects of THC and CBD, when delivered in isolation and in combination (1:1), on TLR3- and TLR4-dependent signalling in peripheral blood mononuclear cells (PBMCs) from people with MS (pwMS) and healthy controls (HCs).

Methods: The viral double stranded RNA mimetic poly(I:C), and endotoxin lipopolysaccharide (LPS), induced viral TLR3 and bacterial TLR4 signalling in PBMCs, respectively. PBMCs from pwMS ($n = 21$) and HCs ($n = 26$) were pre-exposed (45 min) to plant-derived highly purified THC (10 μM), CBD (10 μM) or their 1:1 combination (10 μM THC and 10 μM CBD) (GW Research Ltd, Cambridge, UK), prior to LPS (100 ng/ml) or poly(I:C) (10 μg/ml) exposure. Expression profiles of signalling intermediates were assessed by ELISA and qPCR. Normality testing was conducted using the Shapiro-Wilk test, and data analysed via student's *t*-test, Mann-Whitney U test, ANOVA or Kruskal-Wallis test as appropriate. When analysis indicated significance ($p<0.05$), *post hoc* Dunnett's or Dunn's multiple comparison tests were used.

Results: TLR3 stimulation promoted the protein expression of the chemokine CXCL10 and the type I IFN-β in PBMCs from both cohorts. THC and CBD, delivered in 1:1 combination, attenuated TLR3-induced CXCL10 and IFN-β protein expression in PBMCs from pwMS and HCs, an effect that was not seen consistently when THC and CBD were delivered alone. In terms of LPS, TLR4 activation promoted TNF-α expression in PBMCs from both cohorts, and interestingly CBD, when delivered alone and in combination with THC (in 1:1 combination), exacerbated TLR4-induced TNF-α expression in PBMCs from pwMS and HCs. THC and CBD displayed no evidence of toxicity in primary PBMCs. No significant alteration in the relative expression of *TLR3* and *TLR4* mRNA, or components of the endocannabinoid system including the cannabinoid receptor CB_1 (encoded by *CNR1* gene) and CB_2 (encoded by *CNR2* gene), and endocannabinoid metabolising enzymes, fatty acid amide hydrolase (*FAAH*) and monoacylglycerol lipase (*MGLL*), were observed in PBMCs from pwMS versus HCs.

Conclusions: Given their role in inflammation, TLRs are clinical targets, and data herein identify CBD and THC as TLR3- and TLR4-modulating drugs in primary immune cells *in vitro*. This offers insight on the cellular target(s) of phytocannabinoids in targeting inflammation in the context of MS.

Acknowledgements: Supported by the Irish Research Council (EPSPG/2015/131) and GW Research Ltd.

1. Fitzpatrick, J.M., et al. J Neuroimmunol, 2020. 343: p. 577217.

CANNABIDIOL AMELIORATES CHRONIC HYPERALGESIA IN A MOUSE MODEL OF SICKLE CELL DISEASE

Hemanth M. Cherukury*, Donovan A. Argueta, Natalie Garcia, Raghda Fouda, Stacy Kiven, Jianxun Lei, Varun Sagi, Graham J. Velasco and Kalpna Gupta

Division of Hematology/Oncology, Department of Medicine,
University of California Irvine, Irvine, CA

Introduction: Sickle cell disease (SCD), the most common inherited disease, is associated with unique recurrent and unpredictable episodes of acute pain as well as long-term chronic pain. We observed that vaporized cannabis with 1:1 ratio of tetrahydrocannabinol (THC) and cannabidiol (CBD) reduces pain (though not statistically significant) in subjects with SCD (*Abrams et al., JAMA Netw Open 2020*). CBD has been shown to reduce mast cell activation, inflammation, and oxidative stress, all of which underlie the pathobiology of pain in SCD. Therefore, we examined if CBD attenuates hyperalgesia in a humanized sickle mouse model.

Methods: HbSS-BERK sickle mice were treated intraperitoneally (i.p.) with CBD at 20 or 50 mg/kg/day or vehicle and examined for mechanical and cold hyperalgesia, motor coordination, and cold avoidance. To analyze the effects of CBD on inflammation and oxidative stress, cytokine array for inflammation, tryptase, substance P (SP), and malondialdehyde (MDA) were measured in skin secretagogue, and serum amyloid P (SAP) in plasma. Skin sections (6 μm thickness) were stained with toluidine blue for mast cell degranulation. Behaviors were analyzed by two-way ANOVA with Bonferroni's post-hoc correction and biochemical tests were analyzed by unpaired t-tests ($P \leq 0.05$ considered significant).

Results: Following a single dose of CBD (20-50 mg/kg/day i.p.), mechanical and cold hyperalgesia were significantly reduced compared to baseline and vehicle for 8 hours in 3.5 mo male and female HbSS-BERK sickle mice. The analgesic effect of CBD was sustained for 9 days after daily treatment without causing tolerance. Older 6 mo females over 9-day treatment also showed analgesic effect of CBD was sustained. Males treated with CBD demonstrated reduced cold avoidance compared to vehicle after 9 days of treatment (50 mg/kg/day), while we observed no adverse effects on motor coordination in males or females. We observed a significant reduction in the number of mast cells ($P<0.05$) and degranulating mast cells ($P<0.01$) in the skin of CBD treated mice (20 mg/kg/day) compared to vehicle, which was supported by a reduction in tryptase in the skin secretagogue ($P< 0.05$ Vs. vehicle). Following CBD treatment, markers of inflammation were significantly reduced, including plasma SAP ($P<0.05$), and SP ($P=0.05$) and cytokines IL-3 ($P<0.01$), TNFα ($P<0.05$), MIP-1α ($P<0.001$), and RANTES ($P<0.01$) in the skin secretagogue compared to vehicle. Oxidative stress by measure of MDA was also reduced in the skin secretagogue following CBD treatment ($P<0.05$) compared to vehicle.

Conclusions: Our observations suggest that CBD ameliorates chronic hyperalgesia by reducing inflammation and oxidative stress and by inhibiting mast cell activation in a BERK sickle mouse model. CBD may have a disease modifying effect on SCD as seen from global reduction in inflammation and the sustained analgesic effect in older females following the conclusion of the treatment course. The effects of CBD on SCD pathobiology thus warrants further analysis, including on acute pain. Epidiolex is a readily available FDA-approved CBD extract formulation for the treatment of the Lennox-Gastout and Dravet Syndrome forms of Epilepsy, which may facilitate clinical trials to assess the efficacy of CBD for the treatment of pain in SCD.

Acknowledgments: Funded by NIH R01s HL147562 and CA263806

PALMITOYLETHANOLAMIDE ATTENUATES NEURAL INJURY AND PAIN VIA NOGO-A PATHWAY IN SICKLE CELL DISEASE

Donovan A Argueta, Bryant Avalos, Stacy Kiven, Francisco Aguirre, Reina Lomeli, Yannick Fotio, Daniele Piomelli, Nicholas V DiPatrizio and Kalpna Gupta

Division of Hematology/Oncology, Department of Medicine, University of California Irvine, Irvine, CA

Introduction: Sickle cell disease (SCD) is characterized by chronic pain and recurrent and unpredictable episodes of severe acute pain from vasoocclusive crises (VOC). Sickle pain has both neuropathic and inflammatory features but mechanisms underlying neural injury remain unknown in SCD. From transcriptomic analysis, we found that the protective and nerve repair genes are compromised in the dorsal root ganglion of humanized sickle mice (*Paul et al., Sci Data 2016*), thus allowing for ongoing pain due to neural injury. We therefore examined the mechanisms leading to neural injury and strategies to ameliorate nerve damage. We investigated if neurite outgrowth inhibitor (NOGO-A/reticulon-4) and its receptor NGR1, which contribute to neuronal damage, are activated in a sickle microenvironment and whether inhibition with palmitoylethanolamide (PEA) leads to amelioration of hyperalgesia in humanized BERK sickle mice that recapitulate the clinical characteristics of sickle pain.

Methods: Pheochromocytoma (PC12) cells were cultured in complete media or in a simulated sickle microenvironment with hemin (40 μM) and TNFα (1 ng/ml) (H+T). PC12 cells were treated with PEA (30 μM), NOGO-A inhibitor NEP(1-40) (2 μM), or siRNA targeting NOGO-A receptor NGR1. Neurite outgrowth and Rho Kinase (ROCK) activity were measured. HbSS-BERK sickle mice, expressing >99% human sickle hemoglobin were treated with PEA and ARN19702 a known inhibitor of N-acylethanolamine acid amidase (NAAA), a major degradative enzyme for PEA, leading to increased endogenous PEA. Acute hyperalgesia was evoked by hypoxia reoxygenation (HR; 3 h @ 8% O_2, 92% N_2, followed by 1 h @ normoxia). Data analysis: 2-way ANOVA and post hoc Tukey's test ($P \leq 0.05$ considered significant)

Results: H+T elevated ROCK activity in PC12 cells compared to vehicle (veh) ($P<0.05$). PEA and NEP(1-40) attenuated H+T-induced ROCK activity; co-treatment had no additive effect, indicating a common pathway. As well, siRNA (10 nM) knockdown of NGR1 reversed H+T-induced ROCK activity, which was equally effective with PEA co-treatment. Functionally, treatment with PEA or NEP(1-40) enhanced neurite outgrowth in H+T-treated PC-12 cells ($P<0.001$). We observed increased NOGO-A and NGR1 expression in the dorsal root ganglia ($P<0.05$), as well as increased NOGO-A and ROCK activity in spinal cords of sickle mice compared to control mice (expressing normal human hemoglobin A) ($P<0.05$). NEP(1-40) (5 mg/Kg) reduced mechanical and cold hyperalgesia in sickle mice compared to baseline (BL, before treatment) and veh ($P<0.05$). Additionally, 3-day PEA treatment (i.p. 20 mg/kg/d) reduced spinal NOGO-A expression and ROCK activity in sickle mice ($P<0.05$). Mass spectrometry revealed reduced spinal PEA in female ($P<0.05$) and male ($P<0.001$) sickle mice compared to age/sex-matched control mice. PEA (i.p. 20 mg/Kg/d) reduced cold avoidance over 3-day treatment period, compared to BL or veh at 1 h, 24 h, and 72 h ($P<0.05$). The analgesic effect of PEA was maintained for 9 days of treatment without developing tolerance. We next enhanced endogenous PEA by inhibiting its degradative enzyme, NAAA, with ARN19702 (i.p. 3, 10, & 30 mg/Kg/d), which reduced mechanical and cold hyperalgesia over 72 hours in a dose-dependent manner ($P<0.05$). Since hypoxia and ischemia reperfusion injury contribute to acute VOC pain, we incited HR to simulate acute VOC pain. Five-day pretreatment with PEA (i.p. 20 mg/Kg/d) before HR prevented mechanical and cold hyperalgesia following HR in sickle mice. Moreover, treatment with PEA after HR incitement significantly reduced hyperalgesia for 24 h after HR compared to pre-HR and veh-treated sickle mice ($P<0.001$).

Conclusions: We demonstrate that NOGO-A/NGR1 pathway activation may underlie nerve injury and inhibition of this pathway with a NGR1 antagonist or PEA prevents neural injury in a sickle microenvironment. Interventions targeting NOGO-A pathway may prevent/reduce neuropathic pain and that PEA has the translational potential for the treatment of chronic and acute pain in SCD.

Acknowledgement: NIH R01-HL147562; University of California, President's Postdoctoral Fellowship

DESCENDING MECHANISM BY WHICH MEDIAL PREFRONTAL CORTEX ENDOCANNABINOID SIGNALING CONTROLS NEUROPATHIC PAIN

Hai Tran, Yin Feng, Dongman Chao, Quinn H. Hogan and Bin Pan

Department of Anesthesiology, Medical College of Wisconsin,
8701 Watertown Plank Road, Milwaukee, WI 53226, USA

Introduction: Chronic pain is a major public health challenge that is inadequately addressed. While regulation of nociceptive processes in the dorsal horn by deep brain structures has long been established, the role of cortical structures in pain regulation is minimally explored. The medial prefrontal cortex (mPFC) is considered a key brain area in pain processing that receives ascending nociceptive input and processes pain's emotional and cognitive components. It also exerts important top-down control of pain sensation by innervating periaqueductal gray, the primary relay center for descending pain modulation to the spinal cord. We and others have shown critical changes in mPFC synaptic function and neuronal activity during neuropathic pain, mediated by the endocannabinoid (eCB) signaling system. Therefore, the goal of this study is to test whether mPFC eCB signaling controls neuropathic pain by the descending mechanism.

Methods: First, AM4113, an antagonist of cannabinoid receptor type 1 (CB1R), was microinjected into mPFC on the day of SNI and continued for 21 days to counteract the activated eCB signaling in the early phase of SNI. Second, the CB1R agonist, WIN-55,212-2 (WIN), was microinjected into mPFC in the chronic phase of neuropathic pain by SNI. Pain behavioral tests were performed after these treatments to assess pain-like responses over time (before injury, 7, 14, 21, 28, 35, and 43 days after injury). Third, recordings from dorsal horn wide dynamic range (WDR) neurons were performed to understand whether the analgesic effect of mPFC administration of AM4113 or WIN is by regulating descending pain modulation system.

Results: Intra-mPFC injection of CB1R antagonist AM4113 in the early phase of neuropathic pain stops development of hyperalgesia and allodynia in rats with SNI, while intra-mPFC injection of CB1R agonist, WIN, in the chronic phase of neuropathic pain acutely alleviates evoked and spontaneous pain with weightbearing asymmetry test. SNI reduced mechanical threshold to induce action potential firing of WDR neurons, which was reversed in rats with mPFC injection of AM4113 in the early phase of SNI and WIN in the chronic phase of SNI.

Conclusions: These results suggested that dynamic change of eCB-dependent mPFC activity after SNI contributes to the pain chronification. Both blockade of CB1Rs in the early phase of SNI and activation of CB1Rs in the chronic phase of SNI in mPFC can reverse the pain-like behaviors by regulating descending control of pain, which indicates that different treatment strategies to treat pain by targeting eCB signaling system should be employed in different stages of pain development.

Acknowledgements: Funded by the National Institutes of Health Grant R01NS112194.

THE EFFECTS OF CBD AND CBG OILS ON OSTEOARTHRITIS PAIN, INFLAMMATION, AND DISEASE PROGRESSION

Juliet Chung[1], Vengadeshprabhu Karuppagounder[1], Ahmed Abdeen[1], Amy Thompson[1], Andreas Bouboukas[1], William Pinamont[1], Natalie Yoshioka[1], Wesley Raup-Konsavage[2], Nicholas M. Graziane[2,3], Kent E. Vrana[2], Reyad Elbarbary[1] and Fadia Kamal[1,2]

[1]Center for Orthopaedic Research and Translational Science (CORTS), Department of Orthopaedics and Rehabilitation, [2] Department of Pharmacology, and [3]Department of Anesthesiology and Perioperative Medicine, Penn State College of Medicine, Hershey, PA, USA

Introduction: Osteoarthritis (OA) is a chronic degenerative joint disease that is characterized by pain and disability. There were around 654 million individuals (40 years and older) with knee OA in 2020 worldwide. There are no treatments to prevent or slow down cartilage degeneration in OA, with only pain management to improve physical function. However, approximately 60% of OA patients are unsatisfied with their pain treatment demonstrating the urge to discover non-addictive therapies to improve control of OA pain. We hypothesized that hemp oils with main constituents of cannabigerol (CBG) and/or cannabidiol (CBD) can reduce OA chronic pain, while simultaneously ameliorating joint inflammation and degeneration.

Methods: We induced OA in 12-week-old male C57BL/6 mice by DMM surgery. We tested the efficacy of CBD oil (50 mg/ml CBD) at 20 mg/kg/day and CBG oil (25 mg/ml CBG and 25 mg/ml CBD) at 10 mg/kg/day each, in ameliorating OA pain and disease progression by subcutaneous injection in the knee region every other day, to simulate local application by OA patients. Vehicle-treated mice and sham-operated mice were the disease and normal controls, respectively. Treatment was initiated 3 days following DMM and continued until week 8 (OA development). We performed gait analysis, Von Frey test, acetone test, and open-field test to quantify changes in gait, pain, and locomotor activity. We evaluated OA progression by quantifying: (1) articular cartilage structural changes and synovial inflammation by Saf-O-Fast green staining and histomorphometry; (2) subchondral bone changes using micro-CT imaging; and (3) immunofluorescence staining for the catabolic enzyme MMP13.

Results: Both CBD and CBG oils normalized gait, allodynia, and activity; and attenuated synovial inflammation in DMM mice. However, only CBG oil was chondroprotective, as demonstrated by reduced cartilage degeneration, chondrocyte loss, and MMP13 expression. Lastly, neither CBD oil nor CBG oil had an effect on subchondral bone. To determine whether the chondroprotective effect of CBG oil is through its CBG content, we tested the effect of pure CBG (25 mg/ml) at 10 mg/kg/day in DMM mice. Importantly, pure CBG had similar effects to CBG oil in normalizing gait and cold allodynia, and in chondroprotection, with no effect on synovial inflammation.

Conclusions: Our data demonstrate that both CBD oil and CBG oil exert analgesic effects in OA. Both CBD and CBG oils attenuate synovial inflammation in OA, probably through their CBD content, since pure CBG had no effect. Importantly, only CBG oil is chondroprotective, an effect exerted through its CBG content. Altogether, our findings support the efficacy of CBG oil as a disease modifying treatment in OA that ameliorates pain, inflammation, and cartilage degeneration.

Acknowledgements: PA options for Wellness; Penn State University Medical Marijuana Academic Clinical Research Center

ENDOGENOUS ANALGESIC SYNERGY BETWEEN MU OPIOID AND CANNABINOID CB1 RECEPTOR CONSTITUTIVE ACTIVITY

Christopher Norris*, Dana E. Selley and Bradley K. Taylor*

*University of Pittsburgh, Anaesthesiology, PA, USA
Virginia Commonwealth University, Pharmacology, Richmond, VA, USA

The endocannabinoid system is a promising target for the treatment of chronic pain. We previously reported that μ-opioid receptor constitutive activity (MOR_{CA}) in the dorsal horn of the spinal cord (DH) maintains injury-induced latent pain sensitization (LS) in a state of remission. We hypothesized that pain remission is maintained by cannabinoid CB_1 receptor constitutive activity (CB_{1CA}) as well. C57BL/6 mice were subjected to either plantar skin/muscle incision or sham surgery. Mechanical sensitivity was tested with the application of von Frey filaments. After the resolution of mechanical hypersensitivity (21 days post-surgery), CB_1 inverse agonist AM281 (10μg) or vehicle was injected intrathecally. AM281 but not vehicle reinstated mechanical hypersensitivity in the incision but not sham groups. Dose response studies (10ng-30μg) revealed that AM281 was 100X more potent in female (ED50 4.3ng) versus male (ED50 4.3μg) mice. Next, we calculated equipotent dose ratios of AM281:CTOP (a MOR-selective inverse agonist) to be 4300:1 for males, and 4:1 for females and tested multiple doses of their combinations (10pg-10μg, i.t.). We found a 1000-fold leftward shift to the dose response suggesting dramatic synergy. Notably, the AM281:CTOP combination was 100X more potent in female vs male mice. Next, we collected spinal cords at POD24-25, and conducted agonist-stimulated [^{35}S]GTPγS binding in isolated membranes. Injury did not change CB1 agonist (CP55,940)- or MOR agonist (DAMGO)-stimulated E_{max}, or EC_{50} values, indicating no change in G-protein activation. CP55,940+DAMGO (1:3) combination produced no stimulation greater than the predicted additive effects suggesting that synergy was occurring downstream of G-protein activation. We next studied glutamate (10s 300μM)-evoked Ca^{2+} mobilization in DH transverse slices, 21 days after incision or sham surgery. Pre-application of AM281 (5min 100μM) increased the duration of the glutamate-evoked Ca^{2+} response in female but not male mice. Previous literature suggested that the endocannabinoid system interacts with ryanodine-sensitive internal Ca^{2+} stores, so we combined AM281 with the ryanodine receptor blocker JTV519 (1μM), which blocked the effect if AM281. In summary, we report that: surgery establishes $CB1_{CA}$ that maintains LS in remission with 100X greater potency in females; in the setting of LS, AM281 exerts a sexually dimorphic effect on ryanodine-sensitive internal Ca^{2+}stores; and endogenous analgesic synergy between MOR_{CA} and $CB1_{CA}$ prevents chronic postoperative pain with 100X greater potency in females.

ELEVATED LEVELS OF 2- ARACHIDONOYLGLYCEROL (2-AG) IN PLASMA OF PATIENTS WITH NEUROPATHIC PAIN

Stephanie Bourke[1,2,3], Barira Islam[4,5], Maurizio Manca[4,5], John Stephenson[4,] *David P. Finn[1,2,3] and *Patrick C. McHugh[4,5]

[1]Pharmacology and Therapeutics, School of Medicine, National University of Ireland Galway, Galway, Ireland, [2]Galway Neuroscience Centre and [3]Centre for Pain Research, National University of Ireland Galway, Galway, Ireland, [4]Centre for Biomarker Research, University of Huddersfield, Huddersfield, United Kingdom, [5]Department of Pharmacy, School of Applied Sciences, University of Huddersfield, Huddersfield, United Kingdom, [6]School of Human and Health Sciences, University of Huddersfield, Huddersfield, United Kingdom.*Joint lead/corresponding authors.

Introduction: Chronic neuropathic pain affects 7-10% of the population and has a higher prevalence in women than in men. The endocannabinoid system plays a key role in pain modulation. The aim of the present study was to investigate whether chronic neuropathic pain (NP) is associated with alterations in endocannabinoid-related gene expression and circulating levels of endocannabinoids and *N*-acylethanolamines.

Methods: Male and female patients with NP (n=129) and healthy control (n=129) participants were recruited via pain clinics at Seacroft Hospital, Leeds and Galway University Hospital. Pain scores were measured using Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) and Graded Chronic Pain Scale (GCPS). Blood samples were taken and plasma endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide (AEA), and *N*-acylethanolamines, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), were quantified by HPLC-tandem mass spectrometry. The relative gene expression analysis of *DAGLA*, *FAAH* and *CNR1* was carried out on whole blood RNA using quantitative real-time polymerase chain reaction (qRT-PCR). Appropriate parametric or non-parametric statistical analyses were performed, $p<0.05$ was considered significant.

Results: Plasma 2-AG levels were higher in patients with NP compared to controls ($p<0.0001$). AEA, PEA and OEA did not differ between controls and patients with NP, and were not correlated with S-LANSS. PHQ-9 ($p<0.0001$), state anxiety ($p<0.0001$) and trait anxiety ($p<0.005$) scores were all significantly higher in patients with NP, compared with controls. GCPS and S-LANSS scores were positively correlated to PHQ-9 ($p<0.0001$), state anxiety ($p<0.0001$ and $p<0.05$, respectively) and trait anxiety ($p<0.05$) scores. GCPS and S-LANSS scores were significantly higher in female neuropathic pain patients, compared with males ($p<0.005$). There was no difference in *DAGLA*, *FAAH* or *CNR1* gene expression in patients with NP, compared with healthy control participants. Endocannabinoid-related genes did not correlate with S-LANSS or GCPS scales, except for *FAAH* which was negatively correlated with S-LANSS score ($r_s= -0.193$, $p=0.044$).

Discussion: The findings of this study were consistent with the literature in so far as NP was accompanied with higher anxiety and depression scores, compared with healthy controls. Further work is required to determine the clinical relevance and implications of the increased circulating 2-AG levels in patients with NP.

Acknowledgements: This work was supported by grants from the Pain Relief Foundation, British Pain Society Clulow award, and the Government of Ireland Postgraduate Scholarship.

MONOACYLGLYCEROL LIPASE INHIBITION: A MULTIMODAL APPROACH TO TREAT CHRONIC PAIN IN THE BERKELEY SICKLE CELL MOUSE MODEL

Kennedy Goldsborough[1], Karan Muchhala[1], Atuahene Adu-Gyamfi[1], Donald Jessup[1], Kalpna Gupta[2], Benjamin F. Cravatt[3], Micah Niphakis[4], M. Imad Damaj[1], Joyce Lloyd[5], Hamid Akbarali[1], Wally Smith[6] and Aron Lichtman[1]

[1] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA
[2] Division of Hematology/Oncology, University of California - Irvine, Irvine, CA
[3] The Scripps Research Institute, La Jolla, CA [4] Lundbeck Pharmaceuticals, San Diego, CA
[5] Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA
[6] Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA

Introduction: Sickle Cell Disease (SCD) is a major cause of morbidity and mortality worldwide, and affects more than 100,000 people in the US. Recurrent, acute pain episodes compound and result in chronic pain and physical disability for many patients. Although opioids remain the standard of care to treat SCD chronic pain, their myriad adverse side effects (e.g., constipation, respiratory depression, abuse liability, dependence) as well as the fact that SCD chronic pain requires prolonged opioid treatment that results in tolerance, severely limit their therapeutic utility. Thus, a pressing need exists to identify effective non-opioid analgesic strategies to reduce SCD chronic pain. Humanized mouse models of SCD, such as the Berkeley (BERK) model, provide useful tools to investigate disease pathophysiology and evaluate novel therapeutic targets. Dorsal Root Ganglion (DRG) neurons from rodents in neuropathic pain models show hyperexcitability. Inhibitors of the major degradative enzyme of 2-arachidonoylglycerol, monoacylglycerol lipase (MAGL), reduce nociceptive behavior in neuropathic and inflammatory preclinical models of pain through cannabinoid receptor-dependent and -independent mechanisms. However, MAGL inhibitors have yet to be tested in BERK mice. Thus, we hypothesize that MAGL inhibition will ameliorate hyperalgesic behavior and neuronal hyperexcitability in the BERK mouse model of SCD.

Methods: Male and female HbSS-BERK and HbAA-BERK mice were used as subjects for these experiments. Nociceptive behaviors were assessed using the Von Frey, Hot Plate, and Grip Strength tests. Functional deficits in innate behaviors were assessed using Nesting and Burrowing assays. Neuronal hyperexcitability was assessed using whole cell patch clamp electrophysiology of L4-S1 dorsal root ganglia (DRG) neurons. For the dose-response experiments, MJN-110 (1.25, 2.5, 5, and 10 mg/kg, i.p.) was administered one hour prior to testing. For mechanistic studies, Rimonabant and SR144528 (3 mg/kg, i.p) were injected 30 minutes prior to MJN110 or vehicle administration. Data were analyzed as Student's t-test, one- and two-way ANOVAs followed by Tukey or Sidak post-hoc analysis when appropriate ($p < 0.05$ considered significant).

Results: HbSS-BERK mice exhibit impairments in the burrowing and nest building behaviors. HbSS-BERK mice also possess extremely hyperexcitable DRG neurons. MJN-110 dose-dependently reduced mechanical, thermal and deep tissue hyperalgesia in HbSS-BERK mice. Importantly, daily injections of MJN-110 (5 mg/kg) produces sustained antinociception and ameliorates the hyperexcitability of DRG neurons of HbSS-BERK mice. Ongoing studies are being conducted to determine if MJN110 can restore deficits in innate behaviors.

Conclusion: We demonstrate functional deficits in sickle mice, which recapitulates the functional status of patients with SCD. In particular, this work makes the novel observation that dorsal root ganglia sensory neurons innervating the distal parts of BERK mice are hyperexcitable. Finally, the observation that MJN110 reduces neuronal hyperexcitability and hyperalgesic behaviors without tolerance suggests that MAGL inhibition may represent a viable strategy to reduce chronic pain in sickle cell patients.

Acknowledgements: Mouse breeding provided by the VCU Transgenic/Knockout Mouse Core P30 Grant DA033934 and UC-Irvine Grant R01HL147562. KG funded by the IGNITE KUH Training Grant.

THE IMPACT OF CANNABIS IN THE INTESTINAL IMMUNE SYSTEM TO IDENTIFY NOVEL THERAPEUTIC TARGETS AND BETTER INFORM PATIENTS

Alketbi Mariam S. Gh.[1] Sanctuary MR[2,3], Wilkinson CL[2,3], Jones AJ[2,3], Hoffenberg EJ[2,4], Hillard CJ[4], Romero, J[5] and Collins CB[1,2]

[1]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland; [2]University of Colorado School of Medicine, Department of Pediatrics, Mucosal Inflammation Program, Aurora CO, USA; [3]Children's Hospital Colorado, Aurora CO, USA; [4]Department of Pharmacology and Neuroscience Research Center, Medical College of Wisconsin, Milwaukee, WI, USA; [5]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, Madrid, Spain

Introduction:
Cannabis use is increasingly common among IBD patients despite the lack of empirical evidence supporting its use. Alarmingly, chronic cannabis use has been linked with a five-fold increased surgical risk in Crohn's patients. Our goal was to better understand the impact of cannabis in the intestinal immune system to identify novel therapeutic targets and better inform patients. We have previously demonstrated that the CB2R ligand GP1a can treat established ileitis in a murine model of inflammatory bowel disease (IBD). Questions regarding the agonism or inverse agonism of GP1a, prompted us to examine the contribution of cell-specific deletion of CB2R in our TNF-driven ileitis model.

Methods:
We focused on the contribution of CNR2 expression by CD4+ T cells because of their central role in IBD pathogenesis. We found that CD4-specific CB2R deficiency significantly attenuated intestinal inflammation and decreased central memory T cell frequency in the ileum. Using single-cell RNA sequencing on intestinal CD4+ T cells, we identified a unique metabolic gene signature on CNR2 expressing cells, which we validated with conventional PCR on flow-sorted CB2R-GFP+ cells. Fluorescent glucose analogue was also used to demonstrate the effect on CB2R agonist JWH133 in isolated T cells in vitro. Competitive homing assay was also used where, CB2R-deficient CD4+ T cells were labelled via fluorescently.

Results:
Metabolic gene signature on CNR2 was consistent with activation of the pentose phosphate pathway and suppression of T cell glycolysis, a process that may suppress T cell proliferation in the short term but ultimately promotes the persistence of long-lived memory T cells that contribute to the chronic nature of the disease. Moreover, we demonstrated a direct impact on glucose handling, with CB2R agonist JWH133 increasing uptake of a fluorescent glucose analogue and inverse agonism with GP1a suppressing it in isolated T cells in vitro, similar to what has been reported in microglia. We then recapitulated these findings in vivo using a competitive homing assay in which fluorescently labelled CB2R-deficient CD4+ T cells displayed reduced homing to the ileum relative to littermate controls.

Conclusion:
We conclude that, chronic activation of the CB2R on CD4+ T cells may select for long-lived memory T cells with enhanced invasive potential resulting in the exacerbation of IBD.

Acknowledgements: Funding by Crohn's & Colitis Foundation Senior Research Award.

CANNABINOID CB1 AND GHRELIN GHSR1A RECEPTORS ON SENSORY AFFERENTS OF THE GASTROINTESTINAL TRACT CONTRIBUTE TO THE CONTROL OF VOLUNTARY ALCOHOL DRINKING IN MICE

G. Godlewski, A. Herrerias, A. Oliverio, R. Cinar, J. Liu, M.R. Iyer and G. Kunos

Laboratory of Physiologic Studies,
National Institute on Alcohol Abuse and Alcoholism,
National Institutes of Health,
Bethesda, MD, USA

The vagal gut-brain axis has been recognized as a vital regulator of the metabolic, motivational, and emotional states. These include anxiety, depression, reinforcement, food and alcohol craving, and involve the endocannabinoid system. Our previous observations indicated that the brain non-penetrant CB1 receptor (CB1R) antagonist, JD5037, suppresses alcohol preference in mice by blocking CB1R in ghrelin-producing cells and hampering the permissive role of ghrelin to drink alcohol (Cell Metab 2019; 29: 1320-1333).

Here we evaluate the possible contribution of CB1R located on sensory afferents of the gastrointestinal (GI) tract in the voluntary intake of alcohol. We found that selective deletion of CB1R from ghrelin-producing cells only partially occluded the inhibitory effect of JD5037 in alcohol drinking transgenic (Ghrl-Cre CB1$^{flox/flox}$) mice. We then created transgenic mouse lines to delete CB1R in dorsal root ganglia (Wnt-Cre CB1$^{flox/flox}$) and nodose ganglia (Phox2b-Cre CB1$^{flox/flox}$) and found the inhibitory effect of JD5037 to be occluded in the latter. Likewise, the deletion of GHSR1A in nodose ganglia in the transgenic (Phox2b-Cre Ghsr1a$^{flox/flox}$) mouse line obstructed the inhibitory effect of GHSR1A antagonist PF-52960457 in alcohol preferring mice.

RNA sequencing of nodose ganglia revealed the existence of various clusters of neurons, which differ with respect to genetic markers, function, and innervation (Cell Rep., 2019; 27: 2508-2523). We found that the deletion of CB1R from the subpopulation Trpv1 positive neurons only marginally affected alcohol drinking (Trpv1-Cre CB1$^{flox/flox}$), whereas the deletion of CB1R from advillin positive neurons (Avil-Cre/ERT2 CB1$^{flox/flox}$) abolished the modulatory effect of JD5037 on alcohol drinking.

In conclusion, CB1 receptor on sensory afferents of the GI tract may contribute to alcohol seeking behavior in mice. Ongoing studies further dissect the roles of two major subpopulations of nodose ganglion nerve terminals that project to the muscular vs. mucosal layers of the GI tract by testing the effects of CB1 ligands in Glp1r-Cre CB1$^{flox/flox}$ and GPR65-Cre CB1$^{flox/flox}$ mice, respectively, and examine the nature of interaction between CB1 and Ghsr1a on sensory afferent neurons.

CANNABINOID CB1 RECEPTOR REGULATION OF SALIVATION

Kelsey Andreis, Jenna Billingsley, Kian Naimi Shirazi, Heather Bradshaw and Alex Straiker

Department of Psychological & Brain Sciences, Indiana University

Saliva serves multiple important functions within the body that we typically take for granted, such as helping prepare food for swallowing and defense against oral pathogens. Dry mouth is a primary symptom of Sjögren's syndrome and is a side effect of many drug treatments. Cannabis users frequently report dry mouth, but the basis for this is still unknown. If the effects occur via the endogenous cannabinoid signaling system, then this may represent a novel mechanism for the regulation of salivation. We examined expression of cannabinoid CB1 receptors in submandibular salivary gland using immunohistochemistry and tested regulation of salivation by THC and cannabinoid-related ligands.

We now report that CB1 receptors are expressed in the axons of cholinergic neurons innervating the submandibular gland. No staining is seen in submandibular gland epithelial cells (acinar and ductal), or myoepithelial cells (MECs). Treatment with THC (4mg/kg, IP) or the cannabinoid receptor agonist CP55940 (0.5mg/kg) reduced salivation in both male and female mice one hour after treatment. The CB1 receptor antagonist SR141716 (4mg/kg) had no effect on salivation. The CB2-selective agonist JWH133 (4mg/kg) had no effect on salivation. Interestingly, CB1 knockout mice see a substantial rise in basal salivation over the course of 4-6 weeks in early adulthood. We also tested whether fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide, regulates salivation. We found that salivation was reduced in FAAH knockout mice as well as mice treated with the FAAH blocker URB597 (4mg/kg), in a CB1-dependent manner. FAAH protein is detected intracellularly in acinar but not ductal epithelial cells. Blocking the synthetic enzyme for anandamide NAPE-PLD greatly increased salivation in male, but not female mice.

Our results are consistent with a model wherein the endocannabinoid anandamide activates CB1 receptors on cholinergic axons innervating the submandibular gland. The resultant drop in acetylcholine release reduces salivation. THC likely acts by plugging into this system, activating CB1 receptors to reduce salivation, thus offering a mechanism underlying the dry mouth reported by cannabis users.

BLOCKADE OF CANNABINOID 1 RECEPTOR ARRESTS INSULITIS AND PRESERVES HUMAN ISLET FUNCTION IN A NOVEL *EX VIVO* MODEL OF ISLET INFLAMMATION

Isabel González Mariscal [1], Valery Gmyr [2], Francisco Javier Bermudez Silva [1, 3], Julie Kerr-Conte [2], François Pattou [2], Josephine M Egan [4] and Marisol Ruiz de Adana [1]

[1] Instituto de Investigación Biomédica de Málaga (IBIMA), Malaga, Spain [2] Université de Lille, CHU Lille, INSERM, European Genomic Institute of Diabetes (EGID), Lille, France [3] CIBERDEM, Spain, [4] National Institute of Aging, NIH, Baltimore, MD, USA

Introduction: Type 1 diabetes (T1D) is an autoimmune disease without a cure that results in progressive destruction of insulin-producing beta cells in islets of Langerhans; destruction is preceded by infiltration of immune cells in and around islets, a pathological process known as insulitis, that finally leads to failure of insulin production to maintain normoglycemia. T1D patients require insulin injections for life, which is linked to dangerous hypoglycemia and, often, is inadequate to maintain normal glycemic levels. Moreover, diabetes increases the risk of renal failure, blindness, peripheral neuropathy, and premature death. At onset, there are still some functional beta cells, and efforts to preserve the remaining beta cell pool have been attempted without long-term success. Sequencing data identified the cannabinoid type 1 receptor (CB1R), which is present in beta cells and immune cells, as the most activated regulator in the pancreas from antigen-positive donors compared to controls. Previously we have described that genetic ablation of CB1R in beta cells is sufficient to prevent insulitis in mice. Here we investigated the role of CB1R in insulitis in humans and the capacity of pharmacological intervention to prevent this pathological process by blocking CB1R.

Methods: $CD4^+$ T lymphocytes were isolated from peripheral blood mononuclear cells (PBMCs) from patients at the onset of T1D or non-diabetics (n=11-16), and RNA was extracted. *CNR1* expression was analyzed by real time PCR. Patient-derived human islets from cadaveric donors were 3D-cultured in solubilized extracellular matrix gel. Islets alone or in coculture with same donor PBMCs, were incubated with cytokines (IL-1β, TNF-α, IFN-γ) for 24-48h in the presence of vehicle or increasing concentrations of the CB1R blocker JD5037. Dead-cell protease activity and caspase 3 activity were determined. Production of hydrogen peroxide (H_2O_2) and nitric oxide (NO) in living islets was measured using MitoSOX and DAF-FM probes. Islet function measured as glucose-stimulated insulin secretion (GSIS) was determined in a perifusion system. Infiltration of immune cells into the islets was quantified by cell counting under a fluorescent microscope. Statistics: Student's t-test or one-way ANOVA (Bonferroni post-hoc test).

Results: T1D patients-derived $CD4^+$ T cells expressed 1.5-fold more *CNR1-full length* than those from control patients ($p < 0.05$), and none expressed *CNR1-b*. Cytokines induced H_2O_2 and NO production in islets, triggered cell death and islet dysfunction, leading to a 20% reduction of GSIS ($p < 0.01$). Cytokines also induce islet-directed immune cell infiltration. Treatment with 10-100 nM JD5037 prevented cytokine-induced NO but not H_2O_2 production. JD5037 did not reduce cytokine-induced caspase 3 activity, although 10 nM JD5037 prevented cytokine-mediated cytotoxicity. JD5037 not only preserved islet function in the presence of cytokines, but it also enhanced GSIS by 52% compared to control ($p < 0.001$). JD5037 10 nM significantly reduced immune cell infiltration in and around islets by 43%, and JD5037 100 nM fully abolished immune cell infiltration for up to 6 days after insult with cytokines.

Conclusions: CB1R blockade prevents insulitis in humans, preserving islet viability and function *ex vivo*. Our data describe, for the first time, human CB1R as a therapeutic target for T1D, and shed some light on the regulatory mechanisms of insulitis.

Acknowledgements: Funded by Consejería de Salud y Familias of Junta de Andalucía [PI-0318-2018] co-financed with the European Union FEDER funds, Nicolas Monardes Program [C1-0018-2019], and Sociedad Espanola de Diabetes (SED).

PARASYMPATHETIC SIGNALING CONTROLS BIOSYNTHESIS OF OREXIGENIC ENDOCANNABINOIDS IN THE SMALL-INTESTINE OF WESTERN DIET-INDUCED OBESE MICE

Courtney P. Wood and Nicholas V. DiPatrizio

Division of Biomedical Sciences, School of Medicine,
University of California, Riverside, Riverside, CA, USA

Introduction: The endocannabinoid (eCB) system is an endogenous lipid signaling system that controls food intake and energy balance. In diet-induced obesity (DIO), the eCB system becomes dysregulated in the brain and peripheral organs and contributes to overeating. For example, we reported that the eCB 2-arachidonoyl-sn-glycerol (2-AG) is elevated in the small-intestinal epithelium of mice fed a high-fat/high-sucrose western-style diet (WD) when compared to control mice fed a standard low-fat/no sucrose rodent diet (SD). Overactive eCB signaling drives hyperphagia in DIO animals by activating local cannabinoid receptor subtype-1 (CB1R) via a mechanism that includes inhibiting nutrient-induced release of satiation peptides from the gut. Moreover, efferent vagal cholinergic neurotransmission controls 2-AG production in the small intestine in rats, which drives overeating after a fast. We now tested the hypothesis that cholinergic signaling at muscarinic acetylcholine receptors (mAChRs) leads to increased biosynthesis of 2-AG in the small-intestinal epithelium, which drives hyperphagia in DIO.

Methods: Male 8-week-old C57BL/6 mice were maintained on WD for 60 days. Littermates maintained on SD served as controls. Animals received IP injections of methylhomatropine bromide (a peripherally restricted non-selective muscarinic receptor antagonist), DAU5884 (a selective m_3 mAChR antagonist), pirenzepine (a selective m_1 mAChR antagonist), or vehicle 30 minutes prior to tissue harvest. Levels of 2-AG and its precursor, 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG), in the small intestinal epithelium were quantitated by ultra-performance liquid-chromatography/tandem mass spectrometry (UPLC-MS/MS). In these mice, the ex-vivo activity of the biosynthetic enzyme for 2-AG, diacylglycerol lipase (DGL), was analyzed in the presence of methylhomatropine bromide, DAU 5884, and pirenzepine. Food intake, water intake, and ambulation were recorded with automated feeding chambers.
Results: Mice fed WD exhibited elevated levels of SAG, 2-AG, and DGL activity in the small-intestinal epithelium, which was blocked in mice pre-treated with methylhomatropine bromide, DAU 5884, and pirenzepine. Ex-vivo DGL activity was not inhibited when these drugs were directly included in the assay. Mice fed WD ate significantly more than mice fed SD in 24 hours. Pre-treatment in these mice with methylhomatropine bromide inhibited caloric intake for up to 24 hours and DAU 5884 inhibited caloric intake for two hours.

Conclusions: These results suggest that in DIO, excessive cholinergic activity at m_3 and m_1 mAChRs in the intestinal epithelium drives biosynthesis of 2-AG, which contributes to hyperphagia. Activation of these G_q-coupled mAChRs initiates the PLC-dependent generation of SAG, which is subsequently converted to 2-AG by DGL. Direct roles for the efferent vagus nerve in these processes are currently under investigation.

Acknowledgements: Research is funded by the National Institutes of Health (R01 DK119498 and L30 DK1149978) and the Tobacco-Related Disease Research Program (T29KT0232).

MEASURING CHANGES IN 2-AG SIGNALING WITH THE $GRAB_{ECB2.0}$ SENSOR: CONTROL BY ABHD6 AND ROLE IN THC-MEDIATED INHIBITION OF LOCOMOTION

Anthony English[1,4*], Simar Singh[1*], Dennis Sarroza[1], David J. Marcus[2,4], Ao Dong[5], Yulong Li[5], Larry Zweifel[3,4], Benjamin Land[1,4], Michael R. Bruchas[2,4] and Nephi Stella[1,3,4]

[1]Departments of Pharmacology, [2]Anesthesiology, [3]Psychiatry and Behavioral Sciences, [4]Center for the Neurobiology of Addiction, Pain and Emotion, University of Washington, Seattle, USA; [5]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China. *Equal contribution

Introduction: Activation of cannabinoid 1 receptors (**CB_1R**) by 2-arachidonoyl glycerol (**2-AG**), the most abundant eCB in the brain, controls multiple physiological functions, including neurotransmitter release, neuronal metabolism, and neuronal phenotype. Our understanding of changes in 2-AG levels in cell culture and in vivo model systems has been limited by the low spatiotemporal resolution of mass spectrometry, an approach that require rapid freezing of tissue and lipid extraction. While well suited to measure 2-AG tone and changes in 2-AG levels within minutes, this approach does not resolve cell 2-AG fluctuations occurring at the cellular level and within seconds triggered by changes in neuronal activity. To overcome this limitation, novel genetically encoded sensors, here $GRAB_{eCB2.0}$, are paving the way to study cell specific changes in nanomolar concentrations of 2-AG within sec.

Methods: $GRAB_{eCB2.0}$ was expressed by Neuro 2a cells in culture, treated with cannabinoid agents and neuromodulators, and changes in fluorescence measured by live cell fluorescence microscopy and 96-well fluorescence plate reader. $GRAB_{eCB2.0}$ or GCaMP6f were expressed in mouse prelimbic cortex (**PrL**) and changes in fluorescence were measured by fiber photometry in an open field after the administration of Vehicle or THC.

Results: $GRAB_{eCB2.0}$ expressed by Neuro 2a cells is reliably activated by nanomolar concentrations of 2-AG and with a potency comparable to CB_1R. By contrast, $GRAB_{eCB2.0}$ is 100-1000x less sensitive to anandamide, CP55940, THC, and SR141617 than CB_1R. We discovered that stimulation of Neuro 2a cells with bradykinin (**BK**) acting at metabotropic B_2 receptors and ATP acting at ionotropic $P2X_7$ receptors leads to differential increases in 2-AG production measured by both LC-MS and changes in $GRAB_{eCB2.0}$ fluorescence. Mechanistically, B_2 receptors increase 2-AG production via DAGL, and this response is potentiated by both increases in intracellular calcium and ABHD6 inhibition. By contrast, $P2X_7$ receptors increases 2-AG production via DAGL, but this response depends on extracellular calcium and is insensitive to ABHD6 inhibition. These results suggest that ABHD6 preferentially regulates metabotropic-dependent 2-AG signaling in Neuro 2a cells in culture.

THC, the primary intoxicating ingredient in *Cannabis*, induces behavioral changes in mice, including hypolocomotion characterized by a reduction in the frequency and duration of spontaneous ambulation events. While measuring changes in $GRAB_{eCB2.0}$ fluorescence expressed the PrL under the synapsin promotor, we discovered transient increases in 2-AG production during the initiation of ambulation. Furthermore, transient 2-AG production was paralleled by robust increases in neuronal activity measured with GCaMP6f. Remarkably, both signals were significantly larger in THC-treated mice compared to vehicle-treated mice, while episodes of ambulation were reduced.

Conclusion: Our study outlines the pharmacological profile of the $GRAB_{eCB2.0}$ sensor and leveraged this new tool to show that ABHD6 preferentially regulates metabotropic-dependent 2-AG signaling in Neuro 2a cells in culture. Furthermore, transient 2-AG signaling in the PrL of mouse brain is time locked to initiation of ambulation and this response if increased by THC treatment.

Acknowledgements: Funded by NIH (DA047626, DA051558 and NS118130 to NS) and (R37DA033396 to MRB) and the Pharmacological Sciences Training Program of the University of Washington.

ENDOCANNABINOID CONTROL OF BEHAVIORAL ENGAGEMENT VIA A NEUROPETIDERGIC PVT-NAC CIRCUIT

David J. Marcus[1,2], Emma Seth[1,2], Sabrina Hwang[1,2], Christian E. Pedersen[1,2,3], Avery Hunker[2,4], Selena Schrieber[2,4], Yulong Li[5], Benjamin B. Land[2,4], Larry S. Zweifel[2,4,6], Nephi Stella[2,4] and Michael R. Bruchas[1,2,3,4]

1. Department of Anesthesiology and Pain Medicine, University of Washington School of Medicine, Seattle, WA
2. Center for the Neurobiology of Addiction, Pain and Emotion (NAPE), University of Washington School of Medicine, Seattle, WA
3. Department of Bioengineering, University of Washington, Seattle
4. Department of Pharmacology, University of Washington, School of Medicine, Seattle, WA
5. State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing, China
6. Deparrtment of Psychiatry, University of Washington, School of Medicine, Seattle, WA

Introduction: The Nucleus Accumbens (NAc) represents an integral functional component of the mesocorticolimbic pathway, canonically known as the "reward" pathway. The NAc is highly heterogenous in the expression of neuromodulatory receptors and neuropeptides and it receives dense afferents from the Paraventricular Thalamus (PVT). These neuronal projections regulate the behavioral effects of opiate withdrawal, sucrose seeking/consumption, as well as behavioral responses to painful stimuli. Recent studies examining the PVT-NAc circuit have generated contradictory results with respect to its critical behavioral functions, this is partially driven by a lack of selective genetic and anatomical targeting within the PVT.

Methods: Here, we use molecular, optogenetic, chemogenetic, photometric, and electrophysiologal methods to identify the neuropeptide neurotensin (NTS) neuronal population as uniquely isolated to the anterior portion of the PVT.

Results: We demonstrate that PVT^{NTS} neurons send strong excitatory projections to the NAc, and that this input is biased toward Dopamine Receptor 1 (D1R) expressing medium-spiny neurons (MSNs). We also found that this afferent input bias is mediated via tonic endocannabinoid (eCB) suppression of excitatory input onto Dopamine Receptor 2 (D2R) expressing MSNs. Using fiber photometry in NTS-cre mice expressing DIO-GCaMP6s in PVT, we show that PVT NTS-NAc projections are inhibited during bouts of behavioral engagement and activated upon behavioral disengagement, regardless of stimuli valence. Both engagement in sucrose seeking/consumption and fear-induced freezing inhibit PVT-NAc^{NTS} projections. ChR2-mediated photo-stimulation of PVT-NAc^{NTS} was sufficient to potentiate behavioral disengagement. Next, we used pharmacological and CRISPR-based approaches to determine whether these manipulations depend on eCB signaling and neuropeptide transmission via NTS. Selective antagonism of CB_1R attenuates the behavioral effects of photo-stimulation and alters behavioral engagement and both the novel GRAB-NTS and GRAB-eCB sensors indicated that these two modulators are dynamically altered within this pathway during behavioral engagement.

Conclusions: Our results implicate eCBs in the modulation of a glutamatergic/NTSergic anterior PVT-NAc circuit. This mechanism provides a unique role for regulation of approach and avoidance decision making via a dual eCB- neuropeptide system offering new avenues for treatment of pathopsychological disorders including approach/avoidance, motivation, and drug abuse.

Acknowledgements: University of Washington Center for the Neurobiology of Addiction, Pain and Emotion (P30DA048736), UW Alcohol and Drug Abuse Institute, Scan Design Foundation, and (R37DA033396 to MRB) and (F32DA054709 to DJM and F31DA051124 to CP).

NAPE-PLD REGULATES ANXIETY-LIKE BEHAVIORS, UNCONDITIONED FEAR RESPONSES, AND THE REINFORCING PROPERTIES OF ORAL OXYCODONE CONSUMPTION IN MICE

Taylor Woodward[1], Sarah Stockman[1], Fezaan Kazi[1], Hasaan Kazi[1], Romario Pacheco[1], Wouter Driever[2], Mario van der Stelt[2], Ken Mackie[1,3,4], and Andrea G. Hohmann[1,3,4]

[1]Program in Neuroscience, Indiana University, Bloomington, IN;
[2]Department of Molecular Physiology, Leiden University & Oncode Institute, Netherlands
[3]Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN;
[4]Gill Center of Biomolecular Science, Indiana University, Bloomington, IN

Introduction: Explorations of the endocannabinoid (eCB) system have shown that the eCB ligand anandamide (AEA) is involved in emotional resilience as well as addiction-related behaviors. Until recently, mechanisms underlying biosynthesis of AEA *in vivo* have remained controversial. However, lipidomic analyses employing N-acyl-phosphatidylethanolamine phospholipase D knockout (NAPE-PLD KO) and wildtype mice revealed that NAPE-PLD plays a broad and crucial role in metabolism of not only anandamide (AEA) but other members of the N-acylethanolamine (NAE) family (Leishman, 2016). While NAEs play a known role in affective and reward behaviors, NAPE-PLD's contribution remains poorly explored. In the present study, we asked whether genetic deletion or pharmacological inhibition of NAPE-PLD would produce negative affective states and impact the reinforcing properties of opioids.

Methods: We examined the performance of wild type C57Bl6 (WT) and NAPE-PLD KO mice of both sexes in several assays of affective behavior, including the open field test, the light-dark box test, and the forced swim test. We measured unconditioned freezing in response to exposure to the predator odor behavior in a predator odor exposure assay. For pharmacological experiments, WT mice were challenged 2 hours prior to behavioral testing with either vehicle or the NAPE-PLD inhibitor LEI-401 (30 mg/kg, i.p.), a dose that suppressed NAPE-PLD activity and decreased brain AEA levels (Mock, 2020). A home-cage two-bottle choice assay was used to assess the reinforcing properties of oral oxycodone consumption in WT and NAPE-PLD KO mice.

Results: When compared to wild type (WT) controls, NAPE-PLD KO mice spent less time in the center during the open field test, had longer immobility time and fewer light-side entries in the light-dark box test, and a longer immobility time in the forced swim test ($p<.001$). WT mice treated with LEI-401 prior to testing behaved similarly in each test/ In the freezing assay, WT mice injected with LEI-401 prior to TMT exposure exhibited an exacerbated unconditioned freezing response. In a two-bottle choice test, male WT mice showed strong preference for oral oxycodone and exhibited drug seeking behavior during forced abstinence. Interestingly, male NAPE-PLD KO mice showed no group preference for oxycodone over regular drinking water.

Conclusion: Genetic deletion and pharmacological inhibition of NAPE-PLD exacerbated behavioral responses regarding negative affect and these mice displayed strong increases in anxiety-like behaviors, suggesting that NAPE-PLD deletion detrimentally impacts emotional regulation. Our results demonstrate that functional NAPE-PLD is required for the reinforcing properties of oral oxycodone. Our findings collectively suggest that NAPE-PLD plays a pivotal role in a variety of behaviors relating to affective disorders and opioid addiction.

Acknowledgements: This work was supported by NIH grants DA041229 and DA047858 (AGH and KM), DA042584 and Addiction Grand Challenge Grant (AGH). TW is supported by a NIDA T32 grant DA024628

A NOVEL FABP5 INHIBITOR REGULATES ANXIETY AND FEAR EXPRESSION WHEN ADMINISTERED LOCALLY TO THE BASOLATERAL AMYGDALA

Matthew J. Jones, Taygun C. Uzuneser, Hanna J. Szkudlarek and Steven R. Laviolette

Department of Neuroscience, University of Western Ontario, ON, Canada

Introduction: The endocannabinoid system exhibits large regulatory control over anxiety and fear reactions. Anandamide, a main endocannabinoid ligand, has limited ability for diffusion resulting in reliance on Fatty Acid Binding Proteins (FABPs) for transport back into the synapse to terminate action (Haj-Dahmane et al., 2018). Here, we used a novel pharmacological inhibitor of FABP5 (**SBF I-103**) to study how FABP5 inhibition within the basolateral amygdala (BLA) impacts anxiety and fear-related behavioural processing and neuronal activity states. We hypothesized that intra-BLA FABP5 inhibition would induce reductions in both anxiety and fear expression.

Methods: We investigated the effects of intra-BLA FABP5 inhibition at behavioural and single-neuron levels in adult male Sprague Dawley rats. For behaviour, we surgically implanted guide cannulae targeting the BLA region to enable local acute administration of SBF I-103 (low-dose (0.5 μg/0.5 μL) and high-dose (5 μg/0.5 μL)) prior to examining anxiety and fear expression. We also performed *in vivo* electrophysiology following intra-BLA FABP5 inhibition to monitor neuronal effects in the prefrontal cortex (PFC) and ventral hippocampus (vHipp). With SBF I-103, we co-administered receptor antagonists (targeting CB1r, CB2r, or GPR55) or an acute anandamide-synthesis inhibitor (NAPE-PLD inhibitor). Data were analyzed by one-way and two-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$ considered significant).

Results: We observed significant reductions in anxiety behaviour when animals received the high dose of SBF I-103. Furthermore, we saw significant reductions in fear expression wherein animals that received the high dose of SBF I-103 prior to first fear recall demonstrated faster fear extinction to a conditioned cue, which lasted through all future test days without any further infusions. We also noted strong modulation of pyramidal neurons in the PFC and vHipp, as well as increases in gamma and beta oscillatory patterns. Remarkably, these effects were reversed with co-administration of either a CB1r or CB2r antagonist. These effects were also blocked when NAPE-PLD was inhibited, indicating anandamide availability is responsible for modulating these effects.

Conclusion: Our findings illustrate a novel FABP5-dependent mechanism within the BLA that regulates anxiety phenotypes, fear memory expression, and related neuronal circuitry. Along with advancing knowledge on neurobiological mechanisms of anxiety, this research illuminates FABP5 inhibition as a promising avenue for anxiolytic and post-traumatic stress pharmacotherapies.

Acknowledgements: This work was supported by Artelo Biosciences and MITACs CANADA.

1. Haj-Dahmane, S., Shen, R. Y., Elmes, M. W., Studholme, K., Kanjiya, M. P., Bogdan, D., ... & Kaczocha, M. (2018). Fatty-acid–binding protein 5 controls retrograde endocannabinoid signaling at central glutamate synapses. *Proceedings of the National Academy of Sciences, 115*(13), 3482-3487.

THE UNIQUE EFFECT OF MJN110 (MAGL INHIBITOR) IN HYPERDOPAMINERGIC STATES: IMPLICATIONS FOR 2-AG MODULATION IN PSYCHOSIS

Catharine A. Mielnik[1], Claudia Lutelmowski[1], Clare T. Johnson[2], Julia Zebarth[1,3], Marija Milenkovic[1], Wendy Horsfall[1], Walter Swardfager[1,3], Heather B. Bradshaw[2], Ali Salahpour[1] and Ruth A. Ross[1]

[1]University of Toronto, Faculty of Medicine, Department of Pharmacology & Toxicology
[2]Indiana University Bloomington, College of Arts + Sciences, Psychological and Brain Sciences
[3]Sunnybrook Research Institute, Hurvitz Brain Sciences Program

Background: The endocannabinoid system (ECS) is dysregulated in schizophrenia (SCZ). Enzymes in the biosynthesis pathway of 2-arachidnoylglycerol (2-AG) are shown to be altered in SCZ; DAGL (2-AG synthesis) levels are decreased in patients with first episode psychosis, ABHD6 (2-AG metabolism) mRNA levels are elevated in patients in early stages of disease, while MAGL (primary 2-AG metabolism) expression levels are significantly lower in patients with SCZ. Elevated 2-AG is observed in individuals at high risk of psychosis. Despite this, the elevation of 2-AG is coveted in certain clinical contexts, therefore clinical trials of MAGL inhibitors (MAGLi) are currently underway. However, before wide therapeutic use, it's imperative to fully understand the effect of MAGLi in the aforementioned psychopathologies. A subpopulation with dysregulated 2-AG may be vulnerable to psychiatric effects of MAGLi. Therefore, to address 2-AG modulation in SCZ, we assessed pre-clinical effects of MAGLi in two models of hyperdopaminergia, as it's well established that SCZ is strongly associated with increased subcortical dopamine.

Methods: Genetic (adult DAT-knockout (DATKO) and pharmacological (C57Bl/6J with amphetamine) models of hyperdopaminergia were treated acutely and chronically with a MAGL inhibitor (either MJN110 at 5mg/kg, or JZL184 at 40 mg/kg), and tested on behavioural assays. Lipidomic and molecular analysis was completed (striatal brain samples), and partial correlation networks were generated. Data were analyzed with three-way ANOVA (behaviour) and Student's t-test for lipidomics.

Results: DATKO mice present with subcortical hyperdopaminergia, exhibiting exploratory hyperactivity, anxiety-related changes, impaired sensorimotor gating, and blunted/absent response to psychostimulants. Acute MJN110 treatment exacerbated hyperlocomotion in DATKO mice and led to a deficit in habituation of the acoustic startle response. Furthermore, MJN110 was rewarding in DATKO, but not WT littermates, suggesting exacerbation of hyperdopaminergic states in the presence of acute MJN110. MJN110 effects weren't limited to genetic models; MAGLi exacerbated psychostimulant responses in C57BL/6J mice. Data show that MJN110 effects in both genetic and pharmacological models of hyperdopaminergia are mediated by CB1. Lipidomic and molecular analysis of striatal tissue confirmed that the ECS is dysregulated in DATKO mice. Interestingly, data suggest that, at the dose (5 mg/kg) and time point (30-min pre-treatment) tested, these effects on hyperdopaminergic states are only observed with acute MJN110, since the same is not observed with JZL184, 40 mg/kg with 30-min pre-treatment, nor with chronic MJN110 (5 mg/kg, 14 days) in the DATKO mice.

Conclusion: Our data highlights how states of hyperdopaminergia may be highly sensitive to 2-AG modulation by certain MAGLi; 2-AG elevation may further drive psychopathology in a complicated, and potentially compound-specific, manner. Therefore, MAGLi may be contraindicated in hyperdopaminergic vulnerabilities within neuropsychiatric disorders.

MOLECULAR BASIS FOR SELECTIVE ACTIVATION AND TARGET ENGAGEMENT OF CANNABINOID RECEPTOR 2 AGONISTS

Xiaoting Li[1,2*], Hao Chang[1,2*], Jara Bouma[3*], Laura V. de Paus[4], Cas van der Horst[3], Sanjay S. Kumar[3], Lijie Wu[1,2], Yanan Yu[1,2], Antonius P.A. Janssen[4], Zhi-Jie Liu[1,2#], Mario van der Stelt[4,5#] Laura H. Heitman[3,5#] and Tian Hua[1,2#]

[1]iHuman Institute, ShanghaiTech University, Shanghai 201210, China
[2]School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China
[3]Division of Drug Discovery and Safety, LACDR, Leiden University, the Netherlands,
[4]Department of Molecular Physiology, LIC, Leiden University, the Netherlands [5]Oncode Institute, Leiden, the Netherlands, [*]contributed equally

Introduction: The endocannabinoid system plays an important role in mediating various physiological and pathological processes via the cannabinoid receptors type 1 (CB_1R) and type 2 (CB_2R). Although involved in distinct processes the two receptors share a high sequence similarity, which complicates the development of selective CB_2R agonists for treatment of pain, inflammatory diseases and cancer without inducing psychotropic side effects. Hence, a better understanding of the molecular mechanisms involved in selective CB_2R target engagement and signaling may help to improve drug design.

Methods: Cryo-electron microscopy structures were made of CB_2R in complex with the G_i protein for the non-selective agonist CP55940 and three structurally diverse CB_2R-selective agonists APD371 (Olorinab), HU308 and LEI102 (a novel compound). Furthermore, the interaction of the four agonists with 21 critical amino acids in either the binding pocket or two potential ligand entry pathways of CB_2R and CB_1R were examined in [^{3}H]CP55940 displacement and functional [^{35}S]GTPγS assays by a mutagenesis approach. Moreover, kinetic radioligand binding assays were performed to determine the association (k_{on}) and dissociation rate constants (k_{off}) of the synthetic agonists and endocannabinoids.

Results: Here, we report the successful generation of four cryo-EM structures of the CB_2R-G_i complexes with agonists CP55940, APD371, HU308 and LEI102 with resolutions of 2.94 Å, 3.0 Å, 3.0 Å and 2.9 Å, respectively. Affinity and functional mutagenesis studies showed that the four agonists have distinct interactions in the CB_2R binding pocket, and suggest a selectivity hotspot in CB_2R activation. Our data indicated that highly lipophilic agonist HU308 and endocannabinoids bind and activate the receptor via membrane access, which is not the case for CP55940, APD371 and LEI102. This finding was correlated with slower association of HU308 and the endocannabinoids to the receptor compared to CP55940, APD371 and LEI102.

Conclusions: Structural biology studies combined with site-directed mutagenesis and extensive molecular pharmacology research have revealed the molecular basis of target engagement and selectivity of CB_2R agonists. This provides a framework for the rational design and understanding of experimental drugs targeting the CB_2R.

CELL SPECIFIC ROLES OF THE CB2R IN THE ACUTE LOCOMOTOR RESPONSE TO COCAINE

Christina A. Behlke, Leslie Krause, Jennifer Sterrett, Alison Moe and Cecilia J. Hillard

Department of Pharmacology and Neuroscience Research Center; Medical College of Wisconsin

Introduction: Because of its ability to modulate dopamine transmission, the endocannabinoid system is increasingly gaining interest as a possibility for pharmacological intervention in the treatment of addiction. Several studies have shown that CB2 receptor (CB2R) agonists reduce cocaine-induced hyperlocomotion, however, the cellular identity of the CB2R involved is unclear. Previous studies have identified microglial cells as the primary source of CB2R in the brain; however, there is evidence that CB2R alter neuronal activity directly as well. Our goal was to test the hypothesis that the ability of CB2R agonists to reduce cocaine behavioral effects requires microglial CB2R expression. The alternative hypothesis was that the ability of CB2R agonists to reduce cocaine behavioral effects requires dopaminergic neuron CB2R expression.

Methods: We utilized a transgenic mouse line ($CB2R^{tg/tg}$) containing a floxed CB2R (*Cnr2*) gene. Global CB2R knock out mice ($CB2R^{-/-}$) were created by crossing $CB2R^{tg/tg}$ with mice ubiquitously expressing cre-recombinase. To examine the role of microglial CB2R, we crossed $CB2^{tg/tg}$ with mice expressing cre under the control of the microglial- and macrophage-selective gene for CX3CR1 ($CB2R^{CX3CR1-/-}$). To examine the role of dopamine neuronal CB2R, $CB2^{tg/tg}$ mice were crossed with mice expressing cre recombinase under the control of the gene for the dopamine active transporter (DAT; $CB2R^{DAT-/-}$). Locomotor activity was measured using the open-field assay. Adult male or female mice were given a dose of saline or cocaine (20 mg/kg, i.p.) 30 minutes after pretreatment with vehicle or the CB2 agonist, JWH-133 ("JWH" going forward, 20 mg/kg, i.p.). Ambulation was recorded for 45 minutes. Data were acquired using AnyMaze software and analyzed using ANOVA.

Results: Wild type (WT) vs $CB2R^{-/-}$: In WT mice, 20 mg/kg JWH trends to increase ALA in males and trends to decrease ALA in females, but neither to significance. JWH had no effect in male or female $CB2R^{-/-}$ mice. Interestingly, there was a significant difference in the effect of cocaine between the genotypes of both males and females ($p<0.0001$), post hoc tests indicated that the ALA effect of cocaine was less in $CB2R^{-/-}$ compared to WT mice. CX3CR1 Cre vs. $CB2R^{CX3CR1-/-}$: JWH suppressed cocaine's ALA effects in both male and female CX3CR1 Cre mice, compared to vehicle treated controls of the same genotype and sex. $CB2R^{CX3CR1-/-}$ males show no effect of JWH on cocaine-induced ALA, suggesting that microglial CB2R are required for the effect of JWH in males. In preliminary findings, JWH increases ALA in $CB2R^{CX3CR1-/-}$ females, which suggests that another CB2 receptor pool is a target in female mice. DAT Cre vs. $CB2R^{DAT-/-}$. JWH increases cocaine's ALA effects in both male and female DAT Cre mice, compared to vehicle controls, suggesting that the expression of Cre in these neurons may alter CB2R expression. Cocaine produces greater ALA in male $CB2R^{DAT-/-}$ compared to male DAT Cre and JWH significantly decreases cocaine ALA in male $CB2R^{DAT-/-}$ mice. The effect of JWH had no effect in $CB2R^{DAT-/-}$ females compared to DAT Cre controls (genotype by JWH effect, $p<0.0001$).

Conclusions: These findings demonstrate that CB2R in two different cell types contribute to the effects of JWH-133 to alter cocaine ALA. Activation of CB2R in **microglia** results in **reduced** cocaine ALA. This JWH-133 target predominates in **female mice.** Activation of CB2R in **dopaminergic neurons** results in **increased** cocaine ALA. This JWH-133 target predominates in **male mice.** In addition, CB2R in CX3CR1 expressing cells seem to exhibit constitutive or tonic activity. Because cocaine-induced hyperlocomotion in mice is regulated by cell-specific CB2R, further elucidation of the exact nature of this modulatory activity could reveal CB2R as a potential therapeutic target for psychostimulant abuse treatment.

Supported by NIH grant DA041212 and the Kubly Fund for Depression Research.

EVALUATION OF THE IMPACT OF MICROGLIAL CANNABINOID CB_2 RECEPTOR DELETION IN THE CONTEXT OF ALZHEIMER'S DISEASE

Ana M. Martínez Relimpio[1], Samuel Ruiz de Martín Esteban[1], Irene Benito-Cuesta[1], M. Teresa Grande[1], Diego Herráez-Aguilar[1], Ricardo Mostany[2], Cecilia J. Hillard[3] and Julián Romero[1]

[1]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, 28223, Madrid, Spain;
[2]Department of Pharmacology, Tulane University School of Medicine, 70112, New Orleans, LA, USA;
[3]Neuroscience Research Center, Medical College of Wisconsin, 53226, Milwaukee, WI, USA

Introduction: In recent years the CB_2 cannabinoid receptor has become a promising candidate as a possible diagnostic tool and pharmacological target in the inflammatory processes associated with Alzheimer's disease (AD). The development of neuritic plaques in the brain parenchyma trigger the activation of microglial cells, even in the early stages of pathological development, and as we have previously shown, the induction of the expression of cannabinoid CB_2 receptors. However, their role in the interplay between them and amyloid plaques and the molecular mechanisms involved are not clearly described. Consequently, delving into the functionality of this receptor may be necessary to explore new therapeutic approaches in AD.

Methods: Several lines of transgenic mice, such as 5xFAD/$CB_2^{EGFP;f/f}$/Cx3cr1$^{tm2.1(cre/ERT2)Jung}$ mice or 5xFAD/$CB_2^{EGFP;f/f}$ were used to evaluate the effect of controlled genetic deletion of cannabinoid CB_2 receptors on microglial cells. Here we present data related to the *in vivo* evolution of amyloid neuritic plaques, the ability of microglial cells to phagocytose amyloid peptides, the specific signaling pathways of this receptor or the cytokine production profile.

Results: We have been able to follow the evolution of neuritic plaques in the presence or absence of microglial CB_2, as well to analyze their main features (plaque volume, protein density or skewness). Additionally, our results confirmed that CB_2 deletion caused changes on the ability of microglia to phagocytose amyloid peptides, with a significant decrease in amyloid uptake by CB_2-null mice. The signaling pathways and the production profile of some cytokines were altered after CB_2 deletion.

Conclusions: The deletion of microglial cannabinoid CB_2 receptors altered the amyloid uptake capacity of microglial cells and caused changes in the expression of some cytokines, thus indicating that CB_2 receptor participates in the interplay between these cells and neuritic plaques.

Keywords: neuroinflammation, Alzheimer's disease, cannabinoid CB_2 receptor, microglial activity

Acknowledgements: Supported by grants from the Ministerio de Ciencia e Innovación (PID2019-108992RB-I00, JR) and the Research and Education Component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin to (CJH).

THE CANNABINOID RECEPTOR 2 AGONIST, HU308, MITIGATES OSTEOARTHRITIS VIA CREB-MEDIATED SOX9 ACTIVATION

I. Carmon[1,2], SRK Meka[1,2], J. Elayyan[2], L. Zecharyahu[2], G. Batshon[2], E. Reich[2], Leonid Kandel[3], A. Bilkei-Gorzo[4], A. Zimmer[4], R. Mechoulam[1] and M. Dvir-Ginzberg[1,2]

1. Multidisciplinary Center for Cannabinoid Research, Hebrew University of Jerusalem,
2. Institute of Dental Sciences, Hebrew University of Jerusalem;
3. Orthopedic Complex. Hebrew University- Hadassah Medical Center, Jerusalem, Israel;
4. Institute of Molecular Psychiatry, University of Bonn, Germany

Introduction: Osteoarthritis (OA) is characterized by progressive, irreversible erosion of articular cartilage accompanied with severe pain and immobility. This study aimed to assess the effect and mechanism of action of HU308- a selective cannabinoid receptor type 2 (CB2) agonist, in potentially preventing OA-related joint damage.

Methods: Wild type (WT) mice were induced to develop OA via aging to reach 16 or 21 months, or subjected to post-traumatic induction (i.e. Destabilization of the Medial Meniscus; DMM) at 12 weeks of age. Age-induced mice were systemically (i.e IP) administered with HU308 for 4 weeks (twice weekly), while PTOA mice where administered HU308 intra-artcularly for 4 weeks (twice weekly). Furthermore, we examined joints of Cnr2 null mice vs WT mice, which were aged to 20 months. For all experiments we examined joint pain, and carried out histopathological analysis including OA severity, osteophyte formation, synovitis, to assess joint structural damage. Additional analysis of time and dose courses for HU308 were carried out in human primary chondrocytes, analyzed by RT-PCR, chromatin immunoprecipitation (ChIP), immunoblotting and cell proliferation.

Results: Cnr2 null mice exhibited spontaneous age-related OA severity and synovitis vs. WT mice. Systemic (IP) administration of HU308 to 16-month mice improved local pain thresholds and maintained cartilage integrity, while 21-months mice showed only reduction in local pain. Consistently, assessing local (IA) administration of HU308 following post-traumatic DMM induction, exhibited a significant reduction in synovitis, OA severity, osteophyte formation and local pain thresholds, vs vehicle control. Assessing human chondrocytes treated with HU308 (0-200nM), display a dose- and time related increase in ACAN and COL2A1 expression, which temporally preceded by increased SOX9 expression. Further ChIP analysis revealed that phosphorylated CREB (pCREB) was enriched on the SOX9 promoter 4h after HU308 stimulation of chondrocytes.

Conclusions: Collectively, the results show that HU308 reduced trauma and age-induced OA, via activation of SOX9 and its target genes in a pCREB-dependednt manner.

THE ANTI-TUMORIGENIC ROLE OF THE CANNABINOID RECEPTOR 2 IN COLON CANCER

Jennifer Ana Iden, Bitya Raphael-Mizrahi, Zamzam Awida*, Aaron Naim, Dan Zyc, Tamar Liron, Marilena Vered** and Yankel Gabet

Department of Anatomy and Anthropology
*Department of Cell and Developmental Biology
**Oral Pathology and Oral Medicine
Tel Aviv University, Israel

Introduction: Endocannabinoids are endogenous ligands that bind to the differentially expressed cannabinoid receptors. Cannabinoid receptor 2 (CB2) is predominantly expressed in immune and bone cells. The exact role of CB2 in colon cancer development remains unclear. Evidence from both *in vitro* and *in vivo* studies has established diametric roles of CB2, e.g. both pro-inflammatory and anti-inflammatory, associated with cancer severity as well as anti-proliferative and pro-apoptotic in cancer cells.

Methods: Wildtype (WT), systemic CB2 knockout ($CB2^{-/-}$), $Apc^{Min/+}/CB2^{+/+}$ and $Apc^{Min/+}/CB2^{-/-}$ mice on a C57Bl/6J genetic background were bred in an SPF animal facility. In the spontaneous cancer study, male and female mice were sacrificed at 14 months of age, and tissues were collected for histological analysis. For chemically induced colorectal cancer, 8-week-old WT and $CB2^{-/-}$ female mice were given a single intraperitoneal injection of AOM (12 mg/kg) followed by three cycles of 2.5% DSS in their drinking water for 7 days in weeks 1, 4, and 7. We evaluated the disease activity using colonoscopy after 6 weeks and mice were sacrificed at week 11. $Apc^{Min/+}/CB2^{+/+}$ and $Apc^{Min/+}/CB2^{-/-}$ mice were sacrificed at 9 weeks of age with no treatment throughout. Analyses of the colons included tumor count, histopathological evaluation, and large intestine length. After weighing distal colon tumors, we cultured 1mm fragments of the tumors in RRMI supplemented with 10% FBS for eight hours and measured nitric oxide (NO) in the supernatant using the Griess reagent. We also analyzed spleen cells for the presence of T cells and myeloid cells using flow cytometry. Data were analyzed by Student's t-test or Mann-Whitney U test for continuous variables and Chi-Square test for categorical variables.

Results: Initially, we found higher incidence of spontaneous precancerous lesions in multiple organs, including the colon, in aging $CB2^{-/-}$ mice. For female mice administered with AOM/DSS, we found that the $CB2^{-/-}$ mice had higher disease activity, increased presence of dysplastic polyps, and a higher number of tumors compared to WT. Additionally, compared to $Apc^{Min/+}/CB2^{+/+}$ mice, $Apc^{Min/+}/CB2^{-/-}$ mice showed more aggressive cancer development. They displayed a shorter colon and increased number of tumors in the small and large intestine. In addition, $Apc^{Min/+}/CB2^{-/-}$ mice exhibited a strikingly different immune profile: we observed enhanced splenic population of immunosuppressive and tumor-promoting cells called granulocytic-myeloid derived suppressor cells (G-MDSC), and a decrease in anti-tumor $CD8^+$ T cells and eosinophils. Accordingly, tumors from $Apc^{Min/+}/CB2^{-/-}$ mice secreted more NO, a critical player in T cell suppression produced by G-MDSCs.

Conclusions: Taken together, these results suggest that endogenous CB2 activation suppresses colon cancer development likely by altering the balance between pro-tumorigenic and anti-tumorigenic immune cells in lymphoid organs and attenuating immunosuppressive factors in the tumor microenvironment.

A HUMAN LABORATORY STUDY ON THE INTERACTION BETWEEN INHALED THC AND LIMONENE

Ryan Vandrey[1], Tory Spindle[1], George Bigelow[1], Austin Zamarripa[1] and Ethan Russo[2]

[1]Behavioral Pharmacology Research Unit, Johns Hopkins University School of Medicine
[2]CReDO Science

Introduction: The concept of the cannabis entourage effect, that the acute effects of "full spectrum" cannabis are substantively different than the acute effects of delta-9-tetrahydrocannabinol (D-9-THC) by itself, is widely accepted as true despite little empirical evidence to support it. Moreover, few controlled human studies have tested isolated constituents of the cannabis plant believed to directly impact targeted pharmacodynamic outcomes related to the cannabis entourage effect. The present study was conducted to test the entourage hypothesis that the naturally occurring terpene limonene can mitigate the acute anxiogenic effects that often occur with the acute administration of high doses of inhaled D-9-THC.

Methods: Study participants were 16 healthy adults who used cannabis <2/week and reported experiencing anxiety following cannabis use in the past. Participants completed nine double-blind outpatient experimental test sessions, each separated by at least 48 hours, in which they inhaled vaporized D-9-THC (15 and 30mg), limonene (1 and 5mg), D-9-THC and limonene together (15mg D-9-THC/1mg limonene, 30mg D-9-THC/1mg limonene, 15mg D-9-THC/5mg limonene, and 30mg D-9-THC/5mg limonene), or placebo. A subset of participants (N=9) completed an optional 10th session in which 30mg D-9-THC and 15mg limonene were vaporized. Drug administration was completed using the Might Medic vaporizer (Storz and Bickel). Subjective drug effects and measures of anxiety were assessed at baseline, immediately post-dose, and repeatedly for 6 hours after dosing was completed using a 100pt Visual Analog Scale. Vitals were also collected.

Results: Both 15mg and 30mg D-9-THC increased ratings of self-reported drug effect, anxiety, heart racing, paranoia and other common D-9-THC effects compared with placebo (*ps*<.05). Co-administration of limonene attenuated the anxiogenic effects of D-9-THC in a dose-orderly manner (i.e. the higher the limonene dose, the lower the self-reported rating of anxiety, paranoia, and subjective heart racing). At the 30mg D-9-THC dose, co-administration of 1mg, 5mg, and 15mg limonene reduced anxiety by 14%, 27% and 57% respectively, but these differences are not statistically significant. Limonene coadministration with D-9-THC did not alter subjective ratings of overall drug effect, effects of D-9-THC not associated with anxiety, nor did limonene affect vital signs. Acute administration of limonene in the absence of D-9-THC did not produce discriminable drug effects on any study outcome measures compared with placebo.

Conclusions: D-9-THC-induced anxiogenic effects were attenuated by co-administration of limonene in a dose-orderly manner. Limonene did not alter other subjective effects synonymous with D-9-THC exposure, which indicates that limonene selectively mitigates the anxiogenic effects of D-9-THC. This observation provides empirical support for this specific cannabis entourage theory and should be incorporated into clinical decision making related to medicinal cannabis/cannabinoid product selection and future cannabinoid drug development efforts.

Support: National Institute on Drug Abuse (NIDA); R01-DA043475 and T32-DA07209

EVALUATION OF CYTOCHROME P450-MEDIATED CANNABINOID-DRUG INTERACTIONS IN HEALTHY ADULT PARTICIPANTS

C. Austin Zamarripa[1], Elise M. Weerts[1], Catherine F. Moore[1], Sumit Bansal[2], Tory R. Spindle[1], Jashvant D. Unadkat[2], Mary F. Paine[3] and Ryan Vandrey[1]

[1]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, WA; [3]Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, WA.

Introduction: Cannabidiol (CBD) and delta-9-tetrahydrocannabinol (Δ9-THC) have been shown to inhibit several cytochrome P450 (CYPs) in preclinical studies, including rodent studies conducted by our team, and are predicted to precipitate pharmacokinetic (PK) and pharmacodynamic (PD) drug interactions. However, cannabis-drug interactions have not been carefully studied in controlled human studies. This study evaluated the impact of a Δ9-THC dominant cannabis extract and a CBD dominant cannabis extract on the PK of 5 CYP probe drugs, Δ9-THC-CBD interaction, and the impact of Δ9-THC-CBD interaction on PD outcomes.

Methods: Healthy adult participants (n=15) completed three, randomized, drug administration sessions. Each participant consumed a brownie containing a Δ9-THC dominant cannabis extract (20mg Δ9-THC with no CBD), a CBD dominant cannabis extract (640mg CBD + 20mg Δ9-THC), or no cannabis extract (placebo), followed 30 min later by an oral CYP probe drug cocktail: caffeine 100mg (CYP1A2), losartan 25mg (CYP2C9), omeprazole 20mg (CYP2C19), dextromethorphan 30mg (CYP2D6), and midazolam 2mg (CYP3A). PK of these probes act as reporters of targeted CYP enzyme activity *in vivo*. Plasma samples were collected, and PD measures (vital signs, subjective drug effects, cognitive task performance) were obtained 0-24 hours after cocktail administration. PD outcomes were analyzed using 2-way ANOVA with condition and time as repeated measures (n=15). Plasma samples from the first 6 participants were analyzed using noncompartmental PK analysis to estimate area under the curve (AUC_{0-t}); the geometric mean ratio of AUC_{0-t} was computed relative to the placebo or Δ9-THC condition.

Results: CBD+Δ9-THC increased Δ9-THC AUC_{0-t} and 11-OH-THC/Δ9-THC AUC_{0-t} ratio by 2.3- and 3.9-fold, respectively, due to inhibition of CYP2C9-mediated THC metabolism to 11-OH-THC and UGT-mediated 11-OH-THC metabolism. CBD inhibition of THC metabolism significantly increased the magnitude and time course of heart rate, subjective effects, adverse events, and cognitive impairment. CBD+Δ9-THC, but not Δ9-THC, inhibited CYP1A2, 2C9, 2C19, and 3A activities as the AUC_{0-t} of caffeine, losartan, omeprazole, and midazolam increased by 1.4-, 1.7-, 3-, and 1.7-fold, respectively. Cannabis extracts did not affect CYP2D6.

Conclusions: High dose CBD+Δ9-THC increased PD effects compared with Δ9-THC alone at the same Δ9-THC dose. This contrasts the common conception that CBD attenuates Δ9-THC effects. CBD+Δ9-THC also significantly inhibited metabolism of several CYP probe drugs, indicating that dose adjustment should be considered for many drugs co-consumed with CBD dominant cannabis products. Studies evaluating lower doses of CBD are warranted.

Acknowledgements: NIH grants U54 AT008909 and T32 DA007209

CAN WE MAKE CANNABIS SAFER? AN EXPERIMENTAL STUDY OF FOUR CBD:THC RATIOS IN HEALTHY VOLUNTEERS

Amir Englund[1], Dominic Oliver[1], Edward Chesney[1], Lucy Chester[1], Jack Wilson[2], Simina Sovi[1], Andrea De Micheli[1], John Hodsoll[1], Paolo Fusar-Poli[1], Robin Murray[1], John Strang[1], Tom Freeman[3] and Philip McGuire[1]

1. Institute of Psychiatry, Psychology and Neuroscience – King's College London, UK
2. The Matilda Centre for Research in Mental Health and Substance Use, The University of Sydney, Australia
3. University of Bath, UK

Introduction: Pharmacological investigations of CBD and THC have found these two cannabinoids to be vastly different in terms of mechanism of action and some studies have found CBD may offset some of the negative impact of THC. It has been suggested that the addition of CBD to cannabis may reduce the harms of cannabis use and some retailers are marketing CBD-containing cannabis in this way. Here we investigate the different effects of the four CBD:THC ratios most commonly available on legal recreational markets.

Methods: Healthy volunteers (N=46) attended 5 study visits, 1 baseline and 4 experimental drug administration visits. On each visits participant were administered 10mg intrapulmonary THC, co-administered with either 0mg, 10mg, 20mg, 30mg CBD (CBD:THC ratios – 0:1, 1:1, 2:1 and 3:1). Standardised assessments were used to study changes to cognitive performance, psychopathology, subjective effects, physiological, as well as pharmacokinetic data.

Results: THC alone reliably produced cognitive impairment, psychotic-like symptoms, and subjective effects compared to baseline. There were no significant differences between THC alone and any of the CBD-ratios on these outcomes. THC induced significant increased heart rate following administration, but no overall change to blood pressure – CBD did not alter these effects. There was a significant dose response relationship between CBD dose and CBD plasma (peak and AUC) with no difference in THC plasma across the four ratios. CBD did not influence THC induced subjective effects.

Conclusions: The most commonly available CBD:THC ratios on legal recreational markets offer no protection compared to THC alone (when THC dose remains constant) on cognitive, psychopathological or subjective outcomes.

Acknowledgements: This study was funded by the Medical Research Council, UK

CANNABIDIOL ALLEVIATES CUE-INDUCED ANXIETY LINKED TO ALTERATIONS IN CNR1 AND LIPID LEVELS IN THE NUCLEUS ACCUMBENS SHELL

Jacqueline-Marie N. Ferland, Randall J. Ellis, Joseph A. Landry, James E. Callens, Alexandra Chisholm, Micah D. Frier, Heather B. Bradshaw and Yasmin L. Hurd

Icahn School of Medicine at Mount Sinai, New York, NY USA

Introduction: Anxiety disorders affect an estimated 18% of the US population every year. Cues previously associated with a fearful event have been shown to precipitate acute anxiety experiences, especially for conditions like post-traumatic stress disorder (PTSD). New treatments are needed that not only alleviate general feelings of anxiety but that can serve as tools to help the individual manage the conditioned cues that trigger the acute feelings of fear and anxiety. Cannabidiol (CBD) is currently being explored for its anxiolytic effects, though reports on its efficacy are mixed, with some studies reporting little to no effect on trait anxiety but stronger effects in pathological anxiety. CBD has also been shown to reduce stress-induced social anxiety and cue-induced drug-seeking and craving, suggesting it may be effective in mediating behavioral responses to cues. These data indicate CBD might be particularly effective as a treatment for triggered or cue-induced anxiogenic behavior.

Methods: To test the efficacy of CBD on cue-precipitated anxiety-like behavior, male rats underwent a footshock protocol with the presence of a distinct lemon oil or control, neutral odor cue. Light/dark box and open field were used to assess anxiety-like behavior in the presence of the cue associated with the shock. Rats were given either 10 mg/kg CBD or vehicle 1 hr prior to the behavioral tests. In a second experiment, CBD was given 1 hr before the shock sessions to determine if administration would influence the conditioning to the cue itself. We then used biochemical and molecular assays to determine the effects of CBD, with and without cue exposure, on the nucleus accumbens shell, a mesocorticolimbic region shown to play a role in Pavlovian fear conditioning.

Results: CBD did not reduce anxiety-like behavior in rats in the neutral condition, nor did CBD pre-exposure block the encoding of the cue. However, CBD reversed the heightened avoidance behavior in animals exposed to the lemon oil cue, but only after repeated shock-pairings known to potentiate conditioned fear. qPCR analyses revealed CBD/cue rats had altered expression of several putative CBD targets including Cnr1, GPR55, and 5HT1a. Interestingly, CBD specifically reversed reduced Cnr1 as well as altered levels of linoleic acid and N-acylethanolamines in the nucleus accumbens shell of cue rats with correlations to behavior.

Conclusions: CBD showed anxiolytic properties, but only in the presence of a conditioned cue repeatedly associated with an aversive stimulus. CBD also affected a variety of neurobiological systems known to be implicated in anxiety. These results suggest that CBD may be uniquely suited to alleviate cue-induced anxiety and may serve as a therapeutic for well-learned triggered anxiety and fear including PTSD.

Acknowledgements: "Funding for this project was made possible in part by grant R0DA048613 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."

CANNABIDIOL AND CANNABIGEROL PROMOTE BOTH EARLY AND LATE PHASES OF FRACTURE HEALING

Deepak Kumar Khajuria[a], Vengadeshprabhu Karuppagounder[a], Muhammad Daniyal[a], Wesley Raup-Konsavage[b], Kent Vrana[b], Fadia Kamal[a] and Reyad A. Elbarbary[a]

[a]Department of Orthopaedics and Rehabilitation, [b]Department of Pharmacology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA

Introduction: Bone fractures are among the most prevalent musculoskeletal injuries. Bone healing proceeds through consecutive yet overlapping steps that are categorized into 3 main phases: inflammatory, repair, and remodelling. Inhibition of the initial inflammatory phase results in delayed/defective healing. Consistent with this, nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown in both pre-clinical and clinical studies to significantly inhibit healing when used to manage post-fracture (PF) pain. Opioids are considered analternative line of pain management in fracture patients as they have no reported inhibitory effects on fracture healing. However, opioids addictive and abuse potential is a serious concern that limits their prescription. Accordingly, there is an urgent and unmet clinical need to find non-opioid therapies that can effectively ameliorate PF pain without inhibiting fracture healing. Here, we investigated the efficacy of the FDA-approved epilepsy medication cannabidiol (CBD) and the investigational substance cannabigerol (CBG) as medications for PF pain and comprehensively assessed their impact on different phases of fracture healing.

Methods: We performed mid-diaphyseal tibial fractures in adult male C57BL/6J mice and injected 5 mg/kg daily dose of CBD or CBG, starting from the surgery day until harvest time (14, 21 and 28 days post-fracture). We compared the analgesic effects of CBD and CBG in PF pain to those of the widely used NSAIDs celecoxib and indomethacin using von Frey, acetone, and hot plate tests. We used micro computed tomography (μCT), histological analysis, immunofluorescence (IF) staining, and biomechanical testing to assess the efficiency of fracture healing at different phases. Statistical analysis were performed using one-way ANOVA followed by Tukey's post-hoc test (significance was set as $p<0.05$).

Results: Both CBD and CBG exerted strong analgesic effects in PF pain as reflected in reduced mechanical nociception, thermal nociception, and cold allodynia; their analgesic effects were comparable to those of celecoxib and indomethacin. μCT, histological analysis, and IF staining indicated that CBD and CBG enhanced different phases of bone healing, resulting in increased bone formation and elevated bone mineral density at all analysed PF time points. Biomechanical testing indicated that the stimulatory effects of CBD and CBG on bone healing culminated in formation of stronger bone. Using Flow cytometry, we identified cells within the healing callus that express different cannabinoid receptors, and our studies using primary cultures provide insights into mechanisms by which CBD and CBG promote bone regeneration.

Conclusion: CBD and CBG potently relieve PF pain and, importantly, enhance bone formation during fracture healing through their effects on cells that express cannabinoid receptors. Our data strongly recommend CBD and CBG as potential alternative for NSAIDs in PF pain management.

Acknowledgements: PA options for Wellness; Penn State University Medical Marijuana Academic Clinical Research Center. Research was supported by R01 DK121327 to R.A.E and R01 AR071968 to F.K.

EFFECTS OF TETRAHYDROCANNABINOL (THC) VAPOUR EXPOSURE ON RAT FEEDING BEHAVIOURS AND HOMEOSTATIC APPETITE-REGLATING PATHWAYS

Catherine Hume, Samantha Baglot, Savannah Lightfoot, and Matthew Hill

Cumming School of Medicine, University of Calgary, Alberta, Canada

Introduction: With medical and recreational cannabis being legalised in many parts of the world, there is an urgent drive to investigate the physiological effects of cannabis to assess if it's use is associated with certain health-related effects. It's well established that cannabis acutely drives food intake, commonly referred to as 'the munchies', and that tetrahydrocannabinol (THC), the main psychoactive compound in cannabis, is mainly responsible for driving these feeding effects. Cannabis-induced feeding has been modelled in rodent and human studies. However, the effects of cannabis on eating patterns are not well characterised and the mechanisms underlying cannabis-driven feeding ultimately remains unknown. Therefore, the aim of this project was to use a THC vapour inhalation rat model to characterise the effects of THC on feeding patterns, satiety and macronutrient-specific food preferences, and investigate potential hormonal and neuronal mechanisms underlying THC-driven feeding.

Methods: Adult male and female Sprague Dawley rats were exposed to THC (100mg/ml in vehicle, n=7) or vehicle (polyethylene glycol, n=7) vapour for 15min (10s hit every 2min), to induce blood THC levels comparable to human cannabis use. For behaviour studies, rats were given post-vapour food access (chow, 80% carbohydrate (high-carb) food or 80% fat (high-fat) food), and intake measured over time. For food preference studies, rats were given both high-carb and high-fat foods. For satiety studies, rats were given a 10% sucrose chow mash 'preload' to binge eat prior to vapour exposure. For mechanistic studies, various methods were used. To measure post-vapour appetite hormone profiles, plasma was collected and ran through a magnetic bead panel multiplex assay. To quantify post-vapour brain activity, rats were perfused-fixed, and brains processed for c-Fos immunohistochemistry (indirect marker for neuronal activation). To dissect the role of peripheral and central cannabinoid 1 receptors (CB1R), a universal CB1R antagonist (AM251, 1mg/kg IP) or a peripherally restricted CB1R (AM6546, 1.5mg/kg IP) was administered prior to vapour exposure. At these doses, these drugs alone have no effect on feeding. Differences between treatment group and sex were determined using a two-way ANOVA with Bonferroni post-hoc testing ($p<0.05$ considered significant).

Results: Regardless of which foods are available (chow, high-fat or high-carb), THC significantly increases food intake in the first hour after exposure (all $p<0.05$), and rats compensate for this by reducing subsequent food intake so that 24hr energy intake does not change (all $p>0.05$). THC also significantly drives food intake in satiated rats who otherwise do not eat ($p<0.05$). We found that THC acutely alters macronutrient-specific food choice, and that the preferred food is dependent on the state of the animal, where high-fat food preference is increased by 20-30% in normal conditions and high-sugar food preference is increased by 20-25% in satiated conditions. Interestingly, THC does not appear to act through peripheral mechanisms to drive feeding, as THC does not alter circulating levels of the appetite and metabolic hormones ghrelin, leptin, insulin, GLP-1, PYY, GIP, PP, glucagon, c-peptide, and amylin (all $p>0.05$), and THC-driven feeding is not inhibited by the administration of a peripherally restricted CB1R antagonist (THC vs vehicle $p<0.05$). This points towards THC acting directly on the brain to drive feeding. As expected, THC-induced feeding is inhibited by administration a universal CB1R antagonist (THC vs vehicle $p>0.05$). We also found that THC increases the activity of neurons in the arcuate nucleus of the hypothalamus; the 'hunger hub' of the brain ($p<0.05$). Finally, there were no significant sex differences observed in any of these studies (all $p>0.05$).

Conclusions: We have shown that THC acutely and robustly disrupts homeostatic feeding patterns. However, rats can compensate for acute THC-driven feeding by reducing subsequent intake. Furthermore, THC acutely influences macronutrient-specific food preferences. Therefore, THC may not drive energy overconsumption, but may change the kinds of foods chosen to eat, which could have negative health effects if these foods are high in fat and/or sugar and are eaten regularly. Our findings also imply that THC acts directly on the brain to drive feeding, potentially involving neurons of the hypothalamic arcuate nucleus. Work is currently underway to delve deeper into these underlying mechanisms. Overall, this research sheds light on the physiological effects of THC, information which is critical for the health and well-being of cannabis users.

CANNABIDIOL REDUCES SYMPTOMS OF ANXIETY AND WITHDRAWAL DURING ACUTE E-CIGARETTE ABSTINENCE

Ivori Zvorsky[1], L. Riley Gournay[2], Jordan Petry[3,4], Sarah Bilsky[5], Morgan A. Hill[2], Matthew Feldner[1], Erica Peters[1], Marcel Bonn-Miller [1] and Ellen Leen-Feldner[2]

[1] Canopy Growth Corporation, [2] Department of Psychological Science, University of Arkansas
[3] School of Behavioral and Brain Sciences, University of Texas at Dallas, [4] Steven A. Cohen Military and Family Clinic, [5]Department of Psychology, University of Mississippi

Introduction: Approximately 8.1 million U.S. adults use e-cigarettes, with two-thirds of users in a nationally representative sample reporting interest in quitting and 25% reporting a quit attempt in the past year. Despite this interest in quitting and the negative effects of chronic e-cigarette use, there is a lack of research examining interventions for successful cessation. Cannabidiol (CBD) shows promise as a potential cessation tool by decreasing anxiolytic and somatic symptoms of withdrawal in pre-clinical models; however, these results have not yet been replicated in humans.

Methods: The current study utilized a within-subject, cross-over design to examine the effect of a single dose of oral CBD (320 mg) on withdrawal and anxiety symptoms during acute abstinence from e-cigarettes. Participants (N=20) met DSM-5 nicotine withdrawal criteria and specifically endorsed anxiety as a symptom during nicotine cessation. They were randomly assigned to condition (CBD vs. No CBD) at Time 1 and completed the subsequent condition at Time 2. At each time point, participants abstained from e-cigarettes for four hours and then rated withdrawal symptoms using the Minnesota Tobacco Withdrawal Scale-Revised (MTWS-R) and anxiety symptoms using the State Trait Anxiety Inventory-State (STAI-S). Mixed linear models were run to examine the fixed and random effects of experimental condition, experimental order, and interaction effects.

Results: For MTWS-R scores, the final model was significant and there were significant main effects of both condition and time with no significant interaction. Oral CBD reduced symptom severity on the MTWS-R total score ($M = 6.5 \pm 5.58$) compared to No CBD ($M = 12 \pm 5.65$; $\beta = 5.45$, $t = 4.04$, $p < 0.0001$). MTWS-R scores were lower at Time 2 ($M = 7.75 \pm 6.60$) compared to Time 1 ($M = 10.7 \pm 5.53$, $\beta = -2.95$, $t = -0.52$, $p < 0.01$). For STAI-S scores, the final model was also significant and there were significant main effects of both condition and time with no significant interaction. Oral CBD reduced STAI-S scores ($M = 37.00 \pm 7.17$) compared to No CBD ($M = 48 \pm 9.99$; $\beta = 11.1$, $t = 5.70$, $p < 0.0001$). STAI-S scores were lower at Time 2 ($M = 39.8 \pm 10.7$) compared to Time 1 ($M = 45.2 \pm 9.33$; $\beta = -5.3$, $t = -2.72$, $p < 0.05$).

Conclusions: Oral CBD reduced both acute nicotine withdrawal severity and anxiety symptoms, underscoring the potential utility of CBD to assist e-cigarette users in achieving cessation. Negative mood states and other withdrawal symptoms are often barriers that contribute to failed quit attempts. Future studies can examine the effect of varying doses of oral CBD and the effect of different modes of CBD (e.g., oral vs. vaped) on nicotine cessation outcomes.

Acknowledgements: Funding for this study was provided by the Arkansas Bioscience Institute (grant awarded to last author). Cannabidiol was donated by Canopy Growth Corporation.

THE EFFECTS OF CBD ISOLATE ON MENSTRUAL-RELATED SYMPTOMS

Morgan L. Ferretti[1], Taylor B. Stanley[2], Erica N. Peters[3],
Marcel O. Bonn-Miller[3] and Jessica G. Irons[1]

[1]James Madison University, [2]Auburn University, [3]Canopy Growth Corporation

Introduction: Approximately 75% of individuals who menstruate experience menstrual-related symptoms (MRS; Wakil et al., 2012), which are characterized by both physiological and psychological experiences that result from the menstrual cycle (Yonkers et al., 2008), including sleep disturbance, sadness, nervousness, and exacerbations of mood and anxiety disorders (e.g., Kornstein et al. 2008; Rasgon et al., 2003). Cannabidiol (CBD) has been shown to improve a variety of symptoms and conditions that overlap with MRS (e.g., anxiety, sleep disturbance, pain). In surveys, women have reported using cannabis to manage MRS, but no study has examined the effect of cannabinoids to reduce MRS. The current study aimed to examine the effects of six months of dosing of oral CBD isolate for alleviating both psychological and physical MRS.

Methods: This study was a prospective, pre-post, 7-month randomized clinical trial. Individuals (*N*=40) who experience moderate-to-severe MRS and who do not use any cannabis products were asked to track their MRS for one cycle and were then randomly assigned to consume 160mg or 320mg daily (80mg or 160mg BID) of CBD softgels. This study utilized a novel dosing paradigm in which participants were instructed to consume CBD for 5 consecutive days starting on the first day they believed to be experiencing MRS. Participants consumed CBD for up to 6 months. Participants tracked symptoms and other health outcomes daily and monthly throughout the study.

Results: Mixed effects models revealed meaningful reductions relative to baseline for both CBD doses in monthly ratings of MRS using the Menstrual Related Symptoms Questionnaire (MRSQ; Ferretti et al., in press), subjective severity of symptoms, and global impression of change. For the 160mg condition, mean MRSQ scores (M_{range} = 40.65 – 43.82) during intervention months were meaningfully lower when compared to baseline scores (M = 52.65; all p-values < .01). For the 320mg condition, mean MRSQ scores (M_{range} = 40.80 – 45.65) during intervention months were meaningfully lower when compared to baseline scores (M = 50.61; all p-values $\leq$ .01). Further, the 320mg condition (but not the 160 mg condition) meaningfully reduced irritability and stress during all intervention months, and meaningfully reduced depression and anxiety during months four and six. Sleep quality, physical activity, and alcohol use did not change. Only one participant discontinued from the study because of an adverse event.

Conclusions: In this first study of the effects of CBD on MRS, both studied doses of CBD had immediate and durable effects on MRS, and the higher dose of 320mg meaningfully reduced related psychological symptoms. Both doses of CBD were well-tolerated. Further work is needed to examine the effect of CBD relative to placebo and to investigate other doses of CBD on reducing MRS.

Acknowledgements: CBD donated by Canopy Growth Corporation and funded through James Madison University.

DOES CANNABIS PROTECT AGAINST AGE-RELATED DECLINES IN COGNITIVE FUNCTIONING?

Carrie Cuttler and Marcella Oreta

Department of Psychology, Washington State University, Pullman, WA, USA

Introduction: The aging population is currently at its highest level in human history and continues to grow. Cannabis is the most commonly used recreational drug among older adults, with older adults representing the fastest growing demographic of cannabis users (Lloyd & Striley, 2018). Further, there is evidence that this population experiences enhanced medicinal benefits and decreased negative side effects of cannabis use (Sexton et al., 2019). Moreover, recent emerging evidence utilizing rodent models further indicates that cannabis may protect the aging brain. Specifically, CB1 knock out mice have demonstrated accelerated cognitive decline (Bilkei-Gorzo et al., 2005), while aging mice chronically administered low doses of a CB1 agonist (WIN-55212-2 or tetrahydrocannabinol) have demonstrated restored cognitive functions (Bilkei-Gorzo et al., 2017; Marchalant et al., 2008; Sarne et al., 2018). However, to date there have been no human studies to corroborate the notion that chronic cannabis use may protect against age-related declines in cognitive functioning.

Methods: As part of a larger studying examining the chronic influence of cannabis use on cognition, we had 112 healthy non-cannabis users and 113 healthy cannabis users complete a battery of cognitive tests, including the California Verbal Learning Test (CVLT), the Brief Visuospatial Memory Test (BVMT), two tests of Prospective Memory (PM), a Source Memory Test (SMT), the Digit Symbol Substitution Test (DSST), the Color Word Interference Test (Stroop), and the Zoo Maps test of planning. Cannabis users ranged in age from 18 to 61 (M = 25.62, SD = 8.58), while the non-users ranged in age from 18 to 73 (M = 27.96, SD = 12.02). The difference in the ages of these two groups was not statistically significantly, $t(223) = 1.68$, $p = .10$.

Results: In the non-cannabis users, significant small to moderate sized negative correlations were detected between age and diminished performance on the BVMT, $r(109) = -.43$, $p < .001$; both tests of Prospective Memory, $r(108) = -.25$, $p = .01$, $r(110) = -.20$, $p = .03$; the SMT, $r(110) = -.19$, $p = .04$; the DSST copy trial, $r(109) = -.29$, $p = .002$, and recall trial, $r(109) = -.44$, $p < .001$; the Stroop test, $r(106) = -.31$, $p = .001$; and the Zoo Maps test, $r(107) = -.27$, $p = .005$. In contrast, cannabis users only demonstrated small but significant age-related declines in performance on the CVLT immediate recall trials, $r(111) = -.23$, $p = .02$ and the BVMT, $r(111) = -.23$, $p = .02$.

Conclusions: The present findings revealing fewer age-related declines in cognitive functioning in healthy human cannabis users relative to non-users converge with previous rodent studies demonstrating that cannabinoids may protect the aging brain. To our knowledge, this is the first study to demonstrate this phenomenon in humans. These findings are particularly important given the growing aging population and rapid increases in the prevalence of neurocognitive disorders such as dementia. As the present study focused on healthy adults (those without neurological, psychiatric, and/or serious medical disorders), future research is needed to expand these findings to adults experiencing cognitive impairments (e.g., mild cognitive impairment, dementia).

Acknowledgements: Funded by WSU's Alcohol and Drug Abuse Research Program

ORAL CANNABIS FOR TAXANE-INDUCED NEUROPATHY: A RANDOMIZED PLACEBO-CONTROLLED PILOT STUDY

Margaret Haney and Diana Martinez

Department of Psychiatry, Columbia University Irving Medical Center, New York, NY USA

Introduction: Taxane-induced peripheral neuropathy (TIPN) occurs in about 67% of patients with breast cancer, and there is no effective treatment. The current management of TIPN includes dose reductions, treatment delays, and even discontinuation of chemotherapy. Preclinical data show that two constituents of the cannabis plant, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) reduce TIPN. The goal of this placebo-controlled Phase 2 pilot study was to test the feasibility and tolerability of oral cannabis in patients who have chemotherapy-induced peripheral neuropathy secondary to treatment with paclitaxel or docetaxel treatment for breast cancer.

Methods: Participants (age 21-60) with TIPN $\geq$3 months after completing taxane treatment for breast cancer were randomized to receive active [100 mg CBD: 5 mg THC] or placebo cannabis capsules TID for 8 weeks. Participants were maintained on the highest tolerable dose after dose escalation. To maintain the double-blind, they were told that they would receive capsules varying in THC and CBD, and that only THC might make them feel intoxicated. Participants completed daily and weekly questionnaires that included ratings of pain severity [Brief Pain Inventory-Short Form (BPI-SF)], quality of life and neuropathy [Functional Assessment of Cancer Therapy Gynecologic Oncology Group Neurotoxicity], measures of sleep, a medication log and a rating form to assess abuse liability. Outcome Measures: 1) *Feasibility*: the proportion of participants completing the 8-week study; 2) *Tolerability*: number and severity of adverse events; 3) *Preliminary efficacy*: Average pain severity score, neuropathy, quality of life. Repeated measures ANOVA were conducted that included terms for dose, study week, and the interaction between dose and study week.

Results: We randomized 12 participants (51 ± 6 years of age; 5 White/4 Black/2 Hispanic/1 South Asian) with current pain (BPI-SF; item #5 = 6.4 ± 0.4) to placebo (n=6) or active (n=6) cannabis capsules. All enrolled (n=12) completed the 8-week trial and were compliant with online assessments. The placebo group averaged 2.4 ± 0.4 capsules/day (means ± SEM), while those the active group averaged 2.0 ± 0.4 capsules/day, supporting the maintenance of the double-blind procedures (neither group tolerated 3 capsules/day). There were no severe adverse events. There were reports of sedation, GI disruption, and headache, which were managed by adjusting the dosing regimen by a blinded clinician. Some participants reported mild intoxication, but none had symptoms consisted with abuse liability (drug liking, craving). In terms of preliminary efficacy: Pain significantly decreased over time ($p< 0.001$) but did not vary by dose. By week 8, ratings on the BPI-SF; item #5 were 3.0 ± 0.9 in the placebo group and 2.7 ± 0.6 in the active group. Ratings of neuropathy and quality of life similarly decreased over time for both groups but there was a significant interaction of study week by dose for both measures ($p<0.001$), with the placebo group showing more improvement than the active group. Additional data analysis is ongoing.

Conclusions: The present methodology demonstrates that: (1) double-blind, placebo-controlled testing of cannabis capsules in this dose range is safe, feasible, and well tolerated in women with painful neuropathy, (2) participants showed clinically meaningful changes in pain, neuropathy and quality of life over 8 weeks, with the placebo group having a better response for some outcomes relative to active cannabis. These findings highlight the critical importance of placebo control in assessing the potential therapeutic utility of cannabis. The findings support a fully powered randomized controlled clinical trial testing a range of CBD:THC ratios. Given that most breast cancer patients experience TIPN, which impairs quality of life, reduces adherence to therapy, and worsens prognosis, with no effective treatment, such research is a public health priority.

Acknowledgements: This research was supported by The Thompson Family Foundation. We gratefully acknowledge Tilray for supplying the medication and facilitating its exportation from Canada to the United States for this study.

EFFECTS OF CANNABIDIOL (CBD) AND Δ9-TETRAHYDROCANNABINOL (THC) ON SLEEP AND DAYTIME FUNCTION IN INSOMNIA DISORDER: A RANDOMISED, DOUBLE-BLINDED, PLACEBO-CONTROLLED, PROOF-OF-CONCEPT TRIAL

Anastasia Suraev, Iain S. McGregor, Nathaniel S. Marshall,
Ron R. Grunstein and Camilla M. Hoyos
On behalf of the 'CANSLEEP trial' investigator team

Centre for Sleep & Chronobiology, Woolcock Institute of Medical Research, Sydney, Australia
Lambert Initiative for Cannabinoid Therapeutics, University of Sydney, Sydney, Australia
School of Psychology, Faculty of Science, University of Sydney, Sydney, Australia

Introduction: Medicinal cannabis is often cited as a popular alternative to common sleep aids, and there is increasing clinical interest in the use of cannabinoids to improve sleep. However, there are limited studies using rigorous designs and objective methods of electroencephalography (EEG) and polysomnography (PSG) to examine the nocturnal and next-day residual effects of cannabinoids in clinical insomnia populations. The aim of the present study was to examine the acute effects of a cannabinoid medicine on sleep and daytime function in adults with insomnia disorder using overnight in-laboratory polysomnography.

Methods: Twenty patients (16 female; median [IQR] age, 47 [13.8] years) with clinician-diagnosed chronic insomnia disorder (mean ISI=20.8) and a history of light cannabis use (no cannabis use in the past 3 months) participated in a randomised, placebo-controlled, double-blinded, crossover trial examining the effects of an oral oil formulation ('ETC120') containing 200 mg cannabidiol (CBD) and 10 mg Δ9-tetrahydrocannabinol (THC) on sleep and daytime function. Participants completed two 24-hour in-lab visits separated by a washout period ≥7 days during which they were administered a single dose of ETC120 or placebo. Co-primary outcomes were total sleep time (TST) and wake after sleep onset (WASO). Secondary outcomes included: sleep micro- and macro-architecture as determined via overnight PSG, subjective ratings of sleep-wake behaviour, next-day neurobehavioural function (cognition, alertness, and simulated driving performance), plasma cannabinoid profile (CBD, THC, and metabolites), and adverse event reporting.

Results: All 20 randomised participants completed the protocol. Compared to placebo, ETC120 significantly decreased TST (-24.5 min, p=0.047) with no significant change to WASO (+10.7 min, p=0.422). ETC120 significantly decreased percentage of time spent in REM sleep (-8.1%, p<0.001) and increased REM sleep latency (+65.6 min, p=0.008) relative to placebo. ETC120 did not impair next-day (+12 h post-treatment) cognitive performance on the paced serial addition, divided attention, digit symbol substitution, memory consolidation, psychomotor vigilance, or n-back tasks (all p's>0.05). No significant next-day residual effect of ETC120 was found on standard deviation of lane position (SDLP), a well-established marker of impairment, on a simulated driving performance task (p>0.05). Eighty-five mild, non-serious, adverse events were reported (55 during ETC120; most common being dry mouth, drowsiness, and fatigue).

Conclusions: Compared to placebo, a single dose of ETC120 (200 mg CBD and 10 mg THC) reduced TST and the amount of time spent in REM sleep, and increased REM sleep latency, suggesting a worsening effect on sleep. However, a single dose of 200 mg CBD and 10 mg THC did not appear to produce any next-day residual impairment on neurocognitive function, alertness, or simulated driving performance. Further research is required to determine the impact of chronic dosing on objective sleep outcomes in insomnia disorder.

Acknowledgements: Supported by the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded center at the University of Sydney, Australia.

INCREASING ENDOCANNABINOID TONE ELICITS SEX-SPECIFIC EFFECTS ON BEHAVIOURAL RESPONDING IN A PRENATAL MODEL OF AUTISM

Aoife M. Thornton[1], David. P. Finn[2,3] and Michelle Roche[1,3]

[1]Physiology, [2]Pharmacology and Therapeutics, School of Medicine,
[3]Galway Neuroscience Centre, National University of Ireland Galway, Ireland

Introduction: Given the sex differences evident in the prevalence of autism, there is an increased awareness of the importance of including females in autism research. Cannabinoids and endocannabinoid modulators have been proposed as potential novel treatments for autism-related symptoms however, few studies have examined the impact of sex when evaluating the efficacy of such compounds. Furthermore, although acute endocannabinoid modulation ameliorates autism-related behaviours in several preclinical animal models of autism, few studies have examined the effects of chronic administration. This study used the valproic acid (VPA) model of autism to examine the behavioural responses of male and female rats and the effects of increasing endocannabinoid tone both acutely and chronically.

Methods: Separate cohorts of male and female adolescent rats prenatally exposed to VPA (500mg/kg G12.5) received the FAAH inhibitor PF3845 (10mg/kg i.p once or for 8 days), the MAGL inhibitor MJN110 (5mg/kg; i.p. or 1mg/kg/day for 7 days) or vehicle, followed by behavioural testing for social, repetitive, anxiety nociceptive and cognitive responding. Liquid chromatography mass spectrometry was used to assess endocannabinoid levels, while expression and activity of the endocannabinoid receptors were assessed RT-qPCR and western immunoblotting. Data were analysed by ANOVA followed by Duncan's *post hoc* test. $P<0.05$ deemed significant.

Results: VPA-exposed male, but not female, rats exhibited reduced social responding in the three-chamber, direct social interaction and olfactory habituation/dishabituation tests. In comparison, VPA-exposed female, but not male, rats exhibited anxiety-like behaviour in the elevated plus maze and open field test. In male rats, acute FAAH inhibition attenuated social deficits and repetitive behaviour in VPA-, but not saline-, exposed rats. Acute MAGL inhibition reduced and increased social novelty preference in saline- and VPA-exposed male rats respectively. In female rats, acute FAAH inhibition reduced stress coping behaviour while MAGL inhibition augmented anxiety-like behaviour in VPA-exposed rats. There was no effect of chronic FAAH or MAGL inhibition on any of the behaviours examined in either saline- or VPA-exposed rats. Mass spectrometry confirmed that FAAH or MAGL inhibition increased central anandamide and 2-AG levels, respectively.

Conclusion: These data highlight that prenatal VPA exposure results in sexual dimorphic behaviours in adolescent rodents. Acute, but not chronic, augmentation of endocannabinoid tone attenuates core autistic-like behaviours in male rodents, while exacerbating negative affective behaviours in VPA-exposed females. These data highlight the importance of considering sex-specific effects of cannabinoids for the management and treatment of autism symptoms.

Acknowledgments: Funded by Hardiman Postgraduate Scholarship and CMNHS NUI Galway.

SEX AND HISTORY OF PASSIVE VAPOR EXPOSURE INFLUENCE Δ9-TETRAHYDROCANNABINOL (THC) VAPOR SELF ADMINISTRATION IN RATS

Elise M. Weerts[1], Catherine F. Moore[1], and Catherine M. Davis[2]

[1]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Introduction: The concentration of Δ9-tetrahydrocannabinol (THC), the primary psychoactive constituent in cannabis has been progressively increasing in marketed vape products for recreational and medical cannabis use. To increase our understanding of the vaped cannabis and its rewarding and reinforcing effects, we examined the effects of passive THC vapor exposure in place conditioning and operant self-administration of THC vapor.

Methods: Separate groups of Sprague-Dawley rats (n=24 per group, 50% female) were passively exposed to different vapor conditions in which number of puffs (p) and THC concentration (5p x 100, 5p x 200, 10p x 200 mg/ml) was varied for the 30-min session. One group received only the propylene glycol (PG) vehicle vapor and THC groups received THC and PG vapor, on alternate days, for 16 30-min place conditioning sessions; groups were tested for conditioned place preference (CPP) or aversion (CPA) after 8 and 16-days of conditioning, followed by extinction testing. Rats in the PG and THC exposure groups were assigned to THC (50 mg/ml) or PG vapor self-administration groups, in which vapor puffs were available under FR1 schedule of delivery with 1-min timeout. After acquisition of vapor self-administration, response requirement and THC concentration was varied to assess THC vapor reinforcement.

Results: The lowest THC vapor exposure condition tested (5p x 100 mg/ml THC) did not produce CPP or CPA in either sex. When THC concentration was increased, only males showed a THC-induced CPP after fewer puffs (5p x 200 mg/ml THC) (Sex × THC; $p<0.05$). When number of puffs were also increased (10p x 200 mg/ml THC condition) both sexes showed CPP ($p<0.05$), but females showed greater resistance to extinction of CPP than males. Rats that previously demonstrated CPP to 200 mg/ml THC vapor, self-administered 50 mg/ml THC vapor, and maintained self-administration when FR response costs were increased. When concentration of THC was varied (5-200 mg/kg), rats that had previously been exposured to passive THC vapor altered responding in a dose-dependent manner, and at higher rates when compared PG exposed rats. Females self-administered THC vapor at higher rates than males.

Conclusion: These data provide evidence that THC vapor has reinforcing effects in both male and female rats and is influenced by history of THC vapor exposure. The use of vaporized THC in preclinical models is important for translational value and these experiments provide a foundation for evaluation of the rewarding effects of cannabis constituents and consequences of vapor exposure.

Acknowledgments: Funded by the NIH/NIDA (R21 DA046154) and the Dalio Fund in Decision Making and the Neuroscience of Motivated Behaviors. THC provided by the NIDA drug supply program

SEX AND DOSE-DEPENDENT DELAY IN ANTINOCICEPTIVE TOLERANCE TO CP55,940 IN CB1 DESENSITIZATION-RESISTANT MUTANT MICE DUE TO JNK INHIBITION

Melissa McHann[1], Isabel Castro-Piedras[1], Daniel J. Morgan[2] and Josée Guindon[1,3]

[1]Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, Lubbock, TX, 79430; [2]Department of Biomedical Sciences, Joan C Edwards School of Medicine, Huntington, WV, 25755; [3]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX, 79430.

Introduction: Studies from our group and others have found sex-differences in the response to cannabinoid compounds (Blanton et al., 2021). Chemotherapy-induced peripheral neuropathy (CIPN) is a clinical challenge for cancer patients. The development of novel targeted therapies with long-term efficacy in alleviating CIPN is an ongoing focus of preclinical research. However, our current understanding of the mechanisms underlying tolerance to cannabinoid compounds remains elusive as does the contribution of sex differences to this process.

Methods: The objective of our current work is to assess in S426A/S430A female and male mutant mice whether JNK (SU 3327) inhibitor, CP55,940 (mixed CB_1/CB_2 receptor agonist) alone or in combination demonstrate sex-specific antinociceptive effects and delay in the development of tolerance using cisplatin (5 mg/kg/week) as a CIPN model. We also evaluated co-immunoprecipitation of JNK1 and JNK2 with β-arrestin 2 in HEK293-CB_1 cells.

Results: Our study found that SU 3327 (3 mg/kg i.p.) partially reversed mechanical (von Frey) and cold (acetone) allodynia in male and female KI mice from day 8 to day 35. However, this effect was not observed at a lower dose of SU 3327 (1 mg/kg i.p.). When low dose SU 3327 (1 mg/kg i.p.) was combined with CP55,940 (0.3 mg/kg i.p.), there was a delay in the development of tolerance to the effects of CP55,940 on mechanical and cold allodynia in female mice. Indeed, tolerance to the effects of CP 55,940 on mechanical allodynia developed on day 28 for CP55,940 alone and on day 33 for SU 3327 + CP55,940 for female KI mice. Tolerance to effects of CP55,940 on cold allodynia developed on day 27 for CP55,940 alone and on day 32 for SU 3327 + CP55,940 for female KI mice. For male KI mice, tolerance to the effects of CP55,940 on mechanical and cold allodynia developed on day 33 and on day 32 for CP55,940 alone or combined with SU 3327, respectively. To better understand a possible mechanism for these findings we performed co-immunoprecipitation experiments and found that JNK2 and β-arrestin 2 form a complex in CB_1-espressing HEK293 cells.

Conclusions: Our results illustrate the important role of sex in the development of cannabinoid tolerance in the context of chronic pain and the contribution of sex-specific mechanisms of action.

Acknowledgements: Funded by NIH grants DA044999 (JG, DM) and Texas Tech University Health Sciences Center School of Medicine 121035 (JG).

SEX DIFFERENCES IN CANNABIS WITHDRAWAL AND THE ROLE OF THE ENDOCANNABINOID SYSTEM

Erin L. Martin[1] and Aimee L. McRae-Clark[2,3]

[1]Department of Neuroscience, Medical University of South Carolina, Charleston, SC, USA
[2]Department of Psychiatry & Behavioral Sciences, Medical University of South Carolina, Charleston, SC, USA
[3]Ralph H. Johnson VA Medical Center, Charleston, SC, USA

Introduction: Cannabis Use Disorder affects nearly a fifth of people with past-year cannabis use and is characterized by difficulty controlling use, craving, and withdrawal symptoms. Cannabis withdrawal typically begins within 24 hours of cessation of use and can decrease an individual's quality of life and contribute to relapse. Previous retrospective work indicates that cannabis withdrawal symptoms may be more severe in women, putting them at an increased risk of relapse during a quit attempt. However, the biological basis for this sex difference is presently unknown.

Methods: Ten non-treatment-seeking individuals (5 men, 5 women) with moderate or severe Cannabis Use Disorder were enrolled in a two-week study. During the first week participants were to continue cannabis use as usual, and during the second week participants were asked to remain abstinent. In women, study weeks were each scheduled to occur during the early follicular phase of the menstrual cycle. All participants attended six in-person laboratory visits, three during each week. At each visit, participants completed a mobile assessment battery and provided blood samples to assess endocannabinoid content at 9:00, 12:00, and 13:00. A similar assessment battery examining cannabis use, eating, sleep, affect, and cannabis withdrawal was completed at home at 9:00, 12:00, 17:00, and 21:00 on the remaining days in each study week. Data were analyzed using mixed-effects models.

Results: Men and women were well-matched on demographic and baseline cannabis use characteristics. Sexes did not differ significantly in cannabis use behavior (i.e. reporting of any cannabis use, number of cannabis use sessions since last survey) during the use-as-usual week when controlling for an effect of time (p's > 0.05). However, a significant sex by week interaction was observed in cannabis withdrawal symptom expression when controlling for time, such that women reported a much greater increase in overall symptoms during the abstinent week relative to men ($p = 0.006$). Specifically, significant sex by week interactions were observed for reduced appetite ($p = 0.003$), stomachache ($p < 0.001$), and feeling physically tense ($p < 0.001$). Significant effects of week, but not sex, were observed for subjective sleep quality ($p = 0.028$) and positive affect when controlling for time ($p = 0.018$). Endocannabinoid outcomes have not yet been analyzed.

Conclusions: Significant sex differences can be observed in real-time self-reported cannabis withdrawal symptoms, particularly gastrointestinal symptoms, consistent with previous retrospective research. These symptoms may be associated with sex-specific disruptions in endocannabinoid signaling during an abstinence period. Development of pharmacotherapies that target withdrawal symptoms may improve rates of abstinence in women with Cannabis Use Disorder.

Acknowledgements: Funded by NIH/NIDA U54-DA016511 and T32-DA007288.

EVALUATION OF SEX DIFFERENCES IN ENDOCANNABINOID-MEDIATED MODULATION OF NOCICEPTIVE BEHAVIOUR IN PERIPHERAL NEUROPATHY

Laura Boullon, David P Finn and Álvaro Llorente-Berzal

Pharmacology and Therapeutics, School of Medicine, Galway Neuroscience Centre, Centre for Pain Research, National University of Ireland Galway

Introduction: Clinical evidence supports the existence of sex differences in the cannabinoid modulation of nociception. The phytocannabinoids cannabidiol and Δ^9-tetrahydrocannabinol differentially affect pain-related behaviours between sexes. Despite the description of sex differences in the endocannabinoid tone, density and functionality of endocannabinoid receptors in critical regions of the descending pain pathway, the endogenous mechanisms underlying these sex-specific effects remain unclear. In the present study, we characterize alterations in the endocannabinoid system after chronic peripheral nerve injury; and we investigate the potential antinociceptive effects of the FAAH inhibitor URB597 and the MGL inhibitor MJN110, in male and female animals.

Methods: Mechanical and cold hypersensitivity were regularly assessed in male and female adult Sprague-Dawley rats following Sham or Spared Nerve Injury (SNI) surgery from post-injury day seven to 99. Post-mortem analysis on post-injury day 100 investigated alterations in endocannabinoid ligands (AEA and 2-AG) and related *N*-acylethanolamines levels (PEA and OEA) in the prefrontal cortex (PFC), periaqueductal grey (PAG) and rostroventromedial medulla (RVM) using high-pressure liquid chromatography-tandem to mass spectrometry (HPLC-MS/MS). In addition, protein expression of cannabinoid receptors (CB_1R and CB_2R) and endocannabinoid degrading enzymes (FAAH and MGL) were examined in the same brain regions by Western blot. The therapeutic potential of FAAH and MGL inhibition in SNI-induced mechanical and cold hypersensitivity was assessed in a separate *in vivo* experiment. The pharmacological tools URB597 (0.3mg/kg i.p.) or MJN110 (1mg/kg i.p.), were administered systemically from post-injury day 7 to 22 to inhibit FAAH and MGL enzymes respectively. Post-mortem analysis investigated endocannabinoid and related *N*-acylethanolamine levels in PFC, PAG and RVM using HPLC-MS/MS.

Results: Both male and female animals developed a significant hypersensitivity to mechanical and cold stimulation seven days post-injury. The responses to mechanical and cold stimuli were significantly higher in females than males, suggesting greater sensitivity in this sex. The post-mortem analysis revealed sex-specific alterations across the endocannabinoid system. Male-SNI animals showed higher levels of 2-AG and PEA in PFC and RVM than their control groups, an effect not observed in females. These alterations correlated with decreased mechanical hypersensitivity in this sex. SNI significantly increased MGL expression in the PAG of female rats only. This effect correlated positively with intensified cold allodynia in this sex. URB597 administration attenuated SNI-induced mechanical and cold hypersensitivity, however, the magnitude of this attenuation was greater in females than in males. Administration of MJN110 failed to alter any of the pain-related behaviours analysed. The post-mortem examination of endocannabinoid levels revealed a significant increase of PEA and OEA in the PFC, PAG and RVM of male-SNI animals following URB597 administration. This URB597-effect was only shown in the PAG of female-SNI animals.

Conclusions: SNI induces sex-specific alterations in the brain´s endocannabinoid system, associated with pain sensitivity. Furthermore, inhibition of FAAH had antinociceptive effects in female but not male animals. This antinociceptive effect was accompanied by concomitant alterations in the endocannabinoid levels in the PAG, suggesting a key role for the PAG in sex-specific modulation of nociception by the endocannabinoid system.

Funded by Irish Research Council. Laureate Award (IRCLA/2017/78)

SEX-DEPENDENT CHANGES IN MURINE STRIATAL DOPAMINE RELEASE, SLEEP, AND BEHAVIOR DURING SPONTANEOUS Δ-9-TETRAHYDROCANNABINOL ABSTINENCE

Andrew J. Kesner, Stephanie Ramos-Maciel, Yolanda Mateo,
Alexa L. Gracias and David M. Lovinger

NIAAA, Rockville, MD USA

Introduction: Withdrawal symptoms are observed upon cessation of cannabis use in humans. Known consequences of withdrawal from chronic cannabis use in humans as outlined by DSM-V include irritability, decreased appetite or weight loss, restlessness, depressed mood and hedonics, and more. These symptoms are thought to be driven by physiological changes that correspond with building tolerance to delta-9-tetrahydrocannabinol (THC) during prolonged cannabis use. THC is the major psychoactive ingredient in cannabis and in laboratory animal studies THC withdrawal symptoms are typically precipitated by treatment with a cannabinoid type 1 receptor antagonist or inverse agonist. However, this does not mimic the normal course of withdrawal in human cannabis users. In contrast, spontaneous THC withdrawal symptoms are thought to be difficult to observe in laboratory animal models, and as such, back-translational studies on the physiological mechanism of cannabis/THC withdrawal are lacking. We aimed to fill this knowledge gap by implementing mouse behavioral paradigms that target specific clinical withdrawal symptoms.

Methods: THC tolerance was induced via 10 intraperitoneal injections of 10 mg/kg THC over 6 days. Behavioral measurements from pretreatment, and early and late THC abstinence epochs were obtained, and we investigated between group (THC vs. Vehicle) and within-subject comparisons of changes in behaviors driven by withdrawal from chronic THC, i.e., withdrawal symptoms.

Results: *Sleep.* We observed profound alterations in sleep and vigilance-state architecture in male mice during early THC withdrawal that normalized in late withdrawal. The metrics mimic clinical observations. Conversely, female mice appear resilient to effects of THC withdrawal on sleep. *Reward seeking.* Mice performing an operant-cue discrimination task tended to earn fewer rewards, and responses to reward predictive cues were more prominent in male mice, suggesting motivation and attention is altered in mice during THC abstinence. Conversely, we found no change in sucrose preference, indicating hedonics are not affected by THC withdrawal. *Irritability.* Using the bottle brush test we found specific alterations of irritability-like behaviors in male mice only. *Consumption and Locomotion.* During twenty four-hour home cage food and water intake and locomotion monitoring we observed minimal alterations to intake and locomotion during THC withdrawal. *Plasma corticosterone.* Changes in these behavioral indices do not appear to be mediated by stress per se, as circulating plasma corticosterone was only modestly increased in male but not female mice. *Neurochemistry.* The neurotransmitter dopamine plays a known role in manifestation of withdrawal symptoms, so we performed ex-vivo fast-scan cyclic voltammetry to measure dopamine levels within the striatum male and female mice differed in their spatial and temporal profiles related to alterations in striatal dopamine.

Conclusions: These studies describe several mouse behaviors with translatable relevance that are altered during spontaneous withdrawal from THC. These data open the door for further pre-clinical research efforts to determine the neurobiological bases of, and potentially treat, primary withdrawal symptoms of cannabis use disorder. Male and female mice differed in their expression of THC withdrawal symptoms during both sleep and wake.

This work was supported by the Division of Intramural Clinical and Biological Research of the National Institute on Alcohol Abuse and Alcoholism [grant number ZIA000416 to DML].

TOPICAL CANNABIDIOL IN PATIENTS WITH LOW BACK PAIN SECONDARY TO SPONDYLOLISTHESIS: A RANDOMIZED, DOUBLE-BLIND, 3-ARM, PARALLEL-GROUP PILOT STUDY

T.J. Dowling III, Nicholas Frane, Erik Stapleton, Larry Good, Tim Garret and Thomas Dowling

Zucker School of Medicine at Hofstra/Northwell, Huntington, NY, USA
The CORE Institute Department of Orthopaedic Surgery, Phoenix, AZ, USA
New England Baptist Department of Orthopaedic Surgery, Boston, MA, USA

Introduction: Low back pain is one of the most common reasons for seeing a healthcare professional and represents an opportunistic target for alternative measures for pain management. Few studies exist looking at the effects of topical administration of CBD on low back pain. Furthermore, to our knowledge no previous research has evaluated the cannabinoid CBDA for topical use. With the rising popularity of these products, research is needed now more than ever to help guide physicians when counseling patients on their appropriate use. The purpose of this study is to evaluate the safety and feasibility of our trial using CBD, CBD/CBDA, and placebo for management of pain in patients with low back pain secondary to a diagnosis of lumbar spondylolisthesis.

Methods: This was an IRB approved, randomized, double-blind, placebo-controlled 3-arm parallel-group study that evaluated the safety and efficacy of CBD, CBD/CBDa, and placebo creams in 34 subjects with low back pain secondary to spondylolisthesis. Subjects applied the cream directly to the site of pain three times daily during the 14-day study period. A diary for each subject was maintained to record their pain at time of each application and at scheduled increments thereafter, using a VAS scale from 1-10, as well as the need for any rescue medication (acetaminophen). A quality of life (QOL) survey, Roland-Morris low back pain and disability questionnaire (RMQ), and Oswestry Low Back Pain Disability Questionnaire (ODI) were also administered before and after each study period. Blood samples were drawn after 14 days to evaluate for the presence of cannabinoids or their metabolites. Safety was assessed by considering any adverse events, as well as by comparing screening labs at the beginning and end of the study.

Results: We initially enrolled 40 patients who consented to participating in the study. Out of the 40 patients, 34 had complete participation. Three of the patients declined to participate in the trial after initially agreeing. The remaining three patients who did not complete the study did not consistently apply the topical cream every day and/or did not complete their daily pain diaries.

Of the 34 patients who completed the study, all the patients were compliant with their clinical outcomes' surveys, pre and post laboratory testing, and plasma sample evaluation for detectable cannabinoid metabolites. There were no adverse events from the study. Complete blood counts and complete metabolic panel testing showed no abnormal values before or after the 14 days' of using the different cannabidiol formulations or placebo. Lastly, no patient had detectable levels of systemic cannabinoids or their metabolites.

Pain improvement from day 1-14 was largest in the CBD/CBDA group (25.5% pain reduction), compared to CBD group (17.3% pain reduction) and Placebo group (4.6% pain reduction), however these were not statistically significant differences ($p = 0.41$). In addition, there was no statistically significant difference with regards to their RMQ ($p = 0.31$), ODI ($p = 0.22$), or QOL ($p = 0.74$).

Conclusions: This is the first study to look at topical application of different formulations of CBD and CBD/CBDA, and demonstrated that it is safe, without any adverse events or detectable systemic absorption. While clinical outcomes were not significant, this pilot study was not powered to detect statistical differences in these outcomes, and these results pave the way for larger-scale future studies.

SEX DIFFERENCES IN SUBJECTIVE AND REINFORCING EFFECTS OF SMOKED CANNABIS: A SECONDARY ANALYSIS OF TWO RANDOMIZED CONTROLLED TRIALS

Stephanie Lake,[1,2] Margaret Haney[3] and Ziva D. Cooper[1,2,4]

[1]UCLA Cannabis Research Initiative, Jane and Terry Semel Institute, UCLA, USA
[2]Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, UCLA, USA
[3]Department of Psychiatry, Columbia University Irving Medical Center, New York, USA
[4]Department of Anesthesiology and Perioperative Medicine, David Geffen School of Medicine, UCLA, USA

Introduction: The prevalence of cannabis use among women has grown substantially in recent years concurrent with cannabis law reform. Preclinical studies suggest possible biological sex-based differences in the reinforcing effects of cannabinoid receptor 1 (CB1) agonists such as delta-9-tetrahydrocannabinol (THC). However, findings from the limited number of human experimental trials probing acute subjective drug effects of THC by biological sex are inconsistent, and sex differences in reinforcing effects have not been assessed. This study sought to explore sex differences in subjective and reinforcing drug effects following administration of active cannabis.

Methods: Data from two within-subject randomized controlled trials comparing the subjective and reinforcing effects of smoked active cannabis (~25 mg THC) versus placebo cannabis (0 mg THC) were pooled for this analysis. Participants included healthy males (n = 63) and females (n = 13) who smoked cannabis near-daily. Subjective mood and drug effects of experimenter-administered cannabis were measured via the Subjective Effect-Visual Analog Scale (SE-VAS) and Cannabis Rating Form (CRF). To measure reinforcing effects, participants had an opportunity to self-administer up to 3 puffs (cost: $1/puff) of the experimenter-administered cannabis sampled earlier in the session. We used generalized linear mixed models to examine subjective mood and drug effects as a function of cannabis strength, sex and time, and self-administration as a function of cannabis strength and sex. We tested for sex interactions with time (subjective effects) and cannabis strength (subjective effects, self-administration).

Results: Active cannabis significantly increased subjective ratings of 'High', 'Stimulated', 'Anxious', and 'Good Effect' and significantly decreased ratings of 'Crave cannabis' relative to placebo ($p<0.01$). Females reported greater reductions in cannabis craving following active cannabis administration compared to males ($p<0.05$). Active cannabis increased drug ratings of 'Strong', 'Liking', 'Take again', and 'Good Effect' relative to placebo ($p<0.01$), and females reported significantly higher ratings relative to males on all measures after active cannabis administration ($p<0.05$). Placebo cannabis was self-administered by 12 (19.0%) males and 2 (15.4%) females ($p=0.89$). Active cannabis was self-administered by proportionally more females than males (53.8% versus 31.7%), but this difference was not significant ($p=0.40$). In a generalized linear mixed model accounting for within-subject shared variability, active cannabis was significantly associated with self-administration of the drug ($p=0.02$), but this effect was not sex-dependent ($p=0.18$).

Conclusions: Under active cannabis conditions, females experienced greater reductions in cannabis craving and reported higher ratings of positive subjective drug effects relative to males, replicating our earlier findings. Despite these differences, females were not more likely to self-administer active cannabis. Other sex- or gender-based factors may modify the relationship between heightened sensitivity to certain subjective effects and reinforcing effects. However, given the small number of females in this secondary exploratory analysis, future research with a higher number of females is needed to test sex-based differences in cannabis' acute subjective drug, mood, and reinforcing effects.

Acknowledgements: This research was supported by the US National Institute on Drug Abuse (NIDA: DA047296, DA19239, DA09236, DA027755) and the Semel Charitable Foundation.

AN ASSESSMENT OF CURRENT AND PREVIOUS EXPOSURE TO ILLICITLY OBTAINED CANNABIS IN UK MEDICAL CANNABIS PATIENTS

Lucy J Troup[1], Simon Erridge[2,3], Beata Ciesluk[1]and Mikael H Sodergren[2,3]

1. Division of Psychology, School of Education and Social Sciences, University of the West of Scotland, UK
2. Medical Cannabis Research Group, Imperial College London, UK
3. Sapphire Medical Clinics, London, UK

Introduction: In November 2018, the UK government changed the scheduling of cannabis-based medicinal products (CBMPs) to enable them to be prescribed by specialist physicians, supported by a multi-disciplinary team. Moreover, as CBMPs are unlicensed in the UK, patients must have failed to gain sufficient benefit from first-line licensed therapies. Data from the UK Medical Cannabis Registry has demonstrated that approximately 60% of patients treated with CBMPs in the UK since this change in regulation were consuming illicitly purchased cannabis either at the time initiating therapy, or earlier in their lives. Whilst patients are counselled as to the risks of continuing to source and consume cannabis from illicit sources, the exact extent of prior and current consumption is not known. This study therefore aims to assess the previous and current exposure of those undergoing active treatment with CBMPs.

Methods: This cross-sectional questionnaire study invited 2,319 patients from Sapphire Medical Clinics who were being actively treated with CBMPs. The questionnaire was developed utilising a multidisciplinary group of academic clinicians and psychologist to establish patient demographics, in addition to previous and current cannabis consumption. The Sapphire Medical Clinics Patient and Public Involvement Group (n=7) assessed the content validity and study feasibility. The questionnaire was hosted on Qualtrics (Seattle, Washington, United States). Reponses were assessed utilising descriptive analysis.

Results: 450 (19.4%) active patients completed the questionnaire. 258 (57.3%) identified as male and 176 (39.1%) identified as female. 22.2% (n=100) of patients first started consuming cannabis as a prescription medication under supervision of a doctor. 44.2% (n=199) and 33.6% (n=151) of patients first started consuming cannabis recreationally or self-medicating with illicitly purchased cannabis respectively. The mean age participants started consuming cannabis was 28.0 (±16.3) years old. The mean length of cannabis consumption to date was 12.9 (±11.8) years. 71.8% (n=323) of participants were only consuming CBMPs as prescribed. 13.1% (n=59) and 17.8% (n=80) were continuing to consume illicit cannabis recreationally or source illicit cannabis for self-medication respectively. 4.2% (n=19) of patients were consuming other illicit drugs for either recreational or perceived medical benefit.

Conclusion: This study demonstrates that most patients treated with CBMPs sampled in this study were consuming cannabis prior to initiating treatment. On average this was for almost 13 years. Despite only 22.2% of patients being naïve to cannabis at initiation of therapy over 70% were only consuming CBMPs and no other illicitly sought cannabis. This suggests that, in addition to any derived medical benefits, supervised treatment with CBMPs can reduce the exposure to the associated harms of illegally sourced cannabis.

ORIGINS OF *CANNABIS* IN IRELAND: AN INTERDISCIPLINARY STUDY

John M. McPartland[1,2] and Saoirse E. O'Sullivan[3]

1. University of Vermont, Burlington, VT, USA
2. GW Pharmaceuticals, Sovereign House, Histon, Cambridge CB24 9BZ, UK
3. Arteolo Biosciences, La Jolla, CA, USA

Introduction: Ireland's agriculture has been shaped by innovations brought there by others, with the exception of heather, gorse, kelp, peat, and Guinness Stout. Many people who migrated to Ireland were known to cultivate hemp, *Cannabis sativa* L.: the Iron Age Celts, Romano-British Christians, Vikings, Anglo-Normans, and English. But who introduced hemp? This question will be addressed with data from historical linguistics, palynology (fossil pollen), archaeology, and historical documents.

Methods: Words for hemp/*Cannabis* in six Celtic languages were analyzed to separate cognates (words that share common etymological origins) from loanwords (borrowed from other languages). The European Pollen Database (http://www.europeanpollendatabase.net) was searched for fossil pollen studies conducted in Ireland. Identification of *Cannabis* fossil pollen utilized the "ecological proxy" method (McPartland *et al.* 2018). Archaeological evidence obtained from the literature was stratified by its relative robustness or validity, utilizing a validated algorithm (McPartland and Hegman 2018). Historical documents included Irish glossaries, law tracts, epics, and mythologies, obtained as machine-readable and searchable documents (https://www.ucc.ie/en/smg/cdi/).

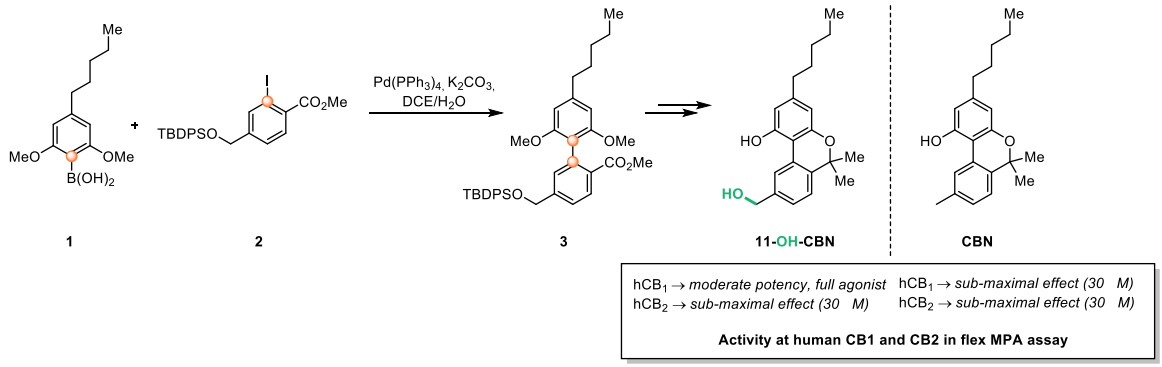
Results: "Hemp" words in Celtic languages are loanwords, not cognates. The Irish word *cnáib* (hemp) is first attested in an epic written 1127-1134 CE, that recorded the deeds of Cellachán Caisil (died 954 CE). Pollen consistent with cultivated *Cannabis* appeared in the Middle Ages, at sites in the vicinity of monasteries, beginning *ca.* 700 CE. Archaeological finds (hemp seeds, fiber) date to later Norse-Viking and Anglo-Norman sites, *ca.* 900-1300 CE.

Discussion: The lack of "hemp" cognates in Celtic languages implies that Celtic people who moved to Ireland and the British Isles did not know hemp. The Irish word *cnáib* is absent in Old Irish glossaries, epics, and mythologies (800-1100 CE) and finally appears *ca.* 1127-1134 CE. The onset of hemp cultivation in Ireland, according to fossil pollen data, corresponded chronologically and geographically with the founding of Romano-British monasteries. Irish *cnáib* was likely a loanword from Clerical Latin, and it was not replaced by the Norse-Viking word *hampr*. The origin of *Cannabis* in Ireland differs from its origin in England, where Anglo-Saxon *hænep* became the English word *hemp*.

A NEW SYNTHESIS OF 11-HYDROXY-CANNABINOL AND PHARMACOLOGICAL CHARACTERISATION AT HUMAN CANNABINOID RECEPTORS

Adam Ametovski,[1] Elizabeth Cairns,[1] Rochelle Boyd,[1] Jonathon Arnold,[1] Iain McGregor[1] and Samuel Banister[1]

[1]Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, The University of Sydney, Camperdown, NSW 2050, Australia



Introduction: Given the increasing interest in cannabinol (**CBN**), our understanding of its mechanism of action, and importantly that of its primary metabolites, is crucial to its development as a therapeutic agent. Despite this, little *in vitro* data are known regarding the pharmacological activity of 11-hydroxy-CBN (**11-OH-CBN**) at human cannabinoid receptors, although nanomolar binding and inhibitory activity of adenylcyclase at low concentrations at rat CB1 and CB2 has been previously reported. Prior syntheses of 11-OH-CBN have been described; however, these are often lengthy, rendering them unsuitable for large scale *in vivo* studies. Thus, we sought to develop a new synthetic approach to access **11-OH-CBN** and characterise functional activity at human CB receptors (hCB).

Methods: We envisaged a 4-step linear sequence to access to **11-OH-CBN** using a Suzuki coupling without the use of silyl protection to optimise synthetic ideality and maximise yield. Efficacy and potency of **11-OH-CBN** at hCB1 and hCB2 in stably transfected AtT20 cells was evaluated using a fluorescence-based membrane potential assay.

Results: While our ideal synthetic design was not viable with the naked benzyl alcohol, silyl protection enabled the synthesis of **11-OH-CBN** through an efficient Suzuki coupling between olivetol derived boronic acid (**1**) and an appropriately functionalised aryl iodide (**2**). The resulting diaryl scaffold (**3**) was converted to **11-OH-CBN** following Grignard addition, cyclisation and deprotection. In the functional assay, **11-OH-CBN** exhibited full agonist activity at hCB1 (EC_{50} = 1.20 µM, E_{max} = 120% of 1 µM CP 55,940), while at 30 µM exhibited sub-maximal efficacy at hCB2 (E_{max} = 55%) Comparatively, **CBN** itself produced a sub-maximal effect at both hCB1 (E_{max} = 10%) and hCB2 (E_{max} = 18%) at 30 µM).

Conclusion: A new synthetic route to **11-OH-CBN** was developed exploiting an efficient Suzuki coupling followed by routine functional group interconversion. In a fluorescence-based membrane potential assay, **11-OH-CBN** was shown to be a moderate potency full agonist of CB1, and display low potency activity at CB2. Given that CBN itself elicits a submaximal response in our assay, these findings could provide rationale for the biological effects associated with CBN through activity of its metabolite.

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

OLEOYL SEROTONIN, AN ACTIVE SMALL MOLECULE IN VENOMS OF *STEPHANOCONUS* SNAILS, IS AN ANTAGONIST OF THE CANNABINOID RECEPTOR TYPE 1

Dongchen An, Steve Peigneur and Jan Tytgat

Toxicology and Pharmacology, University of Leuven (KU Leuven),
Campus Gasthuisberg, O&N2, PO Box 922, Herestraat 49, 3000 Leuven, Belgium

Introduction: The cannabinoid receptor type 1 (CBR1) plays a critical role in numerous human physiological and pathological conditions. Thus, hundreds of CBR1 ligands have been developed, such as phytocannabinoids, synthetic cannabinoids, and peptide-type cannabinoids from animals, with varying affinities relevant for the treatment of diverse diseases. In this context, the venoms of animals can be seen as a tremendous source for finding new drugs to combat the diseases where CBR1 is involved. All cone snails have complex venoms. Each venom has its distinctive chemistry, typically over a hundred bioactive venom components. Oleoyl serotonin (OS) is one of the most abundant compounds discovered in the venoms of *Stephanoconus* snails, certainly exhibiting activity in predation, while it also exists in the gastrointestinal tract of pigs. Though OS is an endocannabinoid-like molecule, it was shown to be an antagonist of transient receptor potential cation channel subfamily V member 1 (TRPV1). In the present study, we have investigated whether OS modulates human CBR1 expressed in *Xenopus laevis* oocytes and have performed selectivity screenings on other ligand-gated receptors.

Methods: We recently reported the oocyte expressing human CBR1 and the G protein-coupled inwardly rectifying potassium (GIRK) channels 1/2, as well as the regulator of G-protein signaling 4 (RGS4), is a robust and reliable system for electrophysiologically characterizing ligands of CB receptors (An, D.; Peigneur, S.; Tytgat, J., *Biomedicines* 2021; 9(5):484.). Oocytes were injected with a mixture of hCB1 or h-μ-opioid receptor (hMOR), mGIRK1, mGIRK2, and RGS4 mRNAs, a mixture of mGIRK1 and mGIRK2 mRNAs, or alpha7 nicotinic acetylcholine receptor (alpha7-nAChR) mRNA, and then were maintained at 16 °C. Currents from oocytes were recorded 2-4 days after injection by two-electrode voltage-clamp (TEVC). The effect of OS in combination with agonists was then assessed on these oocytes. Data were analyzed by GraphPad Prism 9 and shown as mean±standard deviation (SD).

Results: 100 μM OS did not induce K^+ currents in non-injected oocytes and did not alter K^+ current amplitude in oocytes expressing GIRK1/2. However, in oocytes co-expressing CBR1-GIRK1/2-RGS4, OS competitively inhibited K^+ currents induced by WIN55,212-2 (WIN, a CBR agonist) in a concentration-dependent manner. The IC_{50} values of effects of 0.03, 0.1, 0.3, and 1 μM WIN are 2.44, 7.28, 16.1, and 34.1 μM, respectively. Interestingly, 100 μM OS did not alter K^+ currents induced by 0.2 μM morphine (near the EC_{50} of morphine) in oocytes co-expressing MOR-GIRK1/2-RGS4 and Na^+ currents induced by 100 μM acetylcholine (ACh) (near the EC_{50} of ACh) in oocytes expressing alpha7-nAChR.

Conclusions: It is concluded that OS competitively inhibits the activation of CBR1 evoked by WIN, while not showing activity on GIRK1/2, MOR, and alpha7-nAChR. The results open a window for the discovery of new ligands interacting with CBR1 with a focus on animal venoms.

Acknowledgements: Funded by the China Scholarship Council (Grant No. 201907060021) and FWO-Vlaanderen (Grant No. GOA4919N, GOE7120N, GOC2319N and 12W7822N).

FAAH GENETIC INACTIVATION LEADS TO A PRO-INFLAMMATORY, YET NEUROPROTECTIVE, PHENOTYPE IN 5XFAD MICE

M. Andrea Arnanz[1], Kevin Troule[2], Gonzalo Ruiz-Pérez[1], Irene Benito-Cuesta[1], Fernando Puente-Sánchez[3], Ana M. Martínez Relimpio[1], María Posada-Ayala[1], Benjamin F. Cravatt[4], Fátima Al-Shahrour[2], M. Teresa Grande[1], Rocío Palenzuela[1] and Julián Romero[1]

[1]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, Pozuelo de Alarcón; [2]Spanish National Cancer Research Centre (CNIO), Madrid, Spain; [3]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden; [4]The Skaggs Institute For Chemical Biology and Departments of Cell Biology and Chemistry, The Scripps Research Institute, La Jolla, California, USA

Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder that causes the most common form of dementia among the elderly. The presence of extracellular neuritic plaques, mainly constituted by pathogenic amyloid peptides, together with intracellular hyperphosphorylation of tau protein in neurofibrillary tangles are the main pathologic features of AD. We have recently reported a link between the genetic inactivation of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) in the context of amyloidosis and an increased expression of some inflammatory cytokines (such as interleuquin-1 beta and 1 alpha, TNF-alpha, etc.) and glial receptors (such as TREM2 and C3AR). Paradoxically, 5xFAD/FAAH-deficient mice showed improvements in behavioral parameters, recovery of synaptic dysfunction and dendritic spine preservation.

Methods: RNA from 5xFAD and 5xFAD/FAAHKO mice hippocampi was isolated with the RNeasy mini kit (Qiagen). Samples were analyzed by using the NovaSeq 6000 sequencing platform (Macrogen). Data were processed using the Nextpresso protocol, so the quality of the readings was studied with FastQC (v0.11.7) and aligned against the mouse genome (GRCm39) by using TopHat 2.0.10 and Bowtie 1.0.0 and SAMtools 0.1.1.9. Functional analysis was performed with GSEAPreranked and using a limit of FDRq value of <0.25 as statistically significant.

Results: GSEA analysis revealed defined MSigDB gene sets related to the inflammatory response positively linked to FAAH genetic inactivation (TNF-alpha signaling via NFKB, interferon gamma response and IL6/JAK-STAT3 signaling, among others). At the same time, a significant negative enrichment in Alzheimer's and Parkinson's disease gene sets was observed.

Conclusions: These data confirm and expand the notion of a profound change in gene expression linked to the genetic inactivation of FAAH in the context of AD.

Keywords: Alzheimer's disease, FAAH, neuroinflammation, transcriptomic analysis, GSEA, enrichment of gene networks.

Acknowledgements: Funded by Ministerio de Ciencia, Innovación y Universidades (PID2019-108992RB-I00)

CHRONIC EXPOSURE TO *CANNABIS SATIVA* EXTRACTS IMPROVES METABOLIC DYSFUNCTION AND DYSREGULATION OF THE ADIPOINSULAR AXIS IN DIET-INDUCED OBESE MICE

Bryant Avalos, Courtney P. Wood and Nicholas V. DiPatrizio

Division of Biomedical Sciences, School of Medicine, University of California, Riverside

Introduction: The endocannabinoid (eCB) system in the brain and peripheral tissue controls food intake and energy homeostasis. In general, activation of the eCB system increases food intake and storage of energy from food for future use as fuel. In contrast, inhibiting eCB system activity reduces food intake and body weight, and increases energy expenditure in diet-induced obesity (DIO). Similar appetite-stimulating effects occur following acute consumption of *Cannabis sativa* and low doses of its main intoxicating chemical constituent, Δ^9-tetrahydrocannabinol (THC). Conversely, studies in humans suggest that chronic cannabis users display paradoxical improvements in a variety of metabolic parameters when compared to non-users, including decreased risk of developing Type-2 Diabetes (T2D); however, the underlying molecular mechanisms involved in these processes are largely unknown. In the current study, we tested the hypothesis that chronic exposure to THC or whole cannabis oil extracts – matched for THC content by ultra-performance liquid chromatography/mass spectrometry (UPLC/MS^2) – can mitigate metabolic dysfunction in DIO by reversing dysregulation of the adipose-pancreatic (adipoinsular) axis, which includes fat-derived adipokines that control glucose homeostasis.

Methods: Male C57BL/6Tac mice (8 weeks-of-age) were given ad-libitum access to a western-style diet (WD; 40% kcal from fat, 17% kcal from protein, 43% kcal from carbohydrates mainly from sucrose) for a total of 60 days. Beginning at day 30 and for the remainder of the study, animals were administered either pure THC (5 mg per kg) or whole cannabis oil extracts matched for THC content via our UPLC/MS^2 methods (THC at 5 mg per kg). Body weight and food intake were measured daily throughout drug exposure. Intraperitoneal glucose tolerance tests (IP GTT) and insulin tolerance tests (IP ITT) were performed in separate cohorts following the chronic exposure period. Plasma levels of adipokines were measure by enzyme-linked immunoassay (ELISA). Gene expression related to adipose tissue metabolism was assessed in visceral and subcutaneous fat depots via NanoString analysis.

Results: When compared to vehicle treatment, both THC and extracts led to decreases in body weight and white adipose tissue mass in DIO mice, which was accompanied by transient reductions in food intake that lasted for the first 7 days after drug exposure. Drug treatments reversed DIO-associated changes in expression of genes for the adipokines adipsin, leptin, and adiponectin in epididymal fat, with extracts more potently restoring levels of adipsin and leptin to levels found in lean control mice fed a low-fat/no sucrose standard lab chow. Plasma insulin levels were also lower in DIO mice treated with THC or extracts. Moreover, chronic exposure to extracts, but not THC, was associated with improved glucose clearance in DIO mice. Insulin sensitivity, as assessed by IP ITT, indicated no differences across groups in glycemic response to insulin, which suggests that extract-induced improvements in glucose homeostasis were not due to changes in tissue sensitivity to insulin.

Conclusions: These results suggest that chronic cannabinoid exposure in DIO leads to pro-metabolic effects that include transient reductions in WD consumption, decreased body fat mass, and restoration of glucose homeostasis. Moreover, results highlight the ability for cannabis to affect the adipoinsular axis and ameliorate dysfunctional energy metabolism associated with obesity. Cannabis' therapeutic potential in the context of T2D remains unclear; however, other phytocannabinoids aside from THC may be implicated in blood glucose regulation and affect the hormonal feedback loop between adipose tissue and the pancreas. Future studies will aim to further elucidate specific mechanisms in the pro-metabolic effects of cannabis exposure and will focus on identifying differential effects on energy homeostasis for THC and other chemical constituents in cannabis.

Acknowledgements: This research was supported by the Tobacco-Related Disease Research Program (T29KT0232) and the National Institute of Diabetes and Digestive Kidney Diseases (NIDDK R01 DK119498).

THE EFFECTS OF INHALED CANNABIS EXPOSURE DURING PREGNANCY ON ENDOCANNABINOID AND IMMUNE SYSTEM DEVELOPMENT IN MALE AND FEMALE RATS

Samantha L. Baglot[1,2], Catherine Hume[2,3], Lucy Javorcikova[4], Laine M. Grace[2], Robert J. Aukema[1,2], Gavin N. Petrie[1,2] and Matthew N. Hill[2,3]

[1]Graduate Program in Neuroscience, University of Calgary, AB, Canada. [2]Hotchkiss Brain Institute & Alberta Children's Hospital Research Institute, University of Calgary, AB, Canada. [3]Department of Cell Biology & Anatomy and Psychiatry, University of Calgary, AB, Canada. [4]Mount Royal University, AB, Canada.

Introduction: Growing evidence suggests that up to 20% of people report use of cannabis during pregnancy. Cannabis is often considered natural and safe; however, little is actually known about the mechanism through which cannabis may alter neurodevelopment and subsequent behaviour. Clinical studies of prenatal cannabis exposure (PCE) have shown growth retardation, alterations in many neurobehavioural trajectories, increased incidence of autism, and immune changes. However, these studies are often confounded by unknown timing and amount of exposure, as well as exposure to stress and other drugs. Importantly, animal models can control for these variables; in fact, rodent studies of PCE have shown altered locomotion, social behaviour, and cognitive dysfunction in offspring. But, to date much of this rodent work has utilized injectable Δ^9-tetrahydrocannabinol (THC), which results in dramatically different exposure levels compared to inhalation. Our studies aim to perform translational work utilizing the most common route of consumption in humans - inhalation - during pregnancy. THC exerts its effects through acting on the endocannabinoid (eCB) system, which is involved in many processes of brain development. Further, the eCB system is highly interconnected to the immune system, which also has imperative functions during brain development. Thus, THC may exert its effects on neurodevelopment and subsequent behaviour through direct modulation of the eCB system and/or indirect modulation of the immune system. The overall aim of this study is to determine the effects of PCE on eCB and immune system functioning in male and female offspring.

Methods: Utilizing a validated vapor chamber system pregnant rats were exposed to THC-heavy cannabis vapour or vehicle vapour for 15-min a day beginning on gestational day (GD) 1. In aim 1, dams and their fetuses were euthanized on GD15, 17, or 19 to examine fetal brain development across various timepoints. In aim 2, dams gave birth and their offspring were euthanized on postnatal day (PD) 0 and 5 to examine postnatal brain development. Maternal blood and spleen, placenta, and fetal brains were collected via caesarean section surgery from aim 1 and postnatal brains were collected from aim 2. Maternal blood was analyzed for levels of THC and metabolites; fetal and postnatal brains were analyzed for eCB levels via mass spectrometry, eCB and immune-related gene expression via qPCR, and cytokine levels via multiplex assay; placenta was analyzed for eCB and immune-related gene expression; and maternal spleen was analyzed for cytokine levels.

Results and Conclusions: Results from aim 1 have found no impact of prenatal exposure to THC on fetal brain eCB levels or cannabinoid receptor gene expression. Analysis of eCB and immune-related gene expression and cytokine levels are ongoing. In conjunction with ongoing research, our results may help determine the mechanism through which PCE alters neurodevelopment and help determine the safety of cannabis consumption during pregnancy.

Acknowledgments: This research was supported by CIHR Foundation Grant, Operating Grant and Cannabis Research in Priority Areas (Mental Health) Team Grant, as well as NSERC and HBI funding to MN Hill, and a CIHR Vanier Doctoral Scholarship and ACHRI Graduate Award to SL Baglot.

PARADOXICAL PHARMACOLOGICAL PARAMETERS OF CANNABIDIOL IN CB_1 RECEPTOR BINDING AND FUNCTIONAL ASSAYS

Daniel G. Barrus, Jenny L. Wiley and Thomas F. Gamage

Discovery Sciences, RTI International, Research Triangle Park, NC, USA

Introduction: Despite the popularity of cannabidiol (CBD) and broad acceptance of its therapeutic value, its pharmacology remains poorly understood. Numerous pharmacological targets for CBD have been identified, but, generally, CBD appears to possess little affinity or efficacy at these targets, including CB_1. Some evidence indicates that CBD has higher potency as an antagonist of CB_1 agonist effects than would be expected based on its CB_1 affinity,[1] and negative allosteric modulation has been suggested as a mechanism.[2] To further examine CBD's pharmacology at CB_1 receptors, we performed a systematic and kinetic analysis of CBD's binding affinities and impact on a number of agonists' functional effects at the level of the G protein and on cAMP production.

Methods: CBD's hCB_1 binding affinities for the inactive and active conformations were determined using [^{3}H]SR141716A and [^{3}H]CP55,940 competition binding, respectively. CBD's effects were assessed in assays of G protein activation, including 1) agonist-stimulated [^{35}S]GTPγS binding and a novel BRET^2 biosensor (TRUPATH) to measure activation of Gαi3/Gβ3γ9 proteins, and 2) downstream cAMP signaling with the cAMP biosensor CAMYEL. CBD's hCB_1 dissociation was evaluated with [^{3}H]SR141716A saturation binding in membranes treated with 32 μM CBD for 2 h and then rinsed for 30 min or 2 h to allow for dissociation of CBD.

Results: CBD fully displaced 1 nM [^{3}H]SR141716A (K_i = 965 nM) and 1 nM [^{3}H]CP55,940 (K_i = 1560 nM), coming to apparent equilibrium by 1 h. In agonist-stimulated [^{35}S]GTPγS binding, CBD produced probe-dependent reductions in potency (pEC50) and efficacy (E_{max}) for CP55,940, Δ^9-THC, and AEA. Specifically, CBD concentrations above 3.16 μM reduced Δ^9-THC's potency, whereas CBD concentrations below 31.6 μM did not significantly reduce the potency of CP55,940 or AEA, while CBD concentrations above 10 μM reduced the efficacy of CP55,940, compared to 17.8 μM and 56.2 μM for AEA and Δ^9-THC, respectively. Other functional assays yielded similar findings, though CBD's effects other than inverse agonism were not generally significant at CBD concentrations below 31.6 μM. Importantly, CBD's inverse agonism appeared receptor specific – no inverse agonism was observed in untransfected cells or in cells expressing a non-cannabinoid GPCR (hNPBWR1). [^{3}H]SR141716A B_{max} was significantly reduced in CBD-treated (31.6 μM) membranes rinsed for 30 min, an effect that was only partially reversed following 2 h rinse.

Conclusions: CBD reduced the potency and efficacy of CB_1 agonists in all three functional assays, consistent with a non-competitive mechanism, such as negative allosteric modulation,[1,2] though, in the present study, CBD's potency was consistent with its observed CB_1 affinity. Further, CBD's inverse agonism in functional assays was also consistent with its observed greater affinity in [^{3}H]SR141716A competition binding compared to [^{3}H]CP55,940 binding. Surprisingly, reversal of CBD's reduction of [^{3}H]SR141716A's B_{max} was paradoxically slower than expected based on its observed binding affinity and kinetics. In summary, CBD's pharmacological effects at CB_1 are mostly consistent with its calculated orthosteric affinity; however, it remains unclear whether CBD's effects on CB_1 activation can be fully explained by pharmacological mechanisms.

Acknowledgements: R01AT010773, K01DA045752

[1] Thomas et al. (2007), Br. J. Pharmacol. 150, 613-623; [2] Laprarie et al. (2015), Br. J. Pharmacol. 172, 4790-4805

CANNABIDIOL INHIBITS MUSCLE DE NOVO LIPOGENESIS, POSITIVELY INFLUENCING FATTY ACIDS METABOLISM IN A RAT MODEL OF OBESITY INDUCED BY A HIGH-FAT DIET

Patrycja Bielawiec, Karolina Konstantynowicz-Nowicka,
Adrian Chabowski and Ewa Harasim-Symbor

Department of Physiology, Medical University of Bialystok, Bialystok, Poland

Introduction: In obesity, an increased availability of fatty acids (FAs) in the diet leads to excessive storage of lipids in adipocytes and, subsequently, in other metabolically important tissues such as skeletal muscle. For several years, cannabidiol (CBD) has been in the spotlight as a potential therapeutic agent in the treatment of obesity due to its anti-inflammatory and antioxidant properties as well as its excellent safety profile and lack of psychoactive properties. Therefore, our study aimed to investigate the influence of CBD administration on the content of various lipid fractions, de novo lipogenesis, and desaturation process in the red gastrocnemius muscle in a rat model of high fat diet-induced obesity.

Methods: All experiments were performed on Wistar rats fed standard chow or high-fat diet (HFD) for 7 weeks, and injected with CBD (10 mg/kg once daily for the last 2 weeks of a diet regime). Lipid content was evaluated using gas-liquid chromatography (GLC). Based on fatty acid composition, we calculated de novo lipogenesis ratio (16:0/18:2n-3) and desaturation ratio (18:1n-9/18:0), in the selected lipid fractions. The expression of proteins involved in lipid metabolism was measured by Western blotting. Data were analyzed by one-way ANOVA followed by an appropriate post-hoc test ($p < 0.05$ considered significant).

Results: We showed that a HFD resulted in the accumulation of lipids in the red skeletal muscles (in the free fatty acid - FFA, diacylglycerol - DAG, and triacylglycerol - TAG fractions), which was further reduced by the administration of CBD. Similarly, we observed an increased muscular de novo lipogenesis after feeding animals a HFD, which was inhibited by CBD treatment. Moreover, CBD significantly improved the desaturation ratio, which was in line with the changes in the expression of proteins involved in the lipogenesis and desaturation processes.

Conclusions: Our data provide a new insight into the role of CBD as a regulator of lipid metabolism in skeletal muscle and indicate that CBD presents potential therapeutic properties with respect to the treatment of obesity and related disturbances.

Acknowledgements: Study financed under the project No. POWR.03.02.00-00-I051/16 from European Union funds, PO WER 2014-2020 (Grant No. 01/IMSD/G/2019) and supported by the National Science Centre of Poland (Grant No. 2017/26/D/NZ3/01119).

CHANGES IN THE METABOLOME MAY CONTRIBUTE TO THE POSITIVE EFFECTS OF CHRONIC LOW-DOSE Δ^9-TETRAHYDROCANNABINOL ON BRAIN AGEING IN MICE

A. Bilkei-Gorzo[1], B. Schurmann[1], M. Schneider[2], M.Kramer[3], P.Nidadavolu[1], C.Muller[2] and A.Zimmer[1]

1 Institute of Molecular Psychiatry, Medical Faculty, University of Bonn, Bonn, Germany, 2 Pharmaceutical Institute, Department of Pharmaceutical & Medicinal Chemistry, University of Bonn, Bonn, Germany, 3 Institute of Forensic Medicine, Medical Faculty, University of Bonn, Bonn, Germany Bonn, Germany

Introduction: Change in metabolome is one of the primary underlying mechanisms and sensitive indicators of physiological responses. In the brain, change in learning ability or memory is largely dependent on neuronal and glial energy mobilization, on dynamic changes on the number and location of synapses. Age-related cognitive decline is associated with alterations in the metabolome of the brain and the blood plasma. It was previously shown that an increase in the cannabinoid system activity by chronic low dose Δ^9-tetrahydrocannabinol (THC) treatment enhanced synapse densities and improved cognitive abilities in old but not in young mice. It was not known, whether chronic THC treatment and withdrawal influence metabolome in the brain and body and whether THC-induced changes can contribute to the anti-ageing effect of the treatment. Here we show that THC-treatment has a strong, time- and age-dependent effect on the metabolome of hippocampus, blood plasma and adipose tissue.

Methods: Young and old mice were treated with THC or vehicle for 28 days through osmotic minipumps and were killed at day 14, 28 or at day 42, at the 14th day of the withdrawal. Body weight, food consumption, motility was recorded during the treatment period. Global biochemical profiles were determined in male mouse EDTA plasma, adipose tissue and brain tissue using reverse phase UPLC-MS/MS system. Additionally, THC levels and several metabolites in the blood plasma were controlled using LC-MS/MS.

Results: In the hippocampus, 14 days treatment led to a significant increase in the level of metabolites of energy production and several classes of lipids – among others compounds with well-known anti-ageing effects. All these changes were temporary because they disappeared during a longer treatment at day 28. In the blood plasma, THC treatment led to an upregulation of several classes of lipid metabolites at day 14, whereas at day 28, the last day of the continuous THC-treatment though all these changes were normalized but we found a significant decline in the level of several groups of amino acid metabolites. Adipose tissue was only minimally affected by the THC-treatment, whereas withdrawal induced a significant change in metabolites indicating a change in energy production and increased lipid degradation. The metabolic reactivity to THC-treatment was age dependent; THC-treatment at day 28 altered only a small minority of the metabolites in both age groups.

Conclusions: All these results suggest that chronic THC-treatment leads to a temporary increase in energy need and lipid mobilization in the hippocampus of old mice, the same time period where in a previous study THC-treated mice showed an improved memory and enhanced synapse formation. Moreover, THC induced a profound change in plasma metabolome, which may mediate the positive anti-ageing effects of the THC in the brain but also in peripheral organs. Our study suggests that altered metabolome could have a rejuvenating effect on the organism and targeting metabolome is a promising new strategy for anti-ageing medication.

ASSOCIATIONS OF CANNABIS USE TO INSULIN SENSITIVITY, PHYSICAL ACTIVITY AND DIET: IMPLICATIONS FOR OBESITY

Angela D. Bryan[1], Laurel P. Gibson[1], Gregory Giordano[1], Carillon Skrzynski[1], Chasmine Malabanan[1], Jinqiu Yang[1], Madeline Stanger[1], L. Cinnamon Bidwell[1], Kent E. Hutchison[2] and Leigh Perreault[2]

[1]University of Colorado Boulder, Colorado, USA; [2]University of Colorado Anschutz School of Medicine, Colorado, USA

Introduction: The increasing availability of legal market cannabis products has raised many questions about potential harms and benefits of increased use. Concerns have been raised about the possible negative impact of cannabis use on dietary quality and motivation to engage in physical activity, which has obvious negative implications for the obesity epidemic in the USA. Paradoxically, epidemiological data suggest cannabis use may be associated with less risk for type 2 diabetes, lower BMI, and better insulin function. Further, emerging data suggest that cannabis use might actually be *positively* associated with physical activity. Much of this work is based on retrospective or cross-sectional survey data, however, so more rigorous designs are needed to better understand whether there is a relationship between cannabis use and both behavioral and metabolic factors related to obesity.

Methods: As part of a larger ongoing NIH-funded study of the impact of different ratios of exogenous cannabinoids (THC, CBD) on obesogenic metabolic processes, we collected baseline anthropomorphic data, conducted an oral glucose tolerance test to measure insulin sensitivity, and collected 4 weeks of daily diary data from cannabis users and non-users regarding their physical activity and diet. We compared users (n = 88) to non-users (n = 21) at baseline on anthropomorphic measures, insulin sensitivity, habitual physical activity, and diet. Participants were prompted via email with a link to an online survey to report on several behaviors including their cannabis use, minutes of exercise, and dietary quality, on a daily basis.

Results: Participants to date are majority male (60.6%), majority non-Hispanic White (87.2%) and approximately 30 years of age (M = 29.60, SD = 5.49). At baseline, users reported marginally more total minutes of exercise per week than non-users (526.52 vs. 327.59 minutes; $t(108) = 1.65$, p = .10) and greater energy expenditure per day than non-users (29.53 vs. 25.72; $t(108) = 2.00$, p = .05). There were no differences on fat, protein or carbohydrate intake or total energy intake between users and non-users, and BMI was also similar across groups. Also at baseline, cannabis users had marginally higher values on the Matsuda index (i.e., better insulin sensitivity) relative to non-users, $t(62) = 1.80$, p = .07 (Users = 14.94, Non-Users = 11.50). Multilevel modeling with daily data suggested that over the course of 4 weeks, users reported an average of 8.35 more minutes of exercise per day than non-users, although this difference was not statistically significant (B = 8.35, p = .29). Also, users reported exercising an average of 2.37 minutes *less* on days when they used cannabis compared to days when they did not use cannabis, again not a significant difference (B = -2.37, p = .44). Users and non-users reported a similar number of fruit/vegetable servings per day (B = 0.01, p = .98). Also, users reported consuming an average of 0.31 *more* servings of fruits/vegetables on days when they used cannabis compared to days when they did not use cannabis, again not a significant difference (B = 0.31, $p < .001$).

Conclusions: Though the study is ongoing and data are still being collected, these preliminary analyses suggest that the relationship of cannabis use to metabolic factors, physical activity, and diet may be more complex than a simple positive or negative association.

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

GENETICALLY ENCODED FLUORESCENT SENSORS FOR MONITORING ENDOCANNABINOID DYNAMICS *IN VITRO* AND *IN VIVO*

Ruyi Cai[1,2#], Ao Dong[1,2,3#], Kaikai He[1,2], Barna Dudok[4], Jordan S Farrell[4], Jiali Duan[1,2], Lei Wang[5], Shangkun Wang[1,2], Ivan Soltesz[4], Chen Song[3,5] and Yulong Li[1,2,3,6]

[1]State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing 100871, China;
[2]PKU-IDG/McGovern Institute for Brain Research, Beijing 100871, China;
[3]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China;
[4]Department of Neurosurgery, Stanford University, Palo Alto, CA, USA;
[5]Center for Quantitative Biology, Academy for Advanced Interdisciplinary studies, Peking University, Beijing 100871, China;
[6]Chinese Institute for Brain Research, Beijing 100871, China.
# These authors contributed equally

Introduction: Endocannabinoids (eCBs), including anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are retrograde neuromodulators that play important roles in a wide range of physiological processes. However, limited by a lack of suitable probes capable of detecting eCBs with sufficient spatiotemporal resolution, the release and in vivo dynamics of eCBs remain largely unknown.

Results: We developed a genetically encoded eCB sensor $GRAB_{eCB2.0}$ by inserting a circular permutated EGFP into the ICL3 of the human CB1 receptor. This sensor has proper cell membrane trafficking, second-resolution kinetics and a robust fluorescence response at physiological eCB concentrations. Using this sensor, we monitored eCBs dynamics in several biological conditions in vitro and in vivo. Besides $GRAB_{eCB2.0}$ that responses to both eCBs, by structure-guided protein engineering based on $GRAB_{eCB2.0}$, we also developed $GRAB_{AEA1.2}$ and $GRAB_{2\text{-}AG1.2}$. They two showed specific response to AEA and 2-AG respectively in HEK293T cells and cultured neurons. These selective eCB sensors could robustly report electrical stimulation evoked endogenous AEA or 2-AG release in cultured neurons and acute brain slices. Moreover, they can also be used for monitor the dynamics of AEA or 2-AG during foot shock and sleep-wake cycle in vivo.

Conclusion: $GRAB_{eCB}$ sensors are robust probes for measuring the dynamics of eCBs under both physiological and pathophysiological conditions, which could give researchers more details about different dynamics of endocannabinoids during diverse biological conditions in various brain regions, that provide more information for understanding the function of the endocannabinoid system.

CANNABINOID RECEPTOR 2 AGONIST (JWH-133) INCREASES ECTOPIC OVARIAN TUMOR GROWTH AND ENDOCANNABINOIDS (ANANDAMIDE AND 2-ARACHIDONOYL GLYCEROL) LEVELS FOLLOWING CHRONIC ADMINISTRATION IN FEMALE SCID MICE

Isabel Castro-Piedras[1], Melissa C. McHann[1], Henry L. Blanton[1], Haley De Selle[1], Canice Lei Dancel[1], Jose-Luis Redondo[1], Deborah Molehin[2], Nadia German[3], Scott Trasti[4], Kevin Pruitt[2] and Josée Guindon[1,5]

[1]Department of Pharmacology and Neuroscience,
[2]Department of immunology and molecular microbiology,
[3]Department of pharmaceutical sciences,
[4]Department of cell biology and biochemistry,
[5]Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX, 79430.

Introduction: Cannabinoid-based therapies are increasingly being used by cancer patients to treat chemotherapy-induced nausea and vomiting. Recently, cannabinoids have gained increased attention for their effects on cancer growth. Indeed, the effect of CB_2 (JWH-015, JWH-133) agonists on breast cancer models have shown to reduce the size of breast cancer tumors. However, these studies assessing breast cancer progression were using CB_2 agonist administered early into the cancer progression therefore assessing their effects on already established tumors is a critical need.

Methods: In our study, we evaluate tumor growth using an ectopic xenograft ovarian (SKOV-3 and OVCAR-5) cancer model. The impact of chronic (30 days) administration of CB_2 (JWH-133) agonist will be evaluated and started on 30 days of ectopic ovarian tumors. We will then evaluate and determine the mechanisms involved in ovarian cancer tumor growth by measuring levels of anandamide and 2-arachidonoyl glycerol as well as protein levels of CB_1, CB_2, ERα, ERβ, GPER, TNFα, IL-1β and IL-6 in ovarian and tumor tissues.

Results: Our results demonstrate a significant increase in ectopic ovarian tumor growth following chronic administration of JWH-133. Ovarian cancer tumor tissues chronically (30 days) treated with JWH-133 in comparison to vehicle treated groups showed an increase in endocannabinoid (AEA and 2-AG) and protein (CB_2 and TNFα) levels with a decrease in GPER protein levels. Conclusion: Interestingly, our study emphasizes the importance of studying the impact of cannabinoid compounds on already established tumors to improve our understanding of cannabinoid-based therapies and, therefore better address clinical needs in cancer patients.

Acknowledgements: Cancer Prevention and Research Institute of Texas (CPRIT) Award (RR140008) to KP and (RP190524) to ST. Funded by NIH grants DA044999 (JG) and Texas Tech University Health Sciences Center School of Medicine 121035 (JG).

ADVANCES TOWARD THE DISCOVERY OF NOVEL 3-BENZYLQUINOLIN-2(1*H*)-ONES AS POTENT AGONISTS OF THE GPR55 RECEPTOR

Ceni, C.;[1,2] Laprairie, R. B.;[4] Mohamed K.A.;[4] Valoti, M.;[3] Poli, G.;[1] Tuccinardi, T.;[1] Digiacomo M.;[1] Macchia, M.[1] and Bertini, S.[1]

[1] Department of Pharmacy, University of Pisa, Via Bonanno Pisano 6, 56126, Pisa, Italy.
[2] Doctoral school in Life Sciences, University of Siena, Via Aldo Moro 2, 53100, Siena, Italy.
[3] Department of Life Sciences, University of Siena, Via Aldo Moro 2, 53100, Siena, Italy.
[4] College of Pharmacy and Nutrition, University of Saskatchewan, 104 Clinic Place Saskatoon, S7N 2Z4, Saskatchewan, Canada.

Introduction: GPR55 is a G protein-coupled receptor recently proposed as the "type 3" cannabinoid receptor, even if its categorization is still being studied. It is highly expressed in microglia cells, being involved in the regulation of neuroinflammatory processes, even if its molecular mechanisms have not been yet completely understood. Thus, the development of novel and potent GPR55 modulators represents a key step to better understand the peculiar role of GPR55 in neuroinflammation. Starting from 3-benzylcoumarin derivatives recently reported as GPR55 antagonists/inverse agonists, we designed and synthesized a novel series of 3-benzylquinolin-2(1*H*)-ones (**A**, *figure 1*), which we observed to be among the most potent GPR55 agonists developed to date. Moreover, some of these compounds showed also complete selectivity over one or both CBRs. Thus, we expanded the set, by increasing or reducing the length of the *n*-butyl chain in R7 position (**B**, *figure 1*).

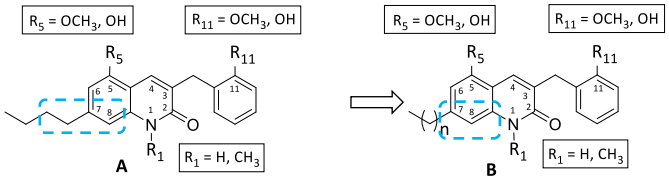


Figure 1. Structural modification leading to the extension of the 3-benzylquinolin-2(1*H*)-ones.

Methods: The purification of final compounds and intermediates was performed through flash liquid column chromatography and the characterization was carried out *via* ^{1}H- and ^{13}C-NMR. Functional activity at *h*GPR55 receptor was investigated in p-ERK activation assay. Binding assays on GPR55 were performed in presence of the orthosteric ligand [^{3}H]CP55940.

Results: We designed and synthesized novel 3-benzylquinolin-2(1*H*)-ones by applying modifications in the length of the *n*-butyl chain. Preliminary data showed that some of the newly synthesized compounds displayed affinity (K_i) values included in the low nanomolar range and either maintained agonist activity at GPR55 receptor or showed some degree of inverse agonism. In particular, a longer side chain seems to increase the affinity toward GPR55, but only when combined with hydroxyl groups in positions 5 and 11.

Conclusion: Starting from a novel series of potent and selective GPR55 agonists based on the 3-benzylquinolin-2(1*H*)-one scaffold, we expanded the set in order to deepen the preliminary outlined structure-activity relationships for this class, finding the influence of these structural modifications on the interaction with the GPR55 binding site.

Acknowledgement: Supported by the University of Pisa under the "PRA-Progetti di Ricerca di Ateneo" (Institutional Research Grants)–Project no. PRA_2020-2021_58. KAM is supported by a graduate scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC). Funding to RBL is from an NSERC Discovery Grant and a GSK-CIHR partnership grant.

AN ELECTROPHYSIOLOGICAL ASSESSMENT OF THE IMPACT OF THE CANNABIS-DERIVED TERPENES ON ACUTE SEIZURE ACTIVITY *IN VITRO*

Amy F. Walsh[1], Hannah MacAulay[1], Antonia Dunbar[2,3],
Helen Sheridan[1] and Mark O. Cunningham[1,3]

1. NatPro Centre, School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin;
2. Department of Biology & Biochemistry, University of Bath; 3. Discipline of Physiology, School of Medicine, Trinity College Dublin.

Introduction: Herbal medicines have been utilised to treat epilepsy for many centuries, although few studies have characterised the individual anti-epileptic activities of plant constituents. Cannabis has received attention for its potential anti-epileptic activity, however studies of cannabis have predominantly focused on the plant's cannabinoid constituents, while terpene constituents have remained largely understudied. This study aims to assess the acute anti-epileptic potential of sesquiterpenes (α-humulene, β-caryophyllene) and monoterpenes (α-pinene, D-limonene, linalool, and myrcene) commonly found in the *Cannabaceae* family of plants using *in vitro* electrophysiological approaches in acute rodent brain slices.

Methods: Extracellular local field potential (LFP) recordings were employed to measure seizure like events (SLEs) in adult horizontal rat brain slices (400 μm thickness). SLEs were elicited using the proconvulsant 4-aminopyridine (100 μM). Following the establishment of baseline seizure activity, various concentrations of the terpenes under examination were added to the circulating artificial cerebrospinal fluid. LFP recordings of SLE activity were recorded from the superficial layers (II-III) of the medial entorhinal cortex (mEC). The effects of each terpene on seizure duration (SD), first spike amplitude (FSA) and spectral power density (SPD) were analysed. DMSO (0.03 % v/v) was used as a vehicle control.

Results: Of all the compounds tested in the study, the acute application of the monoterpenes linalool and myrcene, and the sesquiterpene β-caryophyllene produced significant alterations in the seizure parameters measured. Linalool (300 μM) significantly reduced both SD (n=7, $P<0.01$) and SPD (n=7, $P<0.05$). β-caryophyllene (100 μM) also produced a significant reduction in SD (n=10, $P<0.05$), SPD (n=10, $P<0.05$) and FSA (n=10, $P<0.01$). Finally, myrcene was observed to significantly reduce SD at both concentrations tested (10 μM n=7, $P<0.05$; 30 μM n=7, $P<0.05$). D-limonene, α-humulene and α-pinene all failed to produce a significant change in any of the parameters measured at the concentrations studied.

Conclusions: Our electrophysiological studies demonstrate that certain terpenes known to be commonly found in most cannabis 'strains' are capable of acutely reducing seizure like activity in an *in vitro* model of seizure generation. Specifically, linalool, β-caryophyllene and myrcene are all observed to reduce seizure parameters in an acute manner. This anticonvulsant activity may be attributable to modulation of glutamate neurotransmission, voltage-gated sodium channels or cannabinoid type-2 (CB_2) receptors. Future work will aim to reveal the mechanistic nature of the effect of the terpenes observed in this current study.

GENETIC VARIATION IN ENDOCANNABINOID SIGNALING AND THREAT- AND REWARD-RELATED BRAIN FUNCTIONING IN CHILDREN

Shreya Desai[1], Julia M. Evanski[1], Christine A. Rabinak[1,2] and Hilary A. Marusak[1]

[1]Dept. of Psychiatry and Behavioral Neurosciences, School of Medicine, Wayne State University
[2]Dept. of Pharmacy Practice, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University

Introduction: The endocannabinoid (eCB) plays a key role in modulating neural activity across the lifespan, and disruptions in eCB signaling are implicated in a variety of stress-related psychiatric disorders, including anxiety and depression. Neuroimaging studies in adults link a common genetic variant in fatty acid amide hydrolase (FAAH C385A) — the enzyme that regulates eCB signaling — to lower threat-related amygdala activity but higher reward-related ventral striatal reactivity. Further, FAAH genotype has been shown to modulate the association between amygdala reactivity and anxiety in adults. However, it is unclear whether similar patterns are observed earlier in development, particularly during preadolescence. Indeed, emerging cross-species data suggests that effects of FAAH genotype on anxiety and frontolimbic brain circuitry emerge during adolescence. Here, we leveraged neuroimaging data from the Adolescent Brain Cognitive Development (ABCD) study to test the impact of the FAAH C385A variant on threat- and reward-related neural activity in preadolescent children.

Methods: This study included baseline neuroimaging, genetic, and behavioral data from 10,579 9-10-year-children (M_{age} = 119 months, 48% female) from the ABCD study. We examined the effects of the FAAH C385A variant on threat-related activity in the amygdala and reward-related activity in the nucleus accumbens (NAcc), an area of the ventral striatum implicated in mediating motivational and emotional processes. We also explored whether FAAH genotype modulates the association between neural activity and anxiety or depressive symptoms.

Results: There were no main effects of FAAH genotype on threat-related amygdala reactivity, nor reward-related NAcc activity (p's > 0.3). However, FAAH genotype modulated the association between left amygdala reactivity to threat and withdrawn/depressed symptoms (F[7, 8,223] = 47.44, p < 0.001; t = 2.4, p = 0.016). In particular, there was a positive association between amygdala reactivity to threat and withdrawn/depressed symptoms in youth with the A-allele, and a negative association in those with the CC genotype.

Conclusions: Our findings add to emerging evidence that the effects of the eCB system on the brain vary across development. In particular, main effects of the FAAH C385A variant on threat- and reward-related neural activity may emerge during adolescence or adulthood. However, the FAAH genotype modulates brain-behavior associations during preadolescence. Future studies, including longitudinal studies in the ABCD study, should explore the role of eCB signaling in modulating adolescent neurodevelopment and risk of stress-related disorders.

Acknowledgements: Data were obtained from the Adolescent Brain Cognitive Development (ABCD) Study (abcdstudy.org). The ABCD Study is supported by the National Institutes of Health and additional federal partners. This project is also supported by K01MH119241 to HM.

A NOVEL SYNTHESIS METHOD FOR ALTERING SIDE CHAIN LENGTH ON CANNABIGEROL MOLECULES AND THE IMPACT OF SIDE-CHAIN LENGTH ON PAIN AND CYTOTOXICITY

Wesley M. Raup-Konsavage[1,*], Diana E. Sepulveda[1,2,*], Daniel P Morris[1], Shantu Amin[1], Kent E. Vrana[1], Nicholas M. Graziane[1,2,] and Dhimant Desai[1]

[1]Penn State College of Medicine Department of Pharmacology
[2]Penn State College of Medicine Department of Anesthesiology & Perioperative Medicine
*These authors contributed equally to this work

Introduction: The predominant cannabinoids produced by most *Cannabis* cultivars have a 5-carbon side chain off of the aromatic ring. However, CBD and Δ^9-THC homologs have been isolated, and these homologs can have markedly different pharmacological activities. Cannabigerol, and its respective homologs, serve as the precursor molecule to these cannabinoids; however, in most cultivars cannabigerol is found in low levels, which means that the homolog molecules will be present in very trace amounts. Therefore, we set out to develop a novel synthetic mechanism to produce these side-chain homologs of cannabigerol.

Methods: We have synthesized CBG homologs with 3 (CBGV), 4 (CBGB), 7 (CBGP) and 9 (CBGN) carbon side chains using this method, and compared their activity to that of CBG in a model of chemotherapeutic induced neuropathy and cytotoxicity in colorectal cancer cell lines.

Results: We find a similar effect of CBGV and CBGB (4-carbon) on colorectal cancer cells. We did not find any differences in the anti-nociceptive properties, in neuropathic mice, of any of the side-chain variants compared to CBG, at 10 mg/kg, as assessed by von Frey filaments. We found that CBG homologs with shorter side chains, 3 and 4, were more efficacious at reducing viability in colorectal cancer cell lines compared to homologs with side chains of 5 or more carbons as measured by MTT assay 48 hours after treatment with 10 μM cannabinoid. These data are supported by average IC_{50} values for CBGV of 8.9-11.5 μM and CBGB of 15-15.6 μM, while IC_{50} values for the longer side chain variants were over 20 μM.

Conclusions: Taken together these data are consistent with previous work showing differences in activities between side-chain variants of CBD and THC. The lack of a difference in anti-nociceptive behavior appears to be unique to CBG, as previous work with longer variants of THC have been found to be more efficacious.

Acknowledgements: This project is supported by the NARSAD Young Investigator Award (27364; NMG) and by the Pennsylvania Department of Health using Tobacco CURE Funds (NMG). KEV (and the Penn State College of Medicine) is the recipient of research support from PA Options for Wellness (a state-approved medical marijuana clinical registrant)

LONG-TERM SEX AND DOSE-DEPENDENT NEURODEVELOPMENTAL CONSEQUENCES OF ADOLESCENT EDIBLE THC CONSUMPTION IN RATS

Marieka V. DeVuono[1], Mohammed H. Sarikahya[1] and Steven R. Laviolette[1, 2]

[1]Department of Anatomy and Cell Biology, University of Western Ontario, London, ON, Canada
[2]Department of Psychiatry, University of Western Ontario, London, ON, Canada

Introduction: Adolescent cannabis use may result in an increased risk for neuropsychiatric illness later in life, yet rates of adolescent cannabis use are increasing. Animal models of exposure to Δ^9-tetrahydrocannabinol (THC), the main psychotropic component in cannabis, are helpful to study its neurodevelopmental impact. However, the majority of past research has relied on systemic injections, which is not how humans generally use cannabis. Previous research from our laboratory in male rats has shown that injection of high doses of THC during adolescence increases anxiety-like behaviours, impairs social and object recognition memory, and weakens pre-pulse inhibition of startle in adulthood. These behavioral changes are accompanied by hyperactivity of ventral tegmental area (VTA) dopamine neurons and prefrontal cortex (PFC) glutamatergic neurons in adulthood. To expand on this past work, we investigated the long-term effects of adolescent edible THC consumption, a popular method of human cannabis use, in male and female rats, as THC is known to have sex-specific effects. Additionally, since high potency cannabis is linked to a greater psychiatric risk and THC is known to produce dose dependent responses, we compared a low and high dose edible THC regimen.

Methods: Male and female rats were given edibles containing increasing doses of THC (1-5 mg/kg) mixed in Nutella® either once (low-dose) or twice (high-dose) daily for 11 days during adolescence (Postnatal day 35-45). Thirty days after drug administration, adult rats underwent a battery of behavioural tasks to assess anxiety-like behaviour, memory, sociability, and sensory processing. *In vivo* electrophysiology was then used to determine changes in PFC glutamatergic and VTA dopaminergic activity. Brains were also collected in adulthood to investigate molecular and neurochemical changes for future experiments.

Results: During drug administration, the high dose of THC reduced weight gain in both sexes, but the low dose did not affect weight gain. In contrast to previous findings with injected THC, one edible/day produced an anxiolytic effect in the light-dark box in males, and impaired social recognition memory in females. Preliminary electrophysiology data suggests that the low dose of edible THC decreased firing of PFC glutamate neurons in males, but increased bursting activity of these neurons in females. The effects of twice daily THC edibles on behavioral tasks and neuronal activity are currently being investigated in ongoing experiments.

Conclusions: The long-term effects of edible THC consumption during adolescence are dependent on sex and are likely dose-dependent as well. Differences in neuronal firing patterns in the PFC may partially mediate these effects.

Acknowledgements: This research is supported by the Natural Science and Engineering Research Council of Canada (NSERC), the Canadian Institute of Health Research (CIHR) and Canada First Research Excellence Fund (CFREF) to BrainsCAN at Western University.

ESCALATING Δ^9-TETRAHYDROCANNABINOL (THC) ADMINISTRATION IN ADOLESCENCE LEADS TO PERSISTENT SEX-SPECIFIC DYSREGULATION OF DOPAMINE D1-D2 RECEPTOR HETEROMER

Marie-Eve Di Raddo[1], Marija Milenkovic[1], Yalin Sun[1], Bertha K. Madras[3] and Susan R. George[1,2]

[1]Departments of Pharmacology & Toxicology,
[2]Medicine, University of Toronto, Toronto, ON, Canada
[3]Department of Psychiatry, McLean Hospital, Harvard Medical School, Boston, MA, USA

Introduction: Heavy cannabis use in adolescence is a risk factor for development of long-term psychiatric problems including cannabis use disorder (CUD), cognitive impairment, psychosis, depression, anxiety, and suicidality. The psychiatric symptoms are sexually dimorphic, with females exhibiting higher susceptibility to CUD, anxiety, and dysphoria, whereas males are more vulnerable to cannabis-induced psychosis. These adverse effects of cannabis are largely attributed to the principal psychoactive component in cannabis, Δ9-tetrahydrocannabinol (THC). In adult male but not adolescent male rats, chronic exposure to THC induced anxiety- and depression-like behaviours. Upregulation of the dopamine D1-D2 receptor heteromer correlated with these behaviours, highlighting heteromer dysregulation as potentially mediating some of the aversive effects of THC, especially the negative affective states induced by THC observed in female rats. Accordingly, we sought to determine whether THC exposure during adolescence triggers sex-specific regulation of the D1-D2 heteromer in adulthood.

Methods: Adolescent male and female Sprague-Dawley rats (n=5/group) were treated twice daily with an escalating dose of THC or vehicle for 11 days (day 1-3: 2.5 mg/kg, day 4-7: 5 mg/kg and day 8-11: 10 mg/kg). This treatment regimen previously produced deficits in working memory, sociability, and despair behaviours in adulthood after a 30-day drug-free period. At 30 days, animals were euthanized, and brains harvested for D1-D2 heteromer analysis in striatum and prefrontal cortex (PFC) by *in situ* Proximity Ligation Assay (PLA). Vaginal cytology was evaluated on day of sacrifice to control for potential estrus cycle-dependent fluctuations in the percentage of D1-D2 heteromer positive cells.

Results: Compared to untreated adult males, untreated adult female rats showed elevated expression of the D1-D2 heteromer in the dorsomedial striatum (DMS), dorsolateral striatum (DLS), nucleus accumbens core (NAcCo), prelimbic cortex (PL) and infralimbic cortex (IL), but not nucleus accumbens shell (NAcSh). Adult female rats exposed to THC during adolescence had decreased expression of D1-D2 in the PL and IL, and increased expression of D1-D2 in the DLS compared to vehicle-treated females. In contrast, the density of D1-D2 heteromer remained unchanged in the same brain regions of adult male rats exposed to THC only during adolescence. Preliminary results indicate female treatment groups were balanced by estrous cycle phase, ruling out cycle-dependent alterations in D1-D2 heteromer function.

Conclusions: Dopamine D1-D2 heteromer expression in rats is sex-dependent: in untreated adult animals, heteromer expression is higher in striatum and PFC of female compared with male rats. In adult rats treated with THC only during adolescence, followed by maturation to adulthood in a drug-free state, D1-D2 density was altered in these specific brain regions only in female, but not in male rats. Future experiments will investigate whether behavioural differences between males and females correlate with baseline variations in D1-D2 expression in PFC and striatum and whether any impairments observed in adulthood following adolescent THC exposure are mediated by D1-D2 heteromer activity in these regions.

Acknowledgements: Funded by National Institute of Health NIDA (Grant no. DA042178, BKM, SRG)

LEI-401 IS AN ALLOSTERIC INHIBITOR OF NAPE-PLD

Wouter Driever[1], Berend Gagestein[1], Dirk Minnee[1], Tessa Gote[1], Brenda Pijper[1], Elliot Mock[1], Anthe Janssen[1], Stan van Boeckel[1] and Mario van der Stelt[1]

[1]Department of Molecular Physiology, Leiden University & Oncode Institute, Netherlands

Introduction: *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) is a biosynthetic enzyme for the endocannabinoid anandamide, which can be activated by bile acids [1]. We have previously reported the discovery of the pyrimidine-4-carboxamide LEI-401 as an in vivo active NAPE-PLD inhibitor that modulates emotional behavior in mice [2, 3]. Our aim was to further expand the structure-activity relationship (SAR) study of LEI-401 and to elucidate its molecular mode-of-action.

Methods: 106 novel pyrimidine-4-carboxamides were synthesized and tested in a biochemical assay using a membrane fraction of HEK293T cells that overexpressed recombinant NAPE-PLD. Biochemical studies, photoaffinity labeling and chemical proteomics experiments combined with molecular docking studies were used to identify the binding mode.

Results: Our SAR studies revealed that the hydroxyl-pyrolidine of LEI-401 could be replaced by amine-pyrimidines or alkylcarbamates yielding novel NAPE-PLD inhibitors with $IC_{50} < 10$ nM. Biochemical profiling studies indicated that increasing the substrate concentration did not produce a rightward shift in the K_i, but the V_{max} was decreased upon increasing inhibitor concentrations. This is indicative of a non-competitive mode-of-action with respect to the NAPE-PLD substrate. Interestingly, a rightward shift in the K_i, but no decrease in V_{max}, upon increasing deoxycholic acid concentration was observed. To identify the binding pocket a novel photoprobe (WPF-2-024) was synthesized and used in competitive chemoproteomics experiments. It was found that WPF-2-024 covalently interacted Met260. This observation in combination with molecular docking studies could explain the SAR and the biochemical studies.

Conclusion: LEI-401 is an allosteric inhibitor of NAPE-PLD that binds in the bile acid binding pocket.

[1] Margheritis E.; Castellani B.; Magotti P.; Peruzzi S.; Romeo El.; Natali F.; Mostarda S.; Gioiello A.; Piomelli D.; Garau G, Bile Acid Recognition by NAPE-PLD. *ACS Chemical Biology* **2016** *11* (10), 2908-2914.

[2] Mock, E. D.; Mustafa, M.; Gunduz-Cinar, O.; Cinar, R.; Petrie, G. N.; Kantae, V.; Di, X.; Ogasawara, D.; Varga, Z. V.; Paloczi, J.; Miliano, C.; Donvito, G.; van Esbroeck, A. C. M.; van der Gracht, A. M. F.; Kotsogianni, I.; Park, J. K.; Martella, A.; van der Wel, T.; Soethoudt, M.; Jiang, M.; Wendel, T. J.; Janssen, A. P. A.; Bakker, A. T.; Donovan, C. M.; Castillo, L. I.; Florea, B. I.; Wat, J.; van den Hurk, H.; Wittwer, M.; Grether, U.; Holmes, A.; van Boeckel, C. A. A.; Hankemeier, T.; Cravatt, B. F.; Buczynski, M. W.; Hill, M. N.; Pacher, P.; Lichtman, A. H.; van der Stelt, M., Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. *Nature chemical biology* **2020,** *16* (6), 667-675.

[3] Mock, E. D.; Kotsogianni I.; Driever, W. P. F.; Fonseca, C. S.; Vooijs, J. M.; den Dulk H.; van Boeckel, C. A. A.; van der Stelt, M., Structure–Activity Relationship Studies of Pyrimidine-4-Carboxamides as Inhibitors of *N*-Acylphosphatidylethanolamine Phospholipase D. *Journal of Medicinal Chemistry* **2021** *64* (1), 481-515.

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

Megan Drupals[1,2], Janice Hicks[1], Benjamin Steinberg[1,2] and Michael Salter[1,2]

[1]Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, ON, Canada;
[2]Department of Physiology, University of Toronto, Toronto, ON, Canada

Introduction: Existing human and rodent studies have found some convergent and some contradictory findings on the impact of prenatal cannabinoid exposure on offspring. Overall, the evidence points to early physical consequences, including low birth weight or gestational age in humans. In children and adolescents, behavioural effects which have been reported include a variety of cognitive changes (reviewed in *McLemore and Richardson*, 2016).

Methods: Using a mouse model developed to capture pre- and peri-gestational THC and CBD exposure, we examined the effects on a range of behaviours in adult offspring. Beginning 7 days prior to mating and throughout gestation until E17.5, pregnant mice were subcutaneously injected once daily with either 5mg/kg THC, 60mg/kg CBD, or vehicle (1:4:15 95% EtOH:Tween80:saline). Starting at 9 weeks of age, mice underwent a battery of behavioural tests which included accelerod (motor), elevated plus maze (anxiety), and open field (locomotion and anxiety).

Results: We found no significant differences in the behaviour of THC or CBD-exposed mice in the open field test compared to vehicle controls. Conversely, THC-exposed offspring spent more time in the open arms of the elevated plus maze compared to controls, likely indicating less anxious behaviour ($p<0.05$, ANOVA with Post-Hoc Tukey's multiple comparisons test). We found no differences in performance on the accelerod test between groups across all three experimental trials.

Conclusion: These data suggest that the exposure of CBD in this model had no effect on the behaviours evaluated in adulthood. In the elevated plus maze specifically, offspring of THC-treated dams showed behaviour consistent with a reduction in anxiety. However, this was not recapitulated in the open field test. To investigate the effect of this gestational drug exposure on cognition, a novel object recognition assay is currently underway, with final results pending.

ADOLESCENT THC TREATMENT PROMOTES AN INFLAMMATORY PHENOTYPE IN MALE MEDIAL PREFRONTAL CORTEX

Michaela Dvorakova, Wenwen Du, Catherine S. Wright and Ken Mackie

Gill Center for Biomolecular Sciences,
Department of Psychological and Brain Sciences, Indiana University Bloomington, IN, USA

Introduction: Cannabis use during adolescence increases the risk of developing a psychotic disorder later in life. During adolescence, the prefrontal cortex (PFC), a brain area important in decision making and reward processing, undergoes its final maturation and reorganization. The flexibility of the developing PFC allows it to be wired based on the needs of the environment, but it also offers a vulnerable window to insults in the form of drugs and other negative environmental influences.
Synaptic pruning (i.e., elimination of inactive synapses) by microglia plays a major role in PFC reorganization and maturation. An imbalance in synaptic pruning can negatively affect neural circuits and behavior. For example, synaptic pruning can be negatively affected by stress. Cannabis use during adolescence leads to improper pruning and impaired connectivity in PFC circuits as well. One of the possible mechanisms by which Δ^9-tetrahydrocannabinol (THC), the primary intoxicating component of cannabis, could affect PFC maturation is by promoting a neuroinflammatory state and activating microglia that will subsequently excessively prune synaptic terminals. Impaired synaptic pruning in the PFC can be one of the mechanisms by which THC negatively affects working memory. In adolescent mice, cannabidiol (CBD) has a protective function in working memory tests when co-applied with THC.

Methods: In this study, we are focusing on the morphological changes in PFC microglial cells that accompany the so-called activated state that appears during inflammatory conditions. Activated microglia show decreased branching and increased cell body size. We are employing Sholl analysis as well as other descriptive factors to determine the relative proportions of resting/active state of microglia in brains from mice treated with THC or VEH during adolescence (postnatal day 28-49). To further explore the neuroinflammation caused by THC we are screening the immune-related markers by quantitative PCR to detect changes in their expression within the PFC. We are also exploring whether a co-treatment with CBD is able to rescue the phenotype. As literature indicates, THC effect on brain is often sex-specific, therefore we use cohorts of males and females to describe the consequences of adolescent cannabinoid treatment.

Results: In adolescent males THC causes decreased total area and branching of microglia, increased cell body perimeter and CD68 positive staining within Iba 1 staining. In adolescent females and in adult animals of both sexes the THC treatment does not cause changes in microglial morphology. The activated microglial phenotype is not rescued by co-treatment with CBD.

Conclusions: Adolescent treatment with THC promotes sex-specific activation in microglia in male mice.

EFFECTS OF CHRONIC CANNABIDIOL ADMINISTRATION ON THE *DE NOVO* LIPOGENESIS AND DESATURATION RATIO IN THE WHITE SKELETAL MUSCLE IN HIGH-FAT DIET FED RATS

Sylwia Dziemitko, Patrycja Bielawiec, Klaudia Sztolsztener,
Adrian Chabowski and Ewa Harasim-Symbor

Department of Physiology, Medical University of Bialystok, Poland

Introduction: Sedentary lifestyle and Western diet result in an excessive accumulation of fatty acids in non-adipose tissues, including skeletal and cardiac muscles. In a long-term obesity disrupts various metabolic pathways, leading to the development of lipotoxicity and insulin resistance. Cannabidiol (CBD), a non-psychotropic compound of medical marijuana has been proposed as a potential therapeutic factor in the treatment of obesity. Thus, in the present study we have investigated whether chronic CBD administration influences intramuscular lipid content as well as the de novo lipogenesis and fatty acid desaturation levels in the white skeletal muscle.

Methods: Male Wistar rats were fed a standard or high-fat diets (HFD) for seven weeks. Then, starting from the fifth week, the intraperitoneal injections of CBD or its vehicle were given to the animals. Gas-liquid chromatography (GLC) was performed to examine intramuscular content of lipid fractions. The de novo lipogenesis pathway was measured as a 16:0/18:2n-6 ratio, and the activity of stearoyl-coenzyme A desaturase 1 (SCD1) was determined as a 18:1n-9:18:0 ratio. The expression of proteins involved in lipid metabolism pathways was measured by Western blotting. Obtained data were analyzed by one-way test ANOVA and an appropriate post hoc test. Values $p < 0.05$ were considered statistically significant for all results.

Results: Our study revealed that CBD substantially diminished intramuscular triacylglycerol (TAG) accumulation evoked by HFD in the white skeletal muscle. The de novo lipogenesis pathway in the free fatty acid (FFA), diacylglycerol (DAG) as well as phospholipid (PL) fractions was elevated by HFD, which was attenuated by CBD treatment. Moreover, due to the chronic CBD administration, the SCD1 activity was significantly lessened both in animals fed HFD and standard chow in the white skeletal muscle.

Conclusion: The data showed a favorable effect of CBD, i.e., decreasing TAG content in the white skeletal muscle during feeding rats a high-fat diet. Concomitantly, attenuation of fatty acid synthesis and desaturation pathways by CBD treatment protects the myocytes against the accumulation of biologically active lipid derivatives in fatty acids oversupplied conditions. Therefore, CBD seems to be an attractive therapeutic strategy in obesity.

Acknowledgments: The study was supported by the National Science Centre in Poland (Grant. no. 2017/26/D/NZ3/01119).

ENDOCANNABINOID SYSTEM RECEPTOR EXPRESSION IN THE CONTEXT OF SIV AND cART

Alysha L. Ellison, José-Javier Rosado Franco, Cory J. White, Lisa B. Fridman and Dionna W. Williams

Departments of Molecular and Comparative Pathobiology, Molecular Microbiology and Immunology, and Clinical Pharmacology, Johns Hopkins University School of Medicine and Bloomberg School of Public Health, Baltimore, MD, USA

Introduction: Human immunodeficiency virus (HIV) infects human immune cells throughout the body, resulting in widespread chronic inflammation despite successful treatment with antiretroviral therapy (ART). Prolonged exposure to this inflammatory environment is produced largely by myeloid-lineage cells (monocytes, macrophages, microglia) and causes tissue damage, particularly to delicate tissues of the brain. Manifestations of central nervous system (CNS) damage are associated with poorer daily functioning and affect up to 50% of infected people, a prevalence that is growing due to the survival benefits of ART. Consequently, there is a rapidly growing need for new strategies targeting chronically activated myeloid cells to reduce HIV associated inflammation. Activation of the endocannabinoid system (ECS) is known to elicit immune modulating effects in many inflammatory disorders. However, the ability of the ECS to mediate chronic HIV associated inflammation has not yet been established.

Methods: To evaluate the interplay between the ECS and HIV associated inflammation we employed a virally suppressed rhesus macaque model (SIV/ART) and measured gene expression of ECS receptors (CB1, TRPV1, TRPV2) in tissues of the CNS (cerebellum, frontal cortex, parietal cortex, thalamus).

Results: Expression of the receptors assessed varied across brain regions, but remained consistent between SIV infected and ART groups. CB1 was highly expressed across all brain regions, whereas TRPV1 was lowly expressed in cerebellum and thalamus and not detected in the frontal or parietal cortex. TRPV2 was expressed in the frontal and parietal cortex, as well as thalamus. However, it was not detected in cerebellum.

Conclusions: Novel characterization of endocannabinoid system receptor expression in the context of HIV reveals varying levels of baseline receptor expression between different brain regions and no effect of SIV infection or ART administration on expression levels. These results provide urgently needed information that lays a foundation for assessing the intersection of anti-inflammatory compounds and the endocannabinoid system and their role in mediating neuroinflammation in the context of HIV. Evaluation of additional endocannabinoid system receptors, as well as changes in myeloid cell induced inflammation upon exposure to endogenous endocannabinoid system ligands, are currently underway.

WHAT'S NEW ABOUT THE FIRST CB2R HETEROBIVALENT DUALSTERIC/BITOPIC LIGAND? LATEST RESEARCH ON ITS NEUROPROTECTIVE BENEFITS

Rebecca Ferrisi[1], Francesca Gado[1,3], Kawthar A. Mohamed[2], Caterina Ricardi[3], Vittoria Carnicelli[3], Lorenzo Niccoli[3], Robert B. Laprairie[2], Clementina Manera[1] and Grazia Chiellini[3]

[1]Department of Pharmacy, University of Pisa, Italy; [2]College of Pharmacy and Nutrition, University of Saskatchewan, Canada; [3]Department of Pathology, University of Pisa, Italy

Introduction: The design of dualsteric/bitopic agents as single chemical entities able to simultaneously interact with both orthosteric and allosteric binding sites represents a novel approach in medicinal chemistry. Recently, our research led to the development of the first cannabinoid receptor type 2 (CB2R) heterobivalent dualsteric/bitopic ligand, **FD22a** (*Figure 1*) (Data not published). In *in vitro* assays, **FD22a** displayed very potent anti-inflammatory activity in a human microglial cell inflammatory model without any cytotoxic effects. In this work we present the study of its neuroprotective properties which may be contributing to the induction of autophagy, whose dysfunction plays a key role in neurodegenerative diseases such as Alzheimer's disease.

Methods: MTT vitality assays were initially performed by exposing U87MG human glioblastoma cells to increasing doses of **FD22a** (0.1, 1 and 10 μM). Subsequent experiments examined the ability of this compound to prevent the cytotoxicity produced in these cells from exposure to increasing doses (1, 10, 25 and 50 μM) of Aβ25-35 for time intervals of 24, 48 and 72h. Gene expression analyses have also allowed to highlight the ability of this compound to prevent the negative effects on the induction of autophagy produced in U87MG cells from exposure to Aβ25-35.

Results: The MTT assay revealed the absence of cytotoxicity for compound **FD22a**, tested at concentrations 0.1, 1 and 10 μM in U87MG cells, accompanied by the ability to effectively prevent, when used at 1 μM concentration, the cytotoxic effect produced by the exposure of these cells to Aβ25-35 (10μM, 48h). Gene expression analyses showed the ability of **FD22a** to reverse the negative effects produced by Aβ25-35 (10 μM, 48h) on the expression of genes markers of the autophagic process activation, such as LC-3, SIGMAR-1, SIRT1 and SIRT6, as well as to prevent the increased expression of genes markers of the autophagic process repression, such as MTOR and SIRT5 (*Figure 1*).

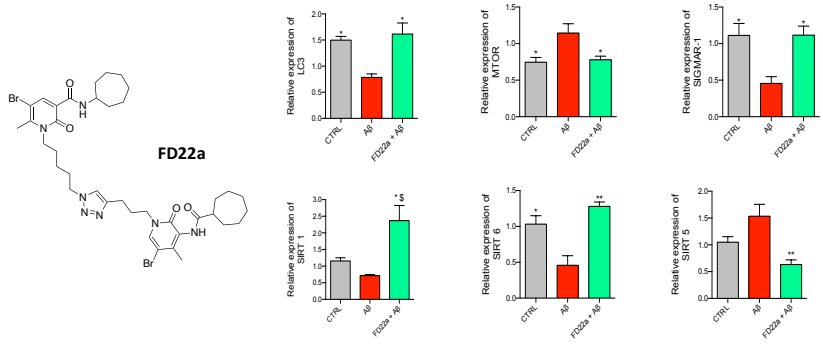


Figure 1. Effect of **FD22a** on the induction of the autophagic process.

Conclusions: The pro-autophagic effects induced in U87MG cells, together with the previously obtained results on human microglial cell inflammatory model, allow to consider the CB2R bitopic ligand, **FD22a**, a promising neuroprotective agent that can be subjected to further studies to confirm its potential as a drug for neurodegenerative diseases.

Acknowledgments: Italian Ministry of Health - Ricerca Finalizzata 2016 - NET-2016-02363765.

PHARMACOKINETIC AND *IN VIVO* BEHAVIORAL CHARACTERIZATION OF A NOVEL CANNABINOID RECEPTOR MODULATOR: GAT1102

Tyson Follack[1], Ayat Zagzoog[1], Vancy To[1], Adam Hupka[1], Deborah Michael[1], Anna-Maria Smolyakova[1], Sumanta Garai[2], Ganesh A Thakur[2] and Robert B Laprairie[1,3]

[1]Department of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada. [2]Department of Pharmaceutical Sciences, Bouve College of Health Sciences, Northeastern University, Boston MA, USA
[3]Department of Pharmacology, College of Medicine, Dalhousie University, Halifax NS Canada.

Introduction: The psychotropic adverse effects of classical orthosteric modulators of cannabinoid type-1 receptors (CB1Rs), pose a barrier-to-benefit from a therapeutic utility in adjacent modalities of analgesia, anxiolysis, sedation, and tremor cessation. We have developed a novel cannabinoid receptor allosteric modulator – GAT1102 – which may provide a novel solution to classical indiscriminate orthosteric-stimulation-related adverse events. GAT1102 enhances endocannabinoid binding affinity to CB1R and potentiates receptor outputs without competing for the orthosteric site. In the present study, we investigate sex differences in the pharmacokinetics and pharmacodynamics of GAT1102 in adult male (M) and female (F) C57Bl/6 mice. This study represents the first such pharmacokinetic exploration of a cannabinoid receptor allosteric ligand.

Methods: Acclimatized and handled M/F C57BL/6 mice received a single dose of 10 mg/kg GAT1102 administered via oral lavage (*p.o.*), intraperitoneal (*i.p.*), or intravenous (*i.v.*) injection. At uniform time points post-administration through a 48-hour time course, mice were euthanized, and blood/brain samples were collected following decapitation. Mice treated with GAT1102 as described above were assessed for cannabinoid-like effects using a triad of catalepsy, internal body temperature, and anti-nociception in the hot water tail-flick assay. GAT1102 levels were quantified by using an FDA-validated HPLC-MS/MS method.

Results: Pharmacokinetic analysis in both M and F mice revealed GAT1102 *i.v./i.p.* bioavailability of 70-100%, and a T_{max} of 0-30 min in the blood and brain. The *p.o.* group displayed a bioavailability of 30-70% in blood, and 40-50% in the brain. The T_{max} for both male and female *p.o.* groups was 30 min in the brain, whereas T_{max} in blood ranged from 10 min for M, and 60 min for F mice. GAT1102 produced anti-nociceptive effects in all dosage forms but is notably most pronounced in the female *p.o.* group. GAT1102 did not produce catalepsy or a hypothermic response.

Conclusion: In oral dosage forms (*p.o.*), GAT1102 produced a comparable anti-nociceptive response in both M and F mouse models regardless of peak blood/brain concentrations and did not produce a cataleptic or hypothermic response observed with classical orthosteric agonists of CB1R. These data support the model that GAT1102 contributes to the pro-analgesic response observed in phytocannabinoids without therapeutically limiting psychotropic adverse events.

This project is supported by a CIHR-GSK Partnership grant and CIHR Project Grant to RBL, and an NIH R01 grant to GAT (EY024717).

EXPLORING THE IMPACT OF CANNABIDIOL ON THE BRAIN TRANSCRIPTOME IN A MILD DISEASE MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Christa M. Frodella, Stephen B. Pruett and Barbara L.F. Kaplan

Mississippi State University, College of Veterinary Medicine, Mississippi State, MS, USA

Introduction: Experimental autoimmune encephalomyelitis (EAE) is a model to study human multiple sclerosis (MS). A mild model of EAE, induced in the absence of pertussis toxin (PTX), was developed to study cannabidiol (CBD) as a treatment. Since CBD might act through Gi-protein coupled receptors and PTX inactivates these receptors, PTX was omitted. While it is an unconventional model, it does mirror slow progression and variability of MS. EAE mice were treated with and without CBD and presented with symptoms that ranged from asymptomatic to symptomatic (i.e., tail and hind limb paralysis). To analyze the robustness of the model compared to MS and the impact of CBD, the brain transcriptome was analyzed and compared to known MS lesions that were obtained from an online repository.

Methods: Female C57BL/6J mice were immunized with Complete Freund's Adjuvant, Heat-killed *Mycobacterium tuberculosis,* and myelin oligodendrocyte glycoprotein. Twenty-four hours after immunization, mice were treated with 75mg/kg CBD or vehicle (corn oil) via oral gavage for 5 days. Brains were harvested on day 18 and RNA sequencing (RNA-Seq) was performed. Reads were assessed for quality, trimmed, and mapped to the GRCm39 genome. Differential expression analysis was performed in CLC Genomics Workbench (Qiagen). Differentially expressed genes (DEGs) with a false discovery rate (FDR) ≤ 0.05 were considered significant. RNA-Seq was analyzed from various MS lesions and healthy controls that was obtained from the gene expression omnibus (GSE138614) and mapped to GRCh38 genome. DEGs were uploaded into Ingenuity Pathway Analysis (Qiagen) to identify canonical pathways.

Results: Canonical pathway analysis revealed that CBD treated EAE mice that presented with tail and/or limb paralysis shared many canonical pathways with MS active lesions. These pathways reflected various aspects active inflammation from innate and adaptive processes. Notably, the number one gene that was increased CBD treated EAE mice that appeared in asymptomatic mice was oxytocin (Fold Change=1,311, FDR=0). Vasopressin, another neuropeptide, was also significantly increased (Fold Change=323, FDR=3.96E-13).

Conclusion: Results show that mild EAE is a valid model that mirrors active lesions in MS. The variability in immunization success and neuroprotection by CBD, may provide insight into mechanisms that are shared between EAE and MS as well as success with CBD treatment. Significantly, oxytocin and vasopressin may be potential biomarkers and differentiate when CBD is effective in some and not others.

A FUNCTIONAL AND ANATOMICAL ASSESSMENT OF ASTROCYTIC CB_1RS IN CANNABINOID ACTION

Briana Hempel, Guo-Hua Bi, Madeline Crissman, Sruti Pari, Ben Klein and Zheng-Xiong Xi

Molecular Targets and Medications Discovery Branch, NIDA IRP, Baltimore, MD

Introduction: Despite the widespread use of cannabis, the neural mechanisms underlying its pharmacological actions and therapeutic benefits are not fully understood. Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive component within cannabis, is a partial agonist at cannabinoid CB_1 receptors (CB_1Rs), which are highly expressed throughout the brain. GABAergic and Glutamatergic CB_1Rs have been the focus of most preclinical work investigating cell type specific cannabinoid action, but little is known regarding the functional role of astrocytic CB_1Rs. Here, we utilized conditional knockout (KO) mice with CB_1Rs selectively deleted from astrocytes (GFAP-$CB_1^{-/-}$) to systemically address whether these receptors are involved in the classic CNS-effects of Δ^9-THC and their distribution pattern in brain tissue.

Methods: In Experiment 1, GFAP-$CB_1^{-/-}$ mice and their wildtype (WT) littermates were treated with Δ^9-THC (0, 10, 30 mg/kg) and tested in the tetrad, a series of 4 assays measuring cannabimimetic effects (hypothermia, analgesia, catalepsy, hypolocomotion). For Experiment 2, we examined the affective properties of Δ^9-THC (3 & 5 mg/kg) using place conditioning and the elevated plus maze. RNAscope *in situ* hybridization was performed in Experiment 3 to evaluate CB_1R RNA distribution in astrocytes across a number of brain regions relevant to our behavioral data. In Experiment 4, GFAP-$CB_1^{-/-}$ mice and controls were administered saline or Δ^9-THC (5 mg/kg) prior to a microPET scan with radiolabeled glucose (^{18}F-FDG) using a between subjects dosing scheme.

Results: Genetic deletion of astrocytic CB_1Rs, significantly reduced Δ^9-THC-induced catalepsy, hypothermia and analgesia at a high dose (30 mg/kg) relative to WT littermates. Gfap-$CB_1^{-/-}$ mice failed to develop a place aversion to Δ^9-THC, whereas Δ^9-THC-induced anxiety was present in both conditional KO mice and controls. Low levels of CB_1R RNA were detected on astrocytes in brain regions implicated in the cataleptic (nucleus accumbens; ~17%), hypothermic (preoptic anterior hypothalamus; ~15%), analgesic (central nucleus of the amygdala or CEA; ~25%) and aversive (ventral tegmental area; ~13%) effects of Δ^9-THC. Moreover, the microPET scan revealed that Gfap-$CB_1^{-/-}$ exhibit significantly less glucose uptake across a number of brain areas relative to WT littermates and their own saline baseline with the most striking differences observed in the amygdala.

Conclusions: These findings implicate a novel astrocytic CB_1R mechanism in the CNS-effects of Δ^9-THC. This receptor population likely works in concert with neuronal CB_1Rs to mediate cannabis action. Our data points to the amygdala as a region of interest for future studies evaluating the functional role of CB_1Rs on astrocytes.

DEVELOPMENT OF HIGHLY POTENT AND SELECTIVE FLUORESCENT MAGL PROBES WITH DRUG-LIKE PROPERTIES

Axel Hentsch[1], Monica Guberman[1], Yelena Mostinski[1], Thais Gazzi[1], Jerome Paul[1], Jörg Benz[2], Bernd Kuhn[2], Uwe Grether[2] and Marc Nazare[1]

[1] Leibniz Research Institute for Molecular Pharmacology, 13125 Berlin (Germany); [2] Roche Pharma Research & Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., 4070 Basel (Switzerland)

Introduction: Monoacylglycerol lipase (MAGL) is a serine hydrolase that metabolizes the endocannabinoid messenger 2-arachidonic acid glycerol (2-AG) into arachidonic acid (AA) and glycerol and is therefore a key mediator at the interface of two major signal transduction pathways. 2-AG is one of the two major endogenous agonists to the cannabinoid receptors 1 (CB1) and 2 (CB2). By regulating the concentration of this neurotransmitter, MAGL thereby greatly influences the retrograde cannabinoid signaling pathways by the CB receptors that are important for learning, mood, appetite and addiction as well as regulation of immune cells. Moreover, the cleavage of 2-AG to AA is the starting point for the biosynthesis of the inflammation- and pain-mediating eicosanoids. The development of small molecular fluorescent probes that target this enzyme could help to visualize and quantify physiological processes *in cellulo* and *in vivo* across species. Enabling the visualization of the MAGL enzyme in native cells, tissues and organisms without the need of genetic engineering would give a clearer view of its functions in a spatiotemporal controlled fashion.

Methods: We designed a set of specific fluorescent MAGL inhibitors with drug-like properties, that allows for investigation in live cells and potentially *in vivo*. The design concept merges the structure of known potent MAGL inhibitors with fluorophore moieties to create ligands that incorporate the fluorophore into the proteins binding site. Both, favorable ADMET and photo-physical properties were simultaneously taken into account for the design process.

Results: The probe ligands show potencies towards MAGL in the sub-nanomolar range, high selectivity over other serine hydrolases as well as high solubility and cell permeability. The probes are active across species isoforms of the enzyme. Several co-crystal structures confirm the proposed binding mode of the novel structures. They are stable towards metabolism as well as photo bleaching and convince with bright fluorescence. The probe can be adapted to special requirements, i.e. red-shifted dyes suitable for super-resolution microscopy or covalent warheads that permanently block and label the proteins catalytic Ser122.

Conclusion: Here we present novel, state-of-the-art fluoroprobes for the key metabolic enzyme of the ECS with drug-like characteristics for applications such as (super resolution) live cell microscopy, TR-FRET & BRET assays, fluorescence polarization binding assays, FACS analysis etc.

CANNABIDIOLIC ACID TREATMENT REVERSES DEFICITS IN SYNAPTIC PLASTICITY IN HIPPOCAMPAL SLICES FROM THE APP/PS1 MOUSE MODEL OF ALZHEIMER'S DISEASE

Beatriz Gil and Caroline Herron

School of Biomolecular and Biomedical Sciences, University College Dublin, Belfield, Dublin 4.

Introduction: Alzheimer´s disease (AD) causes cognitive decline and is diagnosed by the presence of beta-amyloid (Abeta) plaques, and neurofibrillary tangles. Hippocampal long-term potentiation (LTP) is a form of synaptic plasticity linked to cognition and often used to assess potential therapies for AD. In the CA1 region, LTP is attenuated by Abeta and can be reversed by pre-treatment with cannabidiol (CBD) [1]. We have recently shown that treatment of hippocampal slices with cannabidiolic acid (CBDA) can attenuate the neurotoxic effects of Abeta on LTP. [2]. We have also examined LTP recorded from slices of APPswe/PS1dE9 (APP/PS1) mice, an animal model of AD, and shown how the deficit in LTP can be reduced when pre-treating the mice with 30mg/kg CBDA [2]. Here we have examined the effects of lower concentrations of CBDA (1 and 10 mg/kg) on LTP in slices from APP/PS1 mice and vehicle treated control litter mates. In addition, we have investigated alterations in short-term plasticity including post-tetanic potentiation (PTP) and paired pulse facilitation (PPF) in slices from vehicle and CBDA treated mice.

Methods: APP/PS1 mice and control litter mates received CBDA (1 or 10 mg/kg,) or vehicle (i.p) for 25 days, followed by a 2day rest period prior to slice preparation and electrophysiology. Parasagittal hippocampal slices (400 mm thick) were cut from 9month old mice and the Schaffer-collateral pathway was stimulated every 30s (0.033Hz) prior to induction of LTP using high frequency stimulation (HFS; 2x100Hz, 1s). LTP recorded at 60min was compared between groups. PPF (range 10-150ms) was examined in addition to levels of PTP. GraphPad Prism was used for graphing and analysis; paired or unpaired t-test, or ANOVA, were used as appropriate.

Results: In slices from control littermates, pre-treatment with vehicle or CBDA (1 or 10mg/kg) did not significantly alter the level of LTP. There was, however, a significant deficit in LTP in vehicle treated APP/PS1 mice compared with vehicle treated controls ($p \leq 0.05$), which was rescued by treatment with CBDA at 1mg/kg or 10 mg/kg (both $p \leq 0.01$;ANOVA,). When PPF was examined it was found to significantly increase in slices from CBDA treated APP/PS1 mice compared with the vehicle-treated control group. PTP, however, was not changed.

Conclusions Chronic treatment of APP/PS1 mice with 1 and 10mg/kg CBDA can attenuate the impairment in LTP observed in slices from vehicle treated mice however to a lesser extent than that observed with 30mg/kg (2) PTP levels are not altered by CBDA treatment, while PPF is improved across the examined range. Pre-treatment of control littermates with CBDA, however, does not appear to alter levels of LTP compared to vehicle treated mice, nor does it change levels of PTP or PPF. Our data suggests that chronic treatment with lower concentrations of CBDA at 1 and 10 mg/kg can also improve plasticity in slices from APP/PS1 mice.

1. Hughes, B and Herron C.E (2019) Neurochem. Research 44 703-713.
2. Gil, B and Herron, C.E (2019) Brain and Neurosci. Advances; BNA Abstracts PS083
Acknowledgements: Funded by Jazz (formerly GW) pharmaceuticals and University College Dublin

PERIPHERAL CB1 RECEPTOR BLOCKADE REDUCES THE SEVERITY OF HEMORRHAGIC CYSTITIS

Liad Hinden[1], Rami Ludyansky[2], Yoav Harris[2], Sary Leidershnaider[2], Joseph Tam[1] and Guy Hidas[2]

[1]Obesity and Metabolism Laboratory, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel; [2]Pediatric Urology Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

Introduction: Cyclophosphamide (CYP), a chemotherapeutic drug used to treat different types of cancer, can cause hemorrhagic cystitis, defined by dysuria and hematuria in oncological patients. CYP is metabolized to acrolein by the liver and accumulates in the bladder resulting in hemorrhagic cystitis and lower urinary tract symptoms (LUTS), characterized by increased micturition frequency, urgency, nocturia, incontinence, recurrent urinary tract infections, and even renal dysfunction. Recently, the endocannabinoid system (ECS) has been marked as a potential target for relieving LUTS. Whereas most studies indicate that activating the ECS has beneficial effects on bladder function, some studies implied the opposite role. In this study, we investigated the therapeutic potential of peripherally restricted cannabinoid-1 receptor (CB_1R) blockade in ameliorating LUTS, using a CYP-induced cystitis mouse model of bladder dysfunction.

Methods: Female, 12-week-old, C57Bl/6 mice were divided into three groups: 1. Control group, treated with vehicle (Veh; 1% Tween80, 4% DMSO in saline) for three consecutive days; 2. CYP group, treated with Veh for the first day, CYP (300 mg/kg, IP) for the second day, and Veh for the third day; 3. CYP + a peripherally restricted CB_1R blocker group, treated similarly to the 2nd group + JD5037 (3 mg/kg, IP) for three consecutive days. Bladder dysfunction was assessed using the non-invasive voiding spot assay (VSA) on the 3rd day. Following euthanasia, serum and bladders were collected and RT-qPCR, Western blotting, ELISAs, and endocannabinoids assessments were done.

Results: Peripheral CB_1R blockade significantly ameliorated the severity of CYP-induced cystitis assessed by VSA. Specifically, JD5037 reduced urine spots number as well as increased bladder-to-body weight ratio. JD5037 also significantly normalized CYP-mediated bladder endocannabinoid/CB_1R imbalance, resulting in reduced inflammation, demonstrated by the reduction of the pro-inflammatory cytokine TNFα both at the mRNA and protein levels in the bladder and serum.

Conclusions: Collectively, our results highlight the therapeutic relevance of peripheral CB_1R blockade in ameliorating CYP-induced hemorrhagic cystitis, and may further support the preclinical development and clinical testing of peripheral CB_1R antagonism for treating LUTS.

Acknowledgments: This work was supported by the Mentoring Grant (Hadassah University Medical Center, Jerusalem Israel) to Guy Hidas and Joseph Tam.

CANNABINOID RECEPTOR INTERACTING PROTEIN 1A (CRIP1A): FUNCTION AS A Gαi CARRIER

Erin K. Hughes[1,2], Sandra Leone-Kabler[2], Andrew C. England[2,3], Jill C. Clodfelter[1], W. Todd Lowther[1] and Allyn C. Howlett[2]

1. Department of Biochemistry and Center for Structural Biology, Wake Forest School of Medicine, Winston-Salem, NC 20157, USA; 2. Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 20157, USA; 3. Department of Chemistry, Wake Forest University, Winston-Salem, NC 20109, USA.

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

Methods: A myristoylated Gαi N-terminal 9-mer peptide and purified recombinant CRIP1a were used in fluorescent polarization ligand binding studies. Nuclear-free homogenates of cultured N18TG2 neuronal cells were used to activate the CB_1R-G heterotrimer. Immunoprecipitations (IPs) from homogenates or cytosolic and membrane fractions were performed with antibodies to peptide epitopes of CRIP1a. IPs were analyzed by western blot analysis for CRIP1a, Gαi1/2/3 and Gβ. Band densities were quantitated using LI-COR Empiria software for statistical analyses.

Results: Purified recombinant CRIP1a interacts with a myristoylated N-terminal Gαi peptide with μM affinity, but not with the non-myristoylated Gαi peptide. Both CRIP1a and Gαi were detected in CRIP1a co-immunoprecipitations from a nuclear-free homogenate of N18TG2 cells, using an antibody directed to the CRIP1a surface away from the proposed site of Gαi interaction. A prominent shift in mobility for these proteins suggested that CRIP1a and Gαi were co-migrating on the gel as an SDS-resistant unit. The density of the mobility-shifted complex was greater after treatment of the homogenate with GTPγS. Gβ was not demonstrated in this mobility-shifted complex, suggesting that Gαi rather than the G-protein heterotrimer was interacting with CRIP1a. Nevertheless, immunoprecipitation of CRIP1a using an antibody directed to the C-terminal β-strand near the proposed Gαi access site co-immunoprecipitated Gβ, but not Gαi1 or Gαi3.

Conclusions: Collectively these data suggest CRIP1a is a cargo-carrying protein capable of binding Gαi, but also exhibits an interaction with the Gβ-subunit that might be important for the initial contact of CRIP1a with the G-protein heterotrimer. We postulate that CRIP1a might function to bind Gαi when Gβγ is modulating calcium channels. Alternatively, CRIP1a might function to traffic Gαi away from the plasma membrane to another intracellular location. Acknowledgments: Funded by NIH grants R01-DA042157 and T32-GM095440.

CHARACTERISATION OF A NOVEL, POTENT PEPTIDE LIGAND FOR GPR55 THAT MODULATES HIPPOCAMPAL SYNAPTIC PLASTICITY

Andrew J Irving[1], Erica Millet O'Beirne[2], Jenni Harvey[2] and Mark Cunningham[1]

[1] Discipline of Physiology, School of Medicine , Trinity College Dublin, Ireland;
[2] Ninewells Hospital, Dundee University, UK.

The putative novel cannabinoid receptor GPR55 is widely expressed in the body and implicated in a variety of physiological process, both in the periphery and the central nervous system, including modulation energy metabolism and synaptic function. It is also an emerging therapeutic target for a range of diseases, including cancer, diabetes and various CNS disorders. In order to develop GPR55 as a therapeutic target we need a better understanding of its endogenous ligand pharmacology and function. Currently, the most potent endogenous ligand identified for GPR55 is the lysolipid, lysophosphatidylinositol. Here we have used a range of recombinant and native cell approaches to characterize a novel peptide ligand that shows potent activity at GPR55. Moreover, we demonstrate that this peptide can induce synaptic plasticity in the hippocampus and that this effect is mediated by GPR55. Analysis of GPR55 ligand activity was carried out using molecular imaging approaches, involving evaluation of receptor trafficking and high content analysis of protein kinase signaling pathways. Electrophysiological studies evaluated effects of GPR55 activation on synaptic transmission mediated by temporoammonic pathway in the rat hippocampus. Our findings suggest that the endogenous pharmacology of GPR55 is far more complex than previously envisaged showing sensitivity to both lipid and peptide ligands. Furthermore, we have used a new endogenous peptide agonist to demonstrate a role for GPR55 in modulating hippocampal synaptic plasticity.

THE PHYTOCHEMICAL DIVERSITY OF COMMERCIAL CANNABIS IN THE UNITED STATES

Christiana J. Smith, Daniela Vergara, Brian Keegan and Nick Jikomes

Department of Ecology and Evolutionary Biology & Department of Information Science, University of Colorado, Boulder, Colorado USA; Department of Science & Innovation, Leafly Holdings Inc, Seattle, WA

Introduction: The legal status of *Cannabis* is changing, fueling an increased diversity of *Cannabis*-derived products. Because *Cannabis* contains dozens of chemical compounds with potential psychoactive or medicinal effects, understanding this phytochemical diversity is crucial. The legal *Cannabis* industry heavily markets products to consumers based on widely used labeling systems purported to predict the effects of different "strains." We analyzed the cannabinoid and terpene content of tens of thousands of commercial *Cannabis* samples across six US states, finding distinct chemical phenotypes (chemotypes) which are reliably present. By comparing the observed phytochemical diversity to the commercial labels commonly attached to *Cannabis*-derived product samples, we show that commercial labels do not consistently align with the observed chemical diversity. However, certain labels do show a biased association with
specific chemotypes. These results have implications for the classification of commercial *Cannabis*, design of animal and human research, and regulation of consumer marketing—areas which today are often divorced from the chemical reality of the *Cannabis*-derived material they claim to represent.

Methods: We used various computational techniques to analyze a large dataset of legal *Cannabis*-derived product samples tested for multiple cannabinoid and terpene compounds, together with the common industry labels ("strain names" and "Indica/Sativa") associated with these products plus measures of popularity. Using a variety of statistical and machine learning methods, we mapped the phytochemical diversity of legal products across six US states.

Results: The phytochemical diversity of commercial *Cannabis* in the US is highly skewed towards THC-dominant products, with low abundance of most 'minor cannabinoids.' Using unbiased clustering algorithms we defined at least three statistical distinguishable chemotypes of THC-dominant *Cannabis* based on terpene profile composition. Product samples were unevenly distributed across these chemotypes, with over 85% of samples falling into just two chemotypes. A very poor correspondence was observed between the common marketing labels "Indica," "Hybrid," and "Sativa" and product chemistry. For the "strain names" also attached to samples, we found a wide range of variation in how well individual names consistently map to specific chemotypes. Some names map to distinct chemotypes above chance levels, while some do not.

Conclusions: Similar patterns of phytochemical variation are seen across six US states, indicating that *Cannabis*-derived flower products are similar in different states. While there are distinct chemotypes of THC-dominant *Cannabis* present in the US, product samples are highly skewed towards a small number of chemotypes. The common product labels associated with consumer products poorly or inconsistently map to the underlying phytochemistry. More robust product classification and labeling systems, based on product chemistry, are needed in order to accurately segment legal *Cannabis* products.

USE OF HANDHELD FT-NIR SENSORS TO RAPIDLY QUANTIFY CANNABINOIDS OF HEMP *IN SITU*

Cameron Jordan and Luis E. Rodriguez-Saona

Department of Food Science and Technology, The Ohio State University, Columbus, OH, USA

Introduction: Rapidly determining the amount of Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) that is contained in hemp is important for growers, buyers, processors, and analysts alike. Current methods for determining Δ9-THC and CBD include liquid chromatography-mass spectroscopy that requires the destruction of the original hemp flower and can take a long time to receive results (sometimes two weeks) and requires tedious sample preparation. Near-Infrared sensors provide a rapid analysis that does not require sample preparation. **Our objective** was to develop technology for detecting and quantifying the two predominant cannabinoids (THC and CBD) through NIR spectral signature profiles enabling real-time and field-based measurements.

Methods: Hemp samples were scanned using a prototype handheld Fourier Transform Near-Infrared scanner equipped with a rotating stage. These samples were whole and unhomogenized hemp flower samples. Samples were measured in duplicate using a 10 second exposure time. The content of major cannabinoids was determined by separating the compounds by ultrahigh-pressure liquid chromatography, and detection was achieved by LC-MS/MS detector with an electrospray ionization source. The spectral data were then analyzed using chemometric methods to determine the amount of Δ9-THC and CBD in the hemp samples. Spectral data combined with the reference data from LC-MS/MS were analyzed by partial least square regression (PLSR).

Results: NIR spectra obtained from the handheld scanning device for intact hemp flower generated very reproducible fingerprints. uHPLC-MS/MS analysis showed a wide variation of CBD (8 – 11 g/100g hemp) and THC (0.05 – 0.25 g/100g hemp) levels in samples. PLSR results showed excellent signal-to-noise ratios and good linearity, predicting major cannabinoids content with strong correlation (Rpre>0.9) and low standard error of prediction. Our technology can quantify CBD and THC in short measurements (~15 sec) displaying the results wirelessly in an app operated by a tablet.

Conclusions: These NIR sensors could potentially be used to monitor the quantity of CBD and THC during the growing process since it is a non-destructive analysis. The sensors could also be used by growers to quickly quantify their products before they are processed or distributed. The results of the FT-NIR scans (15 sec) require no sample preparation and no use of harsh chemicals. Although the LC-MS/MS quantification is accurate, it is also time-consuming (~2 weeks) and expensive. Handheld FT-NIR technology provides a thorough analysis for a market that requires a faster analysis.

THE CB1R INVERSE-AGONIST INV-202 REDUCES RENAL INJURY IN A MURINE MODEL OF STREPTOZOTOCIN (STZ)-INDUCED TYPE-1 DIABETES

Célia Roger-Villeboeuf[1], Laetitia Jacquot[1], Patricia Passilly-Degrace[1], Chloé Buch[1], Julia Leemput[1], Laurent Demizieux[1], Bruno Vergès[1], Pascal Degrace[1], Glenn Crater[2] and Tony Jourdan[1]

[1] : Inserm-UBFC U1231, Dijon, France ;
[2] : Inversago Pharma, Montreal, QC, Canada

Introduction: Renal diseases remain a burden for Public Health and chronic kidney disease (CKD) affects several millions of individuals worldwide. Diabetic Nephropathy (DN) is one of the major cause of CKD and is characterized by an increase in urinary albumin excretion and progressive loss of renal function associated with glomerular basement membrane thickening, mesangial expansion and tubulointerstitial fibrosis. Current therapeutic approaches including Renin-Angiotensin-Aldosterone System blockers, SGLT2 inhibitors or combination of both, do not fully abrogate DN progression. A growing body of experimental evidence suggests that modulation of the cannabinoid type 1 receptor (CB1R), may be a therapeutic tool in the management of CKD and more particularly DN. Indeed, renal CB1R inhibition promotes a reduction in albuminuria, renal fibrosis and preserves renal function in mouse models of DN mainly through a direct action on podocytes and/or proximal tubules. Here we report the effects of INV-202, a peripherally acting CB1R inverse agonist that is under clinical development for the treatment of diseases associated with diabetes and metabolic syndrome, on renal function and associated parameters in a preclinical model of DN.

Methods: Twelve weeks after the initiation of diabetes using STZ, C57BL6/J mice (8 per treatment group) were randomized to receive a daily oral dose of INV-202, 0.3 mg/kg and 3 mg/kg, or vehicle for 4-weeks. Five mice not treated with STZ were used as a non-diabetic control. At the end of treatment, histological, biochemical and molecular clinical features of DN were assessed.

Results: After 28 days of treatment, there was no significant effect on hyperglycemia nor body weight with either dose of INV-202. Renal and hepatic weight were reduced while heart weight was unaffected. Compared to vehicle-treated diabetic mice, INV-202-treated mice displayed a marked decrease in albuminuria, albumin to creatinine ratio, urinary urea, loss of glomerular filtration rate and renal hypertrophy. Interestingly, we also found an improvement in both podocyte and proximal tubular cells morphology and health markers which were likely responsible for the improvement previously mentioned. Furthermore, INV-202 also led to a dramatic reduction in renal inflammation and oxidative stress markers which are known to be highly involved in the pathogenesis of DN. Finally, despite the limitation of our model in regards of interstitial fibrosis development, we could observe a marked anti-fibrotic effect of INV-202.

Conclusions: Treatment with INV-202 reduced the progression of nephropathy in STZ-induced diabetic mice. Improvements in kidney size, function, and morphology, with corresponding gene expression was noted with both doses of INV-202. Further work to explore the effect of INV-202 in humans is warranted.

Acknowledgment: Funded by Inserm Intramural grant and a collaborative research agreement from Inversago Pharma to TJ.

THC EXPOSURE IN ADOLESCENCE DISRUPTS MICROGLIA HOMEOSTASIS AND DISABLES RESPONSES TO INFECTION AND SOCIAL STRESS IN ADULTHOOD

Kwang-Mook Jung, Hye-Lim Lee, Yannick Fotio, Francesca Palese, Lin Lin, Erica Squire, Alexa Torrens, Faizy Ahmed, Jade Ramirez and Daniele Piomelli

Dept. of Anatomy & Neurobiology, School of Medicine,
University of California, Irvine, USA.

Introduction: During adolescence, microglia are actively involved in the maturation of the neocortex while concomitantly undergoing profound phenotypic changes. We evaluated whether adolescence exposure to Δ^9-tetrahydrocannabinol (THC) might sway the development of microglia and persistently alter their function.

Methods: We administered THC (5 mg/kg, intraperitoneal) once daily to male and female mice from postnatal day (PND) 30 to PND44 and examined the transcriptome of purified microglia in adult animals (PND70 and PND120) under baseline conditions or interventions known to recruit microglia.

Results: Adolescence exposure to THC produced in mice of both sexes a state of microglial dyshomeostasis which persisted until young adulthood (PND70) and receded with further aging (PND120). Key features of this state included transcriptional suppression of genes involved in innate immunity as well as marked impairments in the hormonal, cellular and behavioral responses to LPS injection and RSD-induced social stress. The effects of adolescence THC treatment could be prevented by coadministration of a CB_1 cannabinoid receptor inverse agonist and were not replicated when THC was administered in young adulthood (PND70-84).

Conclusions: The results suggest that daily exposure to low-dose THC during adolescence disables critical physiological functions served by microglia in young adulthood with potentially wide-ranging consequences for brain and mental health.

Acknowledgements: This work was supported by the National Institutes on Drug Abuse (NIDA) (P50DA044118 to DP).

THE CB_1 ANTAGONIST ANEB-001, A CANDIDATE FOR ACUTE CANNABINOID INTOXICATION TREATMENT, BLOCKS CANNABINOID ACTIVITIES *IN VITRO* AND *IN VIVO*

Michael Tagen[2] and Linda Klumpers[1,2,3,4]*

[1]Anebulo Pharmaceuticals Inc., Austin, Texas, USA, [2]Verdient Science LLC, Denver, Colorado, USA, [3]Tomori Pharmacology Inc., Denver, Colorado, USA, [4]Department of Pharmacology, Larner College of Medicine, University of Vermont, Burlington, Vermont, USA

Introduction: Acute cannabinoid intoxication (ACI) is responsible for hundreds of thousands of emergency department (ED) visits per year in the United States. Effects induced by the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (THC), and synthetic cannabinoids can include panic, paranoia, and delusions. No targeted treatment is available that would reduce the socioeconomic burden of ED visits for ACI. In humans, cannabinoid type 1 (CB_1) receptor antagonists block the clinical effects of THC when they are administered beforehand, so this is a promising strategy for ACI treatment. The CB_1 antagonist ANEB-001 (formerly V24343) was studied for weight loss, but its development was halted along with other members of the class due to tolerability issues with chronic dosing. We sought to investigate the potential of ANEB-001 for treatment of ACI through preclinical pharmacology studies.

Methods: The competitive antagonism by ANEB-001 was tested with a cAMP assay using human CB_1 receptor expressing HEK-293 cells. ANEB-001 (3, 10, and 30 nM) was added to the cells prior to addition of the CB_1 agonist, CP-55,940 (0.0001 nM – 1 μM). Antagonism was modeled with a 4-parameter logistic function using R software. Reversal of THC-induced hypolocomotion was also tested *in vivo*. C57 mice were orally administered ANEB-001 (30 mg/kg), the active control rimonabant (3 mg/kg), or vehicle and then administered THC (3 mg/kg) or vehicle intraperitoneally 20 minutes later. Mice were then placed in automated locomotor activity cages 10 minutes after THC/vehicle administration for a duration of 15 minutes.

Results: CP-55,940 inhibited forskolin-stimulated cAMP production in a concentration-dependent fashion. ANEB-001 shifted the concentration-response curve to the right in a manner consistent with competitive antagonism. The EC_{50} of CP-55,940 was shifted to the right 2.3-, 16-, and 61-fold when cells were treated with 3, 10, and 30 nM ANEB-001, respectively. *In vivo* administration of THC to mice resulted in decreased locomotor activity compared to vehicle. Both ANEB-001 and rimonabant reversed the action of THC to a similar extent on the total active time parameter (54% inhibition and 69% inhibition, respectively, $p<0.01$ for both). ANEB-001 appeared to reverse the action of THC on other locomotor parameters, but the effects were not statistically significant.

Conclusions: In preclinical studies, ANEB-001 was shown to have suitable pharmacological properties for clinical development as a reversal agent for ACI. A phase 1b/2a proof-of-concept clinical study with a THC challenge test in healthy cannabis-experienced volunteers is currently underway, with preliminary results expected in mid-2022.

EFFECTS OF ENDOCANNABINOIDS IN MAMMALIAN RESPIRATORY SYSTEM THROUGH COX-DEPENDENT PATHWAYS: A SCOPING REVIEW

Anastasia Kolousek, Ezra Pak-Harvey, Oliver Lam, Mia White and Joshua M Levy

Department of Otolaryngology-HNS, Emory University School of Medicine, Atlanta, Georgia, USA

Introduction: The endogenous cannabinoid (endocannabinoid) system is an emerging target for the treatment of chronic inflammatory disease with the potential to revolutionize the treatment of many respiratory illnesses. However, a strong understanding of the interplay between the eicosanoid and endocannabinoid systems within the airway is key to this development. The vastly different effects of endocannabinoids across tissue types makes it imperative that we explore what is known about the effects of endocannabinoids within the airway. The aim of this review is to explore the impact of endocannabinoids and endocannabinoid hydrolysis inhibitors on cyclooxygenase 2 (COX-2) activity and eicosanoid products in order to understand the physiological impact of the endocannabinoid system within airway tissues.

Methods: A systematic literature review was conducted according to PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews). Search strategies using MeSH terms related to cannabinoids, eicosanoids, cyclooxygenase, and the respiratory system were used to query PubMed, Embase, Chochrane, CINAHL, Web of Science and Biosis Previews in December 2021. 125 records were identified for title/abstract screening. Only studies that investigate the relationship between endocannabinoids and the eicosanoid system in mammalian respiratory tissue after 1992 were included. 16 studies were incorporated in the final qualitative review.

Results: Endocannabinoid system activation increases COX-2 protein expression, potentially through the ceramide-dependent or p38 and p42/44 MAPK pathways, and is associated with a concentration dependent increase in PGE2. Inhibitors of endocannabinoid hydrolysis found either an increase or no change in levels of PGE2 and PGD2 and decreased levels of LTB4, PGI2, and TXA2. Endocannabinoids produced diverse physiological effects. Endocannabinoids were shown to increase bronchial epithelial cell permeability and have vasorelaxant effects in pulmonary arteries in human, as well as contraction of the bronchi and decreased gas trapping in guinea pigs. Tumor response was similarly conflicting showing tumorigenic effect in murine Lewis lung and alveolar carcinoma while showing apoptotic effect in the human A549 lung adenocarcinoma cell line. Finally, inhibitors of endocannabinoid hydrolysis were found to have an anti-inflammatory effect on pulmonary tissue. Airway effects are primarily mediated by COX-2, MAGL and FAAH metabolites and their agonism of various receptors such as the vanilloid and prostanoid receptors while direct agonism of cannabinoid receptors played a minor role.

Conclusion: The effects of the endocannabinoid system within the airway are diverse. For example, while endocannabinoid derived COX-2 metabolites such as PGE-2 can have anti-inflammatory effects, endocannabinoids also produce pro-inflammatory effects such as increased epithelial permeability and bronchial contraction. These conflicting findings suggest that endocannabinoids produce a variety of effects depending on their metabolism and receptor agonism. In addition to direct agonism of the cannabinoid receptors, several endocannabinoid pathways are active within the airway including FAAH, MAGL and COX-2 metabolism. The prevalence of each is highly dependent on tissue type and extrinsic factors and understanding the complex interplay between these pathways is key to leveraging the cannabinoid system as a potential therapeutic target for human airway disease.

PRECLINICAL SAFETY PHARMACOLOGY AND TOXICOLOGY OF FSD201, A PALMITOYLETHANOLAMIDE COMPOSITION

Mohammad Ebrahimzadeh,[1] Ashwini Joshi,[1, 2] Joanne Speed,[1] Nidhi Kanwar,[1] Sandra Lottes,[1] Ram Saralaya,[3] Joga Gobburu,[3] and Lakshmi P. Kotra[1, 2, 4*]

[1]FSD Pharma (Lucid Psycheceuticals), 243 College Street, Toronto, Ontario, Canada M5T 1R5
[2]Krembil Research Institute, University Health Network, Toronto, Ontario, Canada M5G 1L7
[3]Pumas-AI. Inc., 14711 Kamputa Dr, Centreville, Virginia 20120, USA
[4]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 3M3

Introduction: FSD201 (a formulation of palmitoylethanolamide, PEA) is a proprietary molecule under clinical development. PEA, a part of endocannabinoid system (ECS), belongs to the family of *N*-acylethanolamines. PEA is shown to be an agonist to the cannabinoid-like G protein-coupled receptor 55 (GPR55), 119 (GPR119), vanilloid receptor 1 (VR1), and peroxisome proliferator-activated receptor-α (PPAR-α). Furthermore, PEA is proposed to increase the endocannabinoid signaling through cannabinoid CB1 and CB2 receptors by enhancing the levels of endogenous endocannabinoids and the expression of cannabinoid receptors. PEA is being investigated for its anti-inflammatory, analgesic, and neuroprotective effects, and thus FSD201 shows a great promise as a therapeutic agent. Results of the safety pharmacology and toxicology studies on FSD201 in rats and dogs are presented below.

Methods: Safety pharmacology, and single and repeat-dose toxicology studies (at 7, 14, 28, and 90 days) were conducted in Sprague-Dawley rats (bone marrow reticulocytes, respiratory, and central nervous systems) and Beagle dogs (cardiovascular effects) at doses 100-2,000 mg/kg/day of FSD201. Mutagenesis was evaluated using the reverse mutation assay at 30-3,000 µg/plate of FSD201. Clastogenic and aneugenic effects were evaluated *in vitro* using micronucleus test in human peripheral blood lymphocytes at 1,000 µg/mL of FSD201.

Results: Functional Observational Battery, motor activity assessments (CNS), and respiratory monitoring in rats did not show any abnormalities with single-dose *p.o.* administrations of FSD201 up to 1,000 mg/kg. Repeated administration of 125-1,000 mg/kg *p.o.*, *b.i.d.* of FSD201 in dogs did not cause any abnormalities in hematological, hemodynamic, electrocardiographic, and blood chemistry parameters. Necropsy and histopathological examinations did not show any changes following single-dose administrations of FSD201 at 2,000 mg/kg *p.o.* in rats. No mortality and clinical deterioration were observed. Repeat-dose oral administration of 125-1,000 mg/kg *b.i.d.* of FSD201 in rats and dogs did not cause any abnormal changes in the results of microscopic and macroscopic pathology, hematology, ophthalmology, and body weight evaluations. *In vitro* Salmonella typhimurium reverse mutation assay and mammalian cell micronucleus test indicated that FSD201 did not induce mutagenic, clastogenic, or aneugenic effects at 30-3,000 µg/plate and 1,000 µg/mL, respectively. FSD201 did not increase the incidence of rat bone marrow micronucleated reticulocytes at doses 500-2,000 mg/kg/day (*q.d.*). Comprehensive results of FSD201preclinical safetypharmacology ad toxicology will be presented.

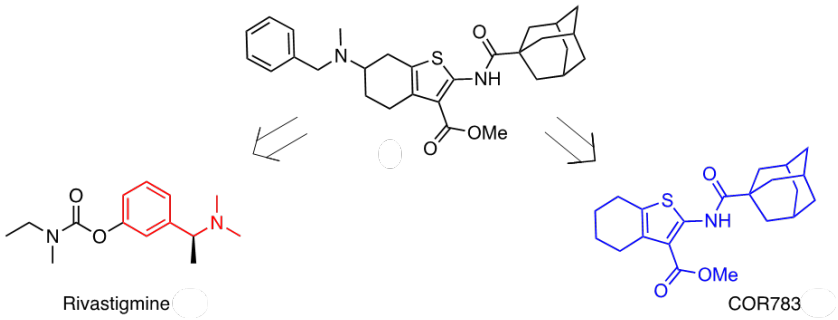
Conclusions: FSD201 is well-tolerated and safe up to 2,000 mg/kg/day in rats and dogs with no significat serious adverse effects.

SELECTIVE CB2 RECEPTOR LIGANDS WITH NOVEL HYBRID MOLECULAR SCAFFOLD AS POTENTIAL TARGET FOR NEURODEGENERATIVE DISEASES OF MULTIFACTORIAL ORIGIN: A MULTI-TARGET-DIRECTED LIGANDS (MTDL) APPROACH

Magdalena Kostrzewa[1], Poulami Kumar[1,2]*, Maria Pina Mollica[2], Rosa Purgatorio[3], Modesto de Candia[3], Claudia Mugnaini[4] and Alessia Ligresti[1]

[1]Institute of Biomolecular Chemistry, National Research Council, Pozzuoli (Naples), Italy
[2]University of Naples "Federico II", Department of Biology, Naples, Italy
[3]University of Bari Aldo Moro, Department of Pharmacy-Pharmaceutical Sciences, Bari, Italy
[4]University of Siena, Department of Chemical Biotechnology and Pharmacy, Siena, Italy

Background: The pathogenesis of neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), Huntington's disease (HD) and Amyotrophic Lateral Sclerosis (ALS) involve more than one molecular factor. In line with their multifactorial nature, besides the combination therapy approaches, the multitarget-directed ligands (MTDL) design strategy has been emerging as a novel route to the development of drugs for treating these neurodegenerative syndromes. This study aims at identifying pharmacophores which can simultaneously modulate targets involved in the multiple aetiologies of such diseases. Rivastigmine is an FDA-approved acetylcholinesterase inhibitor (AChEI) used in the treatment of mild AD. In view of the role of cannabinoid type-2 receptor (CB2R) as a potential target against AD, we focused on integrating the above mentioned pharmacophore features to possibly developing a novel class of MTDL pharmacological hits.
Methods: Based on the MTDL design strategy [1], a series of compounds was synthesized as potential molecular targets to CB2R and AChEI. Their structures were designed by suitably combining moieties derived from rivastigmine, known to covalently bind and inhibit AChE and BuChE, and from COR783, which was described as a selective high affinity CB2R agonist in a recent publication [2].



Results: The binding affinities (K_i values) of nine working compounds yielded higher affinity for CB2R than for CB1R, with K_i(CB2) values ranging from 22.29 to 626.83 nM, while K_i(CB1) is >1000 nM, with the exception of one compound, which was insoluble. In particular, four compounds show noticeable affinity ranging between 22.29 and 48.22 nM with good CB2R/CB1R subtype selectivity with reference to their parental analogue COR783. Upon evaluation for AChEI activity, three out of ten synthesized compounds exhibited inhibitory effects with single digit micromolar IC_{50} values.
Conclusion: The conjugated hybrid of COR783 scaffold and the phenyl-N1 functional subunit from rivastigmine have shown promising findings of concurrent activities – selective CB2R agonism along with AChE inhibitory activity. Further functional assays and *in vitro* on going analyses are expected to provide conclusive inferences regarding the therapeutic potential of the more promising compounds.
Acknowledgements: PK is recipient of a PhD-International program fellowship from UNINA.
References: [1] Cavalli *et al.* (2008) *J. Med. Chem.*, **51:** 347-372 [2] Mugnaini *et al.* (2019) *Eur. J. Med. Chem.*, **161:** 239–251.

INVESTIGATING THE EFFECTS OF ACUTE THC VAPOUR EXPOSURE ON STRESS REACTIVITY AND FEAR CONDITIONING

Savannah H. Lightfoot, Andrei S. Nastase, Samantha L. Baglot, Catherine Hume, Ryan J. McLaughlin[1] and Matthew N. Hill

Hotchkiss Brain Institute, University of Calgary, Calgary, AB, CA
[1]Washington State University, Pullman WA USA

Introduction: Management of stress and anxiety in the general population, and management of PTSD symptoms in patients, are often listed as primary motivations behind cannabis use, yet the understanding of how acute exposure to cannabis modulates the neurobehavioral and endocrine response to negatively valanced stimuli is not well characterized. Preclinical research has demonstrated mixed effects of THC, much of which is suggestive of dose-dependent effects; however, the predominance of this work has employed an injection method to deliver THC. As inhalation is the primary form of consumption in humans, modelling this approach in rodents is important given the robust impact route of administration has on the pharmacokinetics and biodistribution of THC and its metabolites. With the recent development of rodent vapour-based delivery systems for cannabis extracts, we can now establish the impact of acute, inhaled cannabis extracts on the processing of negatively valanced stimuli in a highly translational manner. The aims of the current study are twofold, to examine the impact of acute, controlled passive delivery of THC on stress reactivity and extinction training in a fear conditioning paradigm.

Method: Male and Female rats were exposed to either THC (10%) or vehicle (PEG) for 15 minutes (10 sec puff every 30 secs) and experiments were separated into two distinct aims to investigate the effects of THC on the both the endocrine response and behaviour. For study 1 (THC effects on stress reactivity) sixty-four rats were exposed to vehicle vapour for 9 days, on the tenth day rats were exposed to one of the two vapour conditions (THC or vehicle) then divided into one of two stress conditions: 1) naïve; and 2) stress (30 min restraint stress). Blood samples were taken at several time points and plasma corticosterone (CORT) levels were quantified using an ELISA to determine the impacts of THC on stress reactivity. For study 2 (THC effects on auditory fear conditioning and extinction) fifty rats were habituated to the vapour chambers for three days, following this, animals were randomly assigned to one of two vapour conditions as described above. On the first day rats underwent auditory fear conditioning, on the second day rats were exposed to either THC or vehicle vapour and subsequently underwent extinction, and on the third day extinction retrieval. Both passive (freezing) and active (darting) conditioned responses were quantified.

Results: Exposure to vehicle vapour increases CORT levels in both males and females, however only male rats habituated to exposure by day 9. Acute THC exposure immediately elevates CORT levels in males but not females. Exposure to THC prior to extinction training produced diverging sex differences. While THC prior to extinction training did not alter fear extinction in males, it did however impair fear extinction in females.

Conclusion: This research suggests that acute exposure to THC vapour has sex-dependent effects on both neuroendocrine and behavioural responses to aversive stimuli.

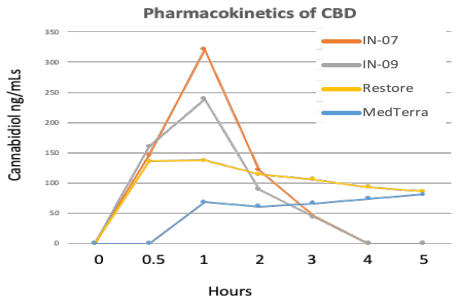
FORMULATION OF CANNABINOIDS PROMOTING TRAFFICKING THROUGH CHYLOMICRONS INCREASES BIOAVAILABILITY IN RATS

James N. Lowder, Patrick Tso, Min Liu, Vassili Kotlov, Chad Pope,
Nobumitsu Sakai and Olapeju Bolarinwa

Impact Naturals, Inc, San Diego, CA, University of Cincinnati,
The Niva Labs, Sylmar, CA, Catalent Pharma Solutions, Tampa FL

Introduction: Lipid soluble substances, such as cannabinoids, have limited bioavailability when administered orally due to lack of solubility in the aqueous environment of the gut and the first-pass metabolism through the liver. In order to avoid the first pass effect cannabinoids are frequently administered via inhalation (smoking or vaping) or mucosal absorption using tinctures. Appropriate formulation, driving the cannabinoids into chylomicrons generated in the intestinal enterocytes preferentially delivers the cannabinoids into the systemic blood via the lymphatics, sparing them the first pass metabolism associated with portal vein transport. I-N's Chylosoma formulation is designed to optimize cannabinoid absorption via this pathway.

Methods: Four 1 mg/mL test articles of CBD were used in this study: 1) IN07 2) IN09 3) I-N Restore powder and 4) MedTerra CBD tincture. The first two were formulated by Catalent/I-N to optimize absorption through chylomicrons. Five rats per group were anesthetized and their jugular veins and stomachs cannulated. The test articles were administered at a dose of 3 mg in 3 mL, through the stomach catheters. Blood samples were collected pre, 0.5, 1, 2, 3, 4, 5 and 6 hours post administration. As no drug was detected in the blood of rates administered #3 and 4 test articles in the first experiment, the gastric tube was advanced into the duodenum in a subsequent experiment the result of which are shown in this abstract.



Results:
The figure shows the mean PK of the 4 preparations. The C_{max}(±SEM) occurred at 1 hr for all 4, 1-320.65(±55.41), 2-239.47(±32.55), 3-137.37(±22.56) and 4-68.16(±8.39). Interestingly, no CBD was detected at 0.5 h for the MedTerra tincture. The shapes of the Restore and MedTerra curves suggest that caches of material were slowly released over time. 2% of the Restore dose and 10.5% of the MedTerra dose was adherent to the catheter post experiment.

Conclusions: The two Catalent formulations showed much greater bioavailability than Restore powder which was greater than the MedTerra tincture. Formulations #3 and #4 had further issues exemplified by possible trapping in the stomach and sticking to the catheter which may account for the continued exposure beyond 4 h. Subsequent experiments are underway to measure relative absorption into the lymphatics and portal vein of all four formulations. This study greatly underscores the various factors affecting absorption variability and the importance of actual experiments to access absorption.

MODULATION OF ENDOCANNABINOID SIGNALING IN HUMAN KERATINOCYTES BY "MINOR" PHYTOCANNABINOIDS

Camilla Di Meo[1], Daniel Tortolani[2], Sara Standoli[1], Clotilde Beatrice Angelucci[3], Salam Kadhim[4], Eric Hsu[4], Cinzia Rapino[3] and Mauro Maccarrone[2,5]

[1]Faculty of Bioscience and Technology for Food Agriculture and Environment, University of Teramo, 64100 Teramo, Italy;
[2]European Center for Brain Research (CERC)/Santa Lucia Foundation IRCCS, Rome, 00143, Italy;
[3]Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy;
[4]InMed Pharmaceuticals Inc., Vancouver BC, V6C 1B4, Canada;
[5]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, 67100 L'Aquila, Italy

Introduction: *Cannabis sativa* contains more than 400 bioactive components, especially phytocannabinoids (pCBs) (Hillig et al., 2004; Elsohly et al., 2005). The legalization of *Cannabis* has opened the way to investigations on the potential of pCBs as natural therapeutics for human diseases. Growing interest has been recently focused on "minor" pCBs, that are non-psychotropic compounds like Cannabigerol (CBG), Cannabichromene (CBC), Tetrahydrocannabivarin (THCV) and Cannabigerolic acid (CBGA). Of note, pCBs can act *via* an endocannabinoid system (ECS), that is an ensemble of bioactive lipids, their receptors and metabolic enzymes involved in the regulation of key physiological and pathological processes, also in the skin. In this study, we used human keratinocytes (HaCaT cells) as an *in vitro* model that expresses all major ECS elements (CB_1, CB_2, GPR55, TRPV1 and PPAR α/γ/δ receptors; NAPE-PLD, FAAH, DAGL α/β and MAGL enzymes), to systematically interrogate the effects of CBG, CBC, THCV, and CBGA on endocannabinoid signaling.

Methods: HaCaT cells were cultured for 24 hours in the presence of various amounts (in the μM range) of CBG, CBC, THCV and CBGA, and cell pellets were collected to analyze gene and protein expression of ECS elements through *quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR)* and *Western blotting*. In addition, functional activity of ECS elements was evaluated through *radioligand binding* and *enzymatic activity assays*.

Results: "Minor" pCBs differently modulate gene and protein expression of distinct ECS elements, and they all significantly increased CB_2 and TRPV1binding, as well as FAAH and MAGL activity.

Conclusions: Our results reveal that the natural cannabinoids CBG, CBC, THCV and CBGA can interact in diverse manners with distinct ECS proteins, thus having distinct effects on endocannabinoid signaling in human keratinocytes. These unprecedented observations should be considered when exploring therapeutic potential of cannabis extracts for human diseases, at least in the skin.

References

Hillig K.W., Mahlberg P.G. A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae). Am J Bot. 91(6) (2004) 966-75.
Elsohly M.A., Slade D. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci. 22, 78(5) (2005) 539-48.

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL IMAGING PROBES FOR THE VISUALIZATION OF THE CANNABINOID RECEPTORS TYPE 1 AND 2

Leonard Mach [1], Malgorzata Wasinska-Kalwa[1], Anahid Omran[1], David Sykes[2,3], Yelena Mostinski[1], Thais Gazzi[1], Wolfgang Guba[4], Arne Rufer[4], Johannes Broichhagen[1], Dmitry Veprintsev[2,3], Uwe Grether[4] and Marc Nazare[1]

[1]Leibniz Research Institute for Molecular Pharmacology, 13125 Berlin (Germany);
[2]Division of Physiology, Pharmacology & Neuroscience,
School of Life Sciences, University of Nottingham, Nottingham, NG7 2UH, UK;
[3]Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham and University of Nottingham, Midlands, UK;
[4]Roche Pharma Research & Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., 4070 Basel (Switzerland)

Introduction: Fluorescent probes are emerging as powerful tools to study G-protein-coupled receptor (GPCR) pharmacology, kinetic ligand binding, visualization of dynamic GPCR processes in living systems such as internalization.[1] The cannabinoid type 1 receptor (CB_1R) and cannabinoid type 2 receptor (CB_2R) are class A GPCRs involved in various pathologies including pain, inflammation, fibrosis, neurodegenerative diseases, anxiety, obesity, and others.[2] Robust and reliable tools that allow *in vitro* and *in vivo* investigation of the CBRs in real-time under different (patho-)physiological conditions across different species are therefore urgently needed. In this work, the design, synthesis, and biological characterization of a toolbox of fluorescent CB_1R and CB_2R probes will be presented.

Methods: Capitalizing on different highly selective and potent synthetic agonists and inverse agonists, drug-derived CBR probes were elaborated by *in silico* modeling and rigorous structure-activity optimization. A general and modular synthetic approach was applied to achieve a versatile toolbox of probes for CB_1R and CB_2R. Different fluorescent dyes allow their application in super-resolution microscopy, FACS, and other imaging techniques.

Results: These probes show exceptional flexibility with regard to the attached fluorophore label. Selected probes show low nanomolar binding affinity for their respective target receptor. Additionally, we have created CBR-subtype specific labeling tools to facilitate subsequent proximity-driven covalent receptor labeling. Their biological and pharmacological action is currently under investigation.

Conclusion: We have established a general modular synthetic approach to produce sub-type specific CBR probes. These are equipped with state-of-the-art fluorophores and labeling modalities as part of a fluorescent CBR probe toolbox. This toolbox allows the investigation of CBR dynamics in different tissues and cellular contexts.

[1] C. Iliopoulos-Tsoutsouvas *et al.*, *Expert Opin. Drug Discov*. **2018**, 10, 933-947
[2] A. Cooper *et al*., *Pharmacol. Rev.* **2017**, 69, 316

CYTOTOXICITY OF CANNABIDIOL IN PANCREATIC DUCTAL ADENOCARCINOMA OCCURS VIA UPREGULATION OF CERAMIDE SYNTHASE 1 AND INDUCTION OF ER STRESS

Nagina Mangal[1,2], Vikash Reebye[1], Barbara Pacchetti[3], Anguraj Sadanandam[2] and Mikael H Sodergren[1]

[1] Imperial College Medical Cannabis Research Group, Department of Surgery & Cancer, Imperial College London, UK
[2] Systems and Precision Cancer Medicine Team, Division of Molecular Pathology, Institute of Cancer Research
[3] Curaleaf International, Guernsey, UK

Introduction: Cannabidiol (CBD) has emerged as a compound with putative anti-neoplastic effects, however, the cytotoxic mechanism of action is not clearly understood. Chemo-resistant pancreatic ductal adenocarcinoma (PDAC) is major barrier to curative treatment. There is a pressing need to identify new therapeutic strategies. Here, we propose that CBD may have a potential role in pro-apoptotic and anti-tumorigenic pathways through its regulation of ceramide synthase 1 (CerS1). In this study we interrogate various oncological signalling pathways to elucidate a cytotoxic mechanism of action of CBD in PDAC. We specifically evaluate whether CersS1 is able to induce growth arrest and apoptosis in PDAC cells.

Methods: A range of PDAC cell lines (human-Panc03.27, Panc1 and mouse-3275) were treated with CBD concentrations alone or in combination with gemcitabine and abraxane. IC_{50} values were calculated for response sensitivity, and a Cancer Inflammation & Immunity Crosstalk PCR Array was used to explore whether CBD influenced immune-oncology signals.

Results: The antagonistic potency of CBD was measured across the PDAC lines. Panc0327 showed the most sensitive response to treatment (CBD, gemcitabine IC_{50} = 15μM, 6.78nM) when compared to Panc 1 cells (CBD, gemcitabine IC_{50} = 25μM, 0.1uM). Single treatment with CBD caused upregulation of CerS1 across all cell lines. A further increase in CerS1 was then observed when CBD was combined with gemcitabine and abraxane. We also observed an increase in GPR78, GPR55, CB_2 and ERK1/2 levels concomitant to this combination treatment in the chemo-sensitive Panc0327 cells only. The response of chemo-resistant Panc1 cells to CBD was distinct to Panc0327. Although Panc1 did not share the same GPR78, GPR55, CB2 and ERK1/2 response, we observed an upregulation of VEGFA, IRF1, CCL2 and BCL2L1 and down-regulation of tumour promoting cytokines.

Conclusions: This study is the first to characterise the signaling pathway that CBD may exert in PDAC. We show evidence of a putative Cers1 dependent pathway driven by CBD in activating ER stress targets (GRP78, p8, CHOP) which in turn leads to GPR55 or CB_2 mediated anti-neoplastic events. CBD may also have an influence on distinct signalling pathways that could potentially predict the differentiation transition of PDAC lines.

THE APPLICATION AND COMPARISON OF VARIOUS COMPUTER-ASSISTED MEDICINAL CHEMISTRY METHODS: INDAZOLE-BASED CB_1 AGONISTS

Jack Markham,[1,2] Lewis J. Martin,[1] Adam Ametovski,[1,2] David E. Hibbs[3] and Samuel D. Banister[1,2]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, NSW, Australia
[2]School of Chemistry, The University of Sydney, NSW, Australia
[3]School of Pharmacy, The University of Sydney, NSW 2006, Australia

Introduction: The cannabinoid type 1 receptor (CB_1) has been an attractive therapeutic target for the development of novel drugs in the treatment of pain, epilepsy, and other conditions, with several Synthetic CB_1 Receptor Agonists (SCRAs) approved by the FDA (e.g. nabilone) or currently undergoing clinical trials. The approved CB_1 targeting drugs currently available are all closely derived from, or directly employ, phytocannabinoids found in *cannabis sativa*. Other drug scaffolds have been investigated, including substantial work by Pfizer on indazole-based CB_1 agonists. Data from these studies are now publicly available and the >1000 structures and accompanying CB_1 binding and functional activity represents a highly valuable dataset that can be employed alongside computational methods to understand and rationalise the Structure-Activity Relationships (SAR) that govern the activity and binding of drugs within this class. Computational analysis is a relatively fast and cost-efficient way to for large-scale, industrial, *in vitro* research to be repurposed for academic research. The consistency of the library also allows us to investigate the utility of various computational and statistical methods in this context.

Methods: Data for this study was obtained from two publicly available Pfizer patents (WO2009106982A1 and WO2009106980A2) and extracted both manually and via supervised machine learning methods. Quantitative Structure-Activity Relationship (QSAR) techniques (Free-Wilson analysis and Molecular Fingerprinting) were initially employed to determine which structural features were associated with favourable *in vitro* metrics. More sophisticated techniques (3D shape-based screening and molecular docking) were also applied to give a greater understanding of specific ligand-receptor interactions and their influence.

Results: The techniques were each performed and evaluated by generation of a Receiver Operating Characteristic (ROC) curve or by ridge regression of the scores against the *in vitro* experimental binding data. The shape-based docking technique performed best (AUC = 0.71) and the Free-Wilson QSAR technique performed the worst (MSE = 0.52). A Multi-Parameter Optimization (MPO) analysis was performed with a combination of scoring techniques and calculated physical properties, outperforming each of the individual measures (AUC = 0.82).

Conclusions: Even at a personal-computer scale, computational analysis and modelling can be applied to historical data to generate new SAR hypotheses and enrich screening libraries. Each of these techniques is sensitive to different factors of the library (e.g. shape), so while their relative agreement with experimental data may be comparable, they each have unique biases which are important to understand in analysis. Further investigation will involve the use of more computationally-intensive techniques using a sub-set of the data, as well as wet-lab experimental validation, with the goal of optimising the virtual screening pipeline for indazole-based SCRAs.

MATERNAL CBD EXPOSURE IN MICE ALTERS MOTOR COORDINATION IN THE OFFSPRING AT ADULTHOOD

César E. Martinez Ramirez, Garrett Sauber, Ashley Doherty, Leslie Krause, Gonzalo Ruiz-Pérez and Cecilia J. Hillard

Department of Pharmacology and Toxicology,
Medical College of Wisconsin, Milwaukee, WI, USA

Introduction: The recreational and medicinal use of cannabis has been rising in the US as a result of legalization and decriminalization: medicinal use is now legal in 38 states and decriminalized in 32 states. Furthermore, data collected from 2002 to 2014 in the U.S. indicate that 7.5% of pregnant women between 18 and 25 years of age use THC, while the rate of use in all pregnant women is approximately 4%. The use of products with low THC/high CBD content are popular given that they produce little dependence and psychoactivity. There is evidence to support that CBD consumption during pregnancy exposes the child to CBD and it is unknown whether this exposure can have negative long-term consequences in the health of the child. In the present study, we have investigated the effects of prenatal CBD exposure on motor coordination in the offspring at adulthood.

Methods: CBD is infused into corn pops which is a breakfast cereal (1mg/ml in ethanol); control pieces are infused with ethanol. The cereal is then dried to evaporate the ethanol. Mouse dams were provided with infused cereal at the beginning of pregnancy and continuing until birth. More than 90% of the cereal is consumed within 1 hour throughout the treatment period. 6 pregnant dams were treated with CBD and 5 with vehicle. Adult offspring (40 weeks of age) were trained on the rotarod for 3 minutes at 4 rpm on day 1. On day 2, mice were placed on the rotarod and the speed was increased from 0 to 45 rpm over 3 minutes then held steady until either the mouse fell off or if they held onto the rod for one full revolution. Data was analyzed using an unpaired t-test ($p<0.05$ considered significant).

Results: Male and female offspring of the CBD treated dams spent on average significantly less time (100.0 sec vs 114.3 sec), fell off at a lower speed (26.903 RPM vs. 30.168 RPM) and traveled a shorter distance on the rotating rod (2.556m vs. 3.236m) when compared to control. $p<0.05$ using an unpaired t-test.

Conclusion: We have discovered a potentially adverse effect of CBD and identified a significant deficit in performance on the rotarod task in adulthood following in utero CBD exposure. These findings support the hypothesis that prenatal CBD exposure can have detrimental effects on brain development and support further studies into the mechanisms involved. Next steps include examining changes in brain morphology with immunohistochemistry studies in mice exposed to CBD *in utero*. We will examine changes in the molecular layer thickness, dendritic spine density in the granular layer, and GPR55 cellular expression in the cerebellar cortex.

Acknowledgements: This research was funded and supported by the Kubly Fund for Depression Research.

NEUROPROTECTIVE EFFICACY OF VCE-004.8 IN A NEWBORN RAT MODEL OF PERINATAL ARTERIAL ISCHEMIC STROKE

María Martinez Vega, María Villa Cruz, Laura Silva Colmenar, María de Hoz Rivera, Ángela Romero Sánchez, Juan Miguel Godoy Corchuelo and José Martínez Orgado

Hospital Clinico San Carlos – IdISSC. Madrid. Spain

Background: cerebrovascular disease (stroke) is a major cause of death and disability in newborns with no current effective treatment. In newborn rat models of ischemic brain damage cannabidiol (CBD) induces neuroprotective effects which were inhibited by CB2 receptor antagonism. We hereby tested the neuroprotective effect of a synthetic CBD aminoquinone derivative (VCE-004.8), known to activate CB2 and the HIF pathway in addition to PPARg receptors.

Aim: to assess the neuroprotective effect of VCE-004.8 after stroke in newborn rats.

Methods: cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) for 3 hours in newborn (P8) rats. 30 min after removing the occlusion, vehicle n= 11) or VCE-004.8 (supplied by Emerald Health Pharmaceuticals, USA) were administered intraperitoneally (i.p.) at three daily doses at 5 mg/kg, starting 0.5 (n=11), 12 (n=9) or 18 h (m=9) post-stroke. Similarly manipulated but non-occluded rats served as controls (n=11). At P14, motor tests assessing coordination (inverse geotaxis) and strength (grasp test) were performed. Then, at P14 brain samples were obtained to assess brain damage using magnetic resonance imaging (MRI) to determine the perilesional hyperintense area, immunohistochemistry (IHC) to quantify cell death (TUNEL) and proton magnetic resonance spectroscopy (^{1}H-NMRS) to assess metabolic damage determining the Lactate/N-acylaspartate (Lac/NAA) ratio, excitotoxicity by determining the Glutamate/NAA ratio (^{1}H-NMRS), and oxidative stress, by determining protein nitrosylation (Oxyblot).

Results: VCE-004.8 administered 30 min after MCAO showed robust neuroprotective effects, reducing the volume of damage, perilesional cell death and excitotoxicity Those effects were associated to the reduction of strength impairment. No differences in coordination performance or Lac/NAA were observed among groups at P14. All protective effects of VCE-004.8 were still observable when drug administration was delayed to 12 h. Beneficial effects on MRI and histological studies, but not on strength an excitotoxicity, were observed when VCE-004.8 administration was delayed to 18 h after MCAO.

Conclusions: VCE-004.8 at 5 mg/kg administered in three doses every day, starting 30 min post-stroke showed robust neuroprotective effects as observed by neuroimaging, histological, biochemical and functional studies. VCE-004.8 showed a therapeutic window of 12 h, which is broader than that reported for most neuroprotective treatments, with some beneficial effects still observable when drug administration was delayed up to 18 h.

Supported by grants from Emerald Health Pharmaceuticals and PI19/00927

EVALUATION OF NOVEL BITOPIC LIGANDS AT THE TYPE 2 CANNABINOID RECEPTOR

Kawthar A. Mohamed,[1] Francesca Gado,[2,3] Rebecca Ferrisi,[2] Beatrice Polini,[2,3] Elena Lucarini,[4] Sara Carpi,[2] Federica Domenichini,[2] Lesley A. Stevenson,[5] Paola Nieri,[2] Roger G. Pertwee,[5] Carla Ghelardini,[4] Lorenzo Di Cesare Mannelli,[4] Grazia Chiellini,[3] Robert B. Laprairie[1] and Clementina Manera[2]

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Canada.
[2]Department of Pharmacy, University of Pisa, Italy.
[3]Department of Pathology, University of Pisa, Italy.
[4]Department of Neuroscience, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Italy.
[5]Institute of Medical Sciences, University of Aberdeen, UK.

Introduction: Of the two cannabinoid receptors that have been identified thus far, the type 2 cannabinoid receptor (CB2R) is of growing interest due to its potential immunomodulatory and anti-inflammatory activities. CB2R-mediated therapeutics may provide safer side effect profiles and avoid CNS-mediated psychoactivity associated with the activation of the type 1 cannabinoid receptor (CB1R). The identification of CB2R-specific ligands has been challenging given the high degree of similarity between CB1R and CB2R; the development of bitopic ligands may represent a novel approach to this challenge. These ligands consist of an orthosteric agonist linked to an allosteric modulator, allowing for simultaneous binding to the receptor's orthosteric and allosteric binding sites.

Methods: In this study, we explored the in vitro pharmacological properties of a novel class of CB2R bitopic ligands using Chinese hamster ovary (CHO)-K1 cells. Enzyme fragment complementation (EFC) assays were used to determine whether downstream signaling involved βarrestin2 recruitment and Gα(i/o)-coupled cAMP inhibition while competitive radioligand binding assays were used to assess binding affinity at the cannabinoid receptors.

Results: The novel bitopic ligands preferentially inhibited cAMP accumulation over βarrestin2 recruitment, suggesting potential signal bias. Furthermore, these ligands induced their actions exclusively through CB2R, as evidenced by lack of binding at CB1R in radioligand binding assays and by our finding that their activity at CB2R was blocked in the presence of the CB2R antagonist/inverse agonist SR144528.

Conclusion: Given CB2R's expression throughout the immune system, these findings may be useful in the development of CB2R-selective therapeutics for illnesses associated with pain and/or inflammation.

Acknowledgements: Funding for this project is from a Natural Sciences and Engineering Research Council (NSERC) Discovery Grant to RBL. KAM is funded by an NSERC Graduate student award.

CANNABIDIOL INCREASES EXCITATORY SYNAPTIC TRANSMISSION AT TEMPOROAMMONIC-CA1 SYNAPSES IN RAT HIPPOCAMPUS

Erica O'Beirne and Jenni Harvey

School of Medicine, University of Dundee, UK

Introduction: Cannabinoids produce their biological effects via activation of cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2), but in the CNS, the predominant receptor is CB_1. Many studies have examined the effects of cannabinoids on excitatory synaptic transmission at hippocampal Schaffer collateral (Sc)-CA1 synapses, with evidence suggesting a role for cannabinoids in learning and memory. However, the effects of cannabinoids on excitatory synaptic function at the anatomically-distinct temporoammonic (TA) input to CA1 neurons is not clear. Here, we examined the effects of cannabidiol (CBD), one of the pharmacologically active components of cannabis, at juvenile hippocampal TA-CA1 synapses.

Methods: Standard extracellular recordings were used to explore the effects of CBD at TA-CA1 synapses in slices from juvenile (P12-24) Sprague Dawley rats. Various concentrations of CBD (3 μM; 10 μM) were applied for 15 min to acute hippocampal slices and the effects on the slope of field excitatory postsynaptic potentials (fEPSPs) were monitored.

Results: Application of CBD induced a significant concentration-dependent potentiation of excitatory synaptic transmission, with 3 μM CBD increasing synaptic transmission to 116 ± 2.5% of baseline ($n=5$; $p<0.05$) and 10 μM CBD causing an increase to 125 ± 3.2% of baseline ($n=5$; $p<0.001$); effects that were sustained on CBD washout. To explore the pharmacology of this CBD effect and possible involvement of CB1R, we administered CBD (10 μM) in the presence of the CB_1R antagonist, O-2050 (50 nM). The ability of CBD to increase excitatory synaptic transmission was not significantly inhibited in the presence of O-2050 (134 ± 5.4% of baseline; $n=6$; $p<0.05$), suggesting the effect of CBD on synaptic transmission is independent of CB_1R. As GPR55 is another putative receptor target for CBD, the possible involvement of GPR55 was also examined. To verify involvement of GPR55, CBD (3 μM) was administered in the presence of the selective GPR55 antagonist, CID16020046 (10 μM). The ability of CBD to increase synaptic transmission was not significantly altered in the presence of CID16020046, such that CBD increased synaptic transmission to 112 ± 3.8% of baseline ($n=6$; $p<0.05$) in the presence of the antagonist. These data indicate that GPR55 is unlikely to contribute to the actions of CBD, and suggest possible involvement of other receptor/s.

Conclusions: These findings suggest that application of CBD results in a persistent increase in excitatory synaptic transmission at TA-CA1 synapses, thereby suggesting potential cognitive enhancing effects of CBD. The synaptic effects of CBD were not inhibited by the CB_1R antagonist, O-2050 or the GPR55 antagonist, CID16020046, indicating that this effect of CBD is likely to be independent of CB1R or GPR55. Further studies are required to identify the receptor mediating CBD-driven effects at hippocampal TA-CA1 synapses.

Acknowledgements: Funded by Medical Research Scotland.

SYNTHESIS OF FLUORINATED CANNABINOID DERIVATIVES; TOWARDS PET RADIOTRACERS FOR IMAGING THE CANNABINOID RECEPTOR

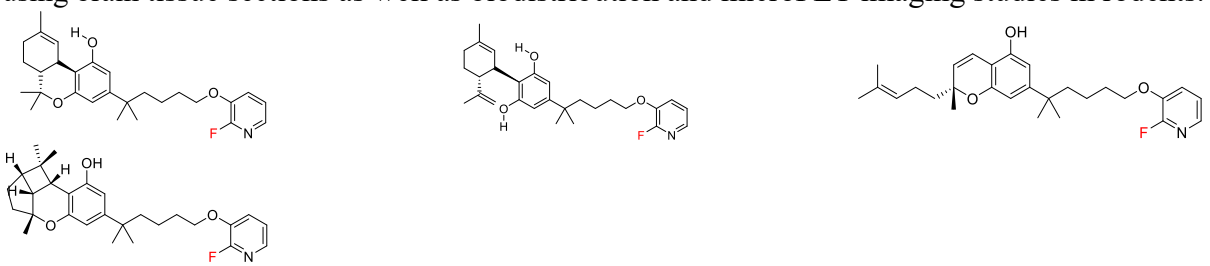
Placid Nnamdi Orji,[1] Asher L. Brandt[2], Ayat Zagzoog[2], John G. Howland[3], Robert B. Laprairie[2,4] and Christopher Phenix[1]

[1]Department of Chemistry, College of Arts and Science, University of Saskatchewan, Saskatoon, SK Canada
[2]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK Canada
[3]Department of Anatomy, Physiology, and Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon SK Canada
[4]Department of Pharmacology, College of Medicine, Dalhousie University, Halifax NS Canada

Introduction: Efforts towards understanding the endocannabinoid system, which is comprised of cannabinoid receptors and endogenous ligands, has rapidly expanded in recent years due to the ability of this complex network to regulate many healthy and disease promoting processes. The endocannabinoid system can modulate the activity of other neurotransmitters through the type 1 cannabinoid receptor (CB_1) which is highly expressed in the human brain. Defects in CB_1 expression or endocannabinoid transmission have been associated with several neuropsychiatric disorders thus establishing CB_1 as a potential drug target. However, tools to study the function of the endocannabinoid system, especially in vivo, are not readily available. Therefore, functional imaging techniques, such as positron emission tomography (PET), may be useful for understanding the complex role of the endocannabinoid system in animals and humans.

Methods: Our major focus was to prepare novel fluorinated analogs of tetrahydrocannabinol-type, cannabidiol-type, cannabicyclol-type, and cannabichromene-type plant cannabinoids from *Cannabis sativa* as PET tracer candidates. Synthetic routes were developed leading to several novel fluorinated cannabinoids (figure below), and their respective precursors, that could be efficiently labeled with ^{18}F for in vivo PET imaging studies. Prior to radiolabeling chemistry, the biological activity of each fluorinated cannabinoid towards CB_1 was assessed in cell models (and in some cases rodents) successfully identifying several promising PET tracer candidates.

Results: In vitro and in vivo biological assays have confirmed that several fluorinated cannabinoid derivatives of tetrahydrocannabinol-type ligands behave as partial agonists in CHO-K1 cells stably expressing CB_1 and evoke tetrad-like responses in C57BL/6 mice at very low doses. Current efforts are focused on labeling the most promising derivatives with ^{18}F for autoradiography experiments using brain tissue sections as well as biodistribution and microPET imaging studies in rodents.



Conclusions: We have identified several novel radiotracer candidates, that bind potently to CB_1 in vitro and in vivo, and may be useful for imaging the endocannabinoid system with PET. We anticipate that these novel radioligands will provide additional information on the pathophysiology of the cannabinoid receptors while also be useful to rapidly identify new ligands that bind to CB_1 in vivo as a part of drug discovery platform.

Acknowledgments: Funding for this project was provided by a CIHR-GSK partnership grant to RBL and a Sylvia Fedoruk Centre project grant to CP, RBL, and JGH. ALB is supported by funding from the National Research Council of Canada. AZ is supported by a scholarship from the University of Saskatchewan College of Pharmacy and Nutrition.

DEFENSIVE ROLE OF CANNABIDIOL (CBD) AGAINST PEST INSECT TOBACCO HORNWORM *MANDUCA SEXTA* THROUGH DISRUPTING EXOSKELETON DEVELOPMENT

Sang-Hyuck Park, Sam Koch and Brian D. Vanden Heuvel

Institute of Cannabis Research, Colorado State University – Pueblo
2200 Bonforte Blvd. Pueblo, CO81001

Cannabidiol (CBD) has received much attention in recent years due to versatile therapeutic potentials. However, little is known about the ecological benefits of the phytocannabinoids in *Cannabis* plants. To investigate the defensive role of CBD, a feeding preference assay was performed with tobacco hornworm *Manduca sexta.* The larvae clearly showed feeding preference towards the *Cannabis* tissue containing low CBD (0.81%/mg of dry weight) over high CBD (3.2%/mg of d.w). While the larvae avoided the high CBD containing tissue, we investigated detrimental effects of CBD dissolved in artificial diet (AD) by comparing to two control groups, AD and AD+0.1% medium chain triglyceride (MCT; vehicle). The feeding assay showed that 2nd instar larvae reared on 1mM CBD were significantly smaller than those of the two control groups. The CBD group had 34% less total crude protein while total glucose and lipid contents were not changed. To further investigate if CBD had any effects on insect's metabolic profiles, gas chromatography time-of-flight mass spectrometry analysis (GC-TOF-MS) was performed. In the CBD-fed insects, three amino acids, asparagine, L-aspartic acid, and β-alanine, involved in dopamine synthesis, were significantly increased by 2.8-, 2.1-, 17-fold, respectively while trehalose was decreased by 5.6-fold. However, 1mM CBD did not influence the diet consumption, body water retention, or mortality in the larvae. Biochemical changes in CBD-treated insects were assessed by RNA-Seq, which yielded 20,898 transcripts. Among them, two genes encoding collagenase-like and six genes encoding cuticle protein are significantly up- and down-regulated, at >2,629-fold and >534-fold (adjusted p-value <0.1), respectively. The drastic changes of the transcripts involved in the exoskeleton formation may disrupt the hardening process of exocuticle (sclerotization). Additionally, electrophysiology analysis was performed on the ventral ganglion to examine if CBD affects the insect's central nervous system. The electrophysiology results revealed that the CBD-treated ganglia had delayed but much larger response with electric stimuli in comparison to the larvae reared on AD only diet. Our results show that high dose CBD (>1mM CBD) negatively affects the growth and development of the pest insect by impacting the central nervous system, as well as disrupting exoskeleton development.

EVALUATING CANNABINOID RECEPTORS AS A THERAPEUTIC TARGET FOR UVEAL MELANOMA

Marzia Pendino[1,2], Sandra Garcia Mulero[3,], Rebeca Sanz Pamplona[3], Simone Marcone[4], Kayleigh Slater[1,2], Josep Piulats [5] and Breandan N. Kennedy[1,2]

[1]UCD School of Biomolecular and Biomedical Science and [2]UCD Conway Institute, University College Dublin, Dublin, Ireland.[3] Oncology data analytics program (ICO-IDIBELL), Hospitalet de Llobregat, Spain, [4]Trinity Translational Medicine Institute, Department of Surgery, Dublin, Ireland [5]Medical Oncology department, Catalan Institute of Oncology (ICO), Hospitalet de LIobregat, Spain

Introduction: This research evaluates the disease relevance of cannabinoid receptors in UM patient samples and the therapeutic potential of synthetic cannabinoids in uveal melanoma (UM) cell lines. UM is a rare cancer, but the most common intraocular malignancy in adults that arises from melanocytes within the uveal tract. Unfortunately, up to 50% of patients develop liver metastases that rapidly progress to mortality. There are no effective therapies available for metastatic UM.

Methods: Cannabinoid receptor gene expression in 80 primary UM samples within The Cancer Genome Atlas was analysed for association with disease-free and overall survival. Gene set variation analysis enriched for molecular functions and biological pathways linked to high or low cannabinoid receptor expression. In vitro assays utilised Mel285 and OMM2.5 human UM cell lines derived from a tumour of the eye and a liver metastasis, respectively. Cell viability was examined at 96 hours after treatment with the synthetic cannabinoid HU210 by measuring metabolic activity. Colony formation assays assessed long-term UM cell proliferation. Multiplex ELISA determined the secreted levels of 10 inflammatory factors at 4 and 24 hours after treatment with HU210 in the OMM2.5 cell line. Western Blot analysed expression of CB_1.

Results: Kaplan-Meier survival curves demonstrate a significant corelation between high CB_1 expression and disease-free survival in UM patients. The CB_1/CB_2 agonist HU210 results in a dose-dependent reduction in Mel 285 and OMM2.5 cell viability with 20 μM HU210 reducing cell viability by around 80%, $p<0.0001$ whereas 150 μM of the more selective CB_2 agonist JWH133 was required to significantly reduced viability by 80%, $p=0.0001$ in both UM cell lines. 10 μM rimonabant hydrochloride and 10 μM SR144528, CB_1 and CB_2 selective antagonists, respectively, were the maximum tolerated concentrations not affecting cell viability. 20 μM HU210 results in a reduction of long-term proliferation of clones in both UM cell lines. Western blot analysis confirmed expression of CB_1 in Mel285, Mel290, OMM2.5 cells.

Conclusions: Significant corelations between high CB_1 expression and disease-free survival in UM patients was identified. HU210 reduces viability and clone proliferation of UM cell lines and modulated inflammatory pathways. Future directions will evaluate the key receptor mediating HU210 effects using antagonists and investigate the mechanism of action.

THE CROSSTALK BETWEEN CANNABINOID 1 RECEPTOR (CB_1R) AND ADENINE NUCLEOTIDE TRANSLOCASE 2 (ANT2) IN THE REGULATION OF MITOCHONDRIAL FUNCTION IN THE KIDNEY

Anna Permyakova, Alina Nemirovski and Joseph Tam

Obesity and Metabolism Laboratory, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Introduction: The endocannabinoid (eCB)/CB_1R system is a ubiquitous ligand-directed signaling system involved in regulating a wide range of physiological processes, including those important for energy homeostasis. Emerging evidence suggests that the eCB/CB_1R system modulates mitochondrial integrity and function, such as oxidative phosphorylation, and ATP production. Recently, we have shown that mitochondrial morphology and shape in the renal proximal tubule cells (RPTCs) is regulated by the eCB/CB_1R system. Adenine nucleotide translocases (ANTs), which transport ADP and ATP through the mitochondrial inner membrane, play an essential role in energy metabolism of eukaryotic cells. As ANT2 is involved in ATP production and oxidative phosphorylation, it is plausible to hypothesize that its function is also regulated, in part, by the eCB/CB_1R system.

Methods: RPTC-$CB_1R^{-/-}$ and RPTC-$ANT2^{-/-}$ mice were generated using the *Cre*/*loxP* system. To generate obesity, C57BL/6J animals and their littermate controls were fed with high-fat diet or standard diet for 24 weeks. Overexpressed CB_1R-TK-d64-transfected HEK293 cells as well as mouse primary RPTCs extracted from the kidney of RPTC-$CB_1R^{-/-}$ and RPTC-$ANT2^{-/-}$ animals were utilized to measure the protein expression levels of ANT2 and CB_1R using western blot or immunostaining. Endocannabinoids were extracted, purified, and quantified from kidney samples of obese and lean human patients as well as RPTC-$ANT2^{-/-}$ animals.

Results: Increased anandamide content, enhanced ANT2, and downregulated CB_1R expression levels were found in kidney samples collected from humans with obesity. Whereas a similar pattern of expression of renal CB_1R was measured also in obese mice, a significant reduction in the levels of ANT2 was found in these animals. Likewise, specific nullification of CB_1R in RPTCs in mice resulted in a dramatic reduction in renal ANT2, while overexpression of CB_1R in HEK293 cells significantly enhanced its levels. Interestingly, genetic deletion of ANT2 in RPTCs in mice dramatically reduced CB_1R expression in RPTCs and increased *N*-palmitoylethanolamide and arachidonic acid.

Conclusions: Our findings reveal a previously undescribed crosstalk between ANT2 and the eCB/CB_1R system in the kidney. Manipulation of RPTC CB_1R differentially affected kidney ANT2 expression in humans and mice. In addition, ANT2 by itself may directly or indirectly regulate eCB/CB_1R 'tone' in the kidney. These findings suggest, at least in part, that CB_1R may affect renal mitochondrial function via regulating ANT2, and that targeted manipulation of RPTC ANT2 may represent a novel approach for the treatment of kidney pathologies caused by an overactive eCB/CB_1R 'tone'.

Acknowledgments: Funded by the ERC-2015-StG (#676841) and Israel Science Foundation (ISF; #158/18) grants to Joseph Tam.

THE EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON MITOCHONDRIAL FUNCTION IN HUMAN EMBRYONIC KIDNEY 293 (HEK 293) CELLS

Pavel Powlowski[1], David Bodenstein[1], Kassandra Zachos[1], Catherine Mielnik[1], Ana Andreazza[1] and Ruth Ross[1]

[1]Department of Pharmacology & Toxicology, University of Toronto, Toronto, ON, Canada

Introduction: In recent years there has been a resurgence in the study of the effects of cannabinoids on mitochondrial function (D. Jimenez-Blasco et al., Nature, 2020, 603-608). As mitochondria produce cellular energy in the form of ATP, they are vital players in overall cell health. Mitochondria have been implicated in a variety of diseases, such as cancer, neuropathic pain, bipolar disorder, schizophrenia (DF. Bodenstein et al, NPJ Schizophr. 2019), and mitochondrial disease, among others. Thus, understanding the pharmacological effects cannabinoids can exert on mitochondria remains important. In this study, we have tested the effects of Δ9-Tetrahydrocannabinol (THC) across a wide dose-response to examine the impact on mitochondrial membrane potential, ATP production, and overall mitochondrial metabolism.

Methods: HEK 293 wild-type and cannabinoid receptor type 1 (CB1) stable cells were tested across a variety of mitochondrial function assays. Cells were treated with a dose-response of THC across ten-fold serial dilutions. JC-1, a carbocyanine dye responsive to changes in mitochondrial membrane potential (ψ_m) was used to qualitatively assess changes in ψ_m induced by THC. CellTiter-Glo, a luciferase assay reliant on the ATP content of cells was used to measure changes to ATP induced by THC. Biolog Mitoplates use a tetrazolium dye to measure electron transport chain activity in the presence of various substrates and were used to assess changes to cellular metabolism in the presence of 1μM THC. JC-1 data was analyzed using a one-way ANOVA with Bonferroni correction, with $p<0.05$ representing significance. CellTiter-Glo data was analyzed using a student's T-test, with $p<0.05$ representing significance.

Results: Membrane potential in response to cannabis was similar across wild-type and CB1 stable cells, with extremely high doses producing a strong decrease in membrane potential, while doses of 1 μM or less were able to slightly increase membrane potential 30 minutes post-THC administration. 1 μM of THC in wild-type cells, but not CB1 cells, was able to produce a small but significant ($p<0.05$, *) increase in ATP content. Mitoplates showed a wide range of changes to cellular metabolism in the presence of THC, however further analysis for pathway effects is necessary to fully understand which changes are induced.

Conclusions: THC shows the ability to produce a wide variety of changes to mitochondrial function. Whether these changes are due to CB1 stimulation or other non-CB1 mediated pathways remains to be determined.

Acknowledgements: PP was funded by a TC3 student fellowship.

BUILDING A GLOBAL CANNABIS SOCIAL NETWORK: DATA FROM THE NEW EcoCaNN MOBILE APPLICATION

Itay Lipka[1], Eliran Malki[2], Nurit Tweezer Zaks[3], Yossi Ben Amram[3], Talma Gotteiner[3] and Neta Rimmerman[3]

[1]EcoCaNN LtD, Kfar Saba, Israel [2]Belong.Life, New York, NY, USA
[3] M.H MediCane Ltd, Kfar Saba, Israel

Introduction: EcoCaNN is a new, user-friendly Cannabis social network mobile application (App) powered by the latest Belong.life patient community and care platform. It was built as a reliable knowledge center to address an unmet need connecting between medical cannabis users, their caregivers, and healthcare professionals. By bridging the knowledge gap, leveraging artificial intelligence, crowds wisdom, and on-line professional supporting tools, EcoCaNN aims to provide significant benefits to its users in order to improve outcomes and quality of life by integrating and analyzing shared data.

Methods: EcoCaNN offers its users (all anonymous) free knowledge in the form of questions & answers with medical experts (e.g. nurse, pharmacist, doctors), article sharing, interviews, regulatory updates, webinars, and updates on clinical trials. The registered anonymous users share their need, and qualitative analysis is continuously run on the data collected through the platform providing up to date information about user's journeys, critical decision points and optimal treatment options. Users occasionally participate in surveys aimed to gather data of interest for their improved treatment options management. The App operates in accordance with privacy regulations in each territory in which it is launched.

Results: In 2022, Israel with a population of approximately 9.2 M has reached nearly 100,000 licensed medical cannabis users. EcoCaNN was first launched in July 2020 and rapidly accumulated more than 50,000 downloads. A recent data analysis performed with approximately 8,500 users revealed that 53% of them reported smoking/vaporizing/using an inhaler, and only 18.6% using cannabis oils. Regarding medical indications, EcoCaNN users reported the largest proportion (20%) receiving medical cannabis for treating non-cancer pain, 16% for sleeping disorders (interestingly, an indication not yet approved by the Israeli Ministry of Health (MoH)), 6% for post-traumatic stress disorder, 5.3% for depression, 4.9% for fibromyalgia, 4.5% for allergy, 4.2% for cancer pain, and 3.6% for anxiety. Data presented will include additional quantitative and qualitative data collected and analyzed from the EcoCaNN App.

Conclusions: Israel is EcoCaNN's first and main market today. Data is therefore biased towards indications approved by the Israeli MoH. EcoCaNN will soon be available in the UK, and additional English-speaking countries. addressing a global unmet need to connect between medical cannabis users and healthcare professionals to improve treatment outcomes by integrating and analyzing shared global data.

CANNABIDIOL NEUROPROTECTION IN INTRAVENTRICULAR HEMORRHAGE-INDUCED BRAIN DAMAGE IN IMMATURA RATS IS RELATED TO ANTI-INFLAMMATORY EFFECTS THROUGH MICROGLIAL MODULATION

A. Romero [(1)], A. Del Pozo Sanz [(1)], M. de Hoz-Rivera [(1)], L. Silva [(1)], M. Villa [(1)], M. Martínez [(1)] and J. Martínez-Orgado [(1)].

[(1)]NEURO-INA-IN, Hospital Clínico San Carlos-IdISSC, Madrid, Spain

Background: Intraventricular Hemorrhage (IVH) is a frequent complication in extremely low gestational age newborns, increasing the risk of Cerebral Palsy (CP) development. Inflammation has been described as a critical contributor to that process due to the particular susceptibility of immature oligodendrocytes. Currently, there is no effective treatment preventing the inflammatory events derived from IVH. Cannabidiol (CBD) has shown neuroprotection linked to anti-inflammatory effects in other animal models of newborn brain damage.

Aim: To assess the effects of CBD on the inflammatory response after IVH insult in newborn rats.

Methods: IVH was induced in newborn Wistar rats (P1) by injecting *Chlostridium* collagenase into the left Germinal Matrix by stereotactic surgery. Animals not exposed to surgery remained as controls (SHAM), CBD or vehicle (VEH) were administered prenatal (10 mg/kg i.p. to pregnant rats at E21) and post-insult (5 mg/kg, i.p. 6h, 24h and 48h after the insult). Neurobehavioral (NB) tests were conducted at medium (P14) and long (P45) term. Brain damage was assessed by MRI and neurobehavioral studies in the short- and long term (P6 to P45). Immunohistochemistry (IHC) was performed to quantify Myelin basic protein (MBP) expression at P45. Western Blot was performed at P6, P14 and P45, to quantify inflammatory markers (TLR-4, NFκβ, and TNFα). Flow cytometry (FC) was used to determine microglial activation and macrophage infiltration [CD11b+/CD45+^{low} (resting cells), CD11b+/CD45+^{high} (activated cells)], as well as their phenotype [M1 (iNOS, pro-inflammatory)/M2 (Arginase: anti-inflammatory) ratio].

Results (Table): CBD reduced brain damage as assessed by MRI and prevented short and long term motor and cognitive deficits secondary to IVH. Those effects were associated with the modulation of inflammation. At P6 (Table), CBD prevented IVH-increase of inflammatory markers such as TLR-4 and NFκβ, and reduced TNFα increase. CBD prevented the increase of activated microglia and infiltrating macrophages in brain tissue and enhanced the polarization of activated and resting microglia to a M2 phenotype. Similar results were obtained at P14, whereas no differences among groups were found at P45. CBD treatment prevented hypomyelination as assessed by MBP and MRI (Corpus callosum area) studies at P45.

Table. Results at P6:

			SHAM (n=16)	IVH-VEH (n=15)	IVH-CBD (n=16)
WB	**P6**	TLR4-4$^{(A.U.)}$	**97.36** (*82.99-101.0*)	**108.8** (*106.7-124.3*) *	**90.71** (*83.72-101.7*) §
		NFκβ$^{(A.U.)}$	**112.9** (*89.78-126.1*)	**125.9** (*108.6-147.3*) *	**106.6** (*88.96-113.5*) §
		TNFα$^{(A.U.)}$	**100.5** (*96.97-104.2*)	**110.1** (*103.1-113.3*) *	**104.9** (*92.27-112.9*)
FC		Resting cells [(1)]	**1.011** (*0.8242-1.154*)	**1.648** (*1.319-2.110*) *	**1.031** (*0.7125-1.109*) §
		Active cells [(1)]	**1.020** (*0,8571-1,149*)	**2.778** (*2.222-3.957*) *	**1.286**(*0.8571-1.531*) §
		M1/M2 Resting [(2)]	**0.9595** (*0.5103-1.607*)	**2.420** (*1.270-2.758*) *	**0.8779** (*0.450-1.187*) §
		M1/M2 Active[(2)]	**93.93** (*64.76-135.2*)	**1322** (*464.3-2580)* *	**93.22** (*31.79-215.7*) §

Median (CI: 95%). [(1)] *CD11b+/CD45+ Cell levels.* [(2)] *iNOS/Arg ratio. Kruskall-Wallis, with Dunn's correction post-hoc: (*) p<0.05 vs SHM; (§) p<0.05 vs IVH+CBD.*

Conclusions: Pre and post insult administration of CBD in a newborn rat model of IVH exerted an anti-inflammatory effect that avoided secondary brain damage, and led to long-term structural and functional protective effects. *Supported by PI19/00927*.

CANNABIDIOL ALTERS ABERRANT IMMUNE CELL POPULATIONS IN A MODEL OF IDIOPATHIC AUTISM SPECTRUM DISORDER

Sarah H. Shrader[1], Nicholas Mellen[2], Gregory Barnes[2] and Zhao-Hui Song[1]

[1]Department of Pharmacology and Toxicology and [2]Department of Neurology, University of Louisville School of Medicine, Louisville, KY, USA

Introduction: Autism Spectrum Disorder (ASD) is rapidly becoming one of the most prevalent neurodevelopmental disorders in the U.S., affecting 1 in 44 children according to the CDC. This heterogeneous condition is characterized by communication deficits, impaired social interactions and restricted interest plus repetitive behaviors. A pharmacological intervention for the treatment of core autistic symptoms has yet to be discovered. While the pathology of idiopathic ASD remains elusive, preclinical and clinical studies have implicated immune cell dysregulation in both the periphery and the brain. Cannabidiol (CBD), the major nonpsychoactive constituent of *Cannabis sativa*, has demonstrated anti-inflammatory and neuroprotective properties in multiple developmental and neurological disorders. However, the phytocannabinoid's immunomodulatory effect(s) in idiopathic autism is unknown. We hypothesized that chronic CBD treatment will ameliorate the aberrant peripheral and central immune cell profiles in BTBR $T^{+}Itpr3^{tf}$/J (BTBR) mice, an established mouse model of idiopathic ASD.

Methods: Flow cytometry techniques were used to investigate the immunomodulatory effects of CBD in BTBR and control C57BL/6J (B6) mice. Male and female BTBR and B6 mice between 8 to 12 weeks old were administered by intraperitoneal injection either vehicle, 20 mg/kg CBD or 50 mg/kg CBD daily for one week. Following injection on the final treatment day, single-cell suspensions were prepared from harvested spleens, mesenteric lymph nodes (MLN), superficial cervical lymph nodes (CLN), peripheral blood and perfused whole brains. FACS staining was performed and flow cytometric analysis was used to identify $CD45^{+}$, $CD3^{+}$, $CD4^{+}$, and $CD8^{+}$ cell populations. An equal number of male and female mice were used per dosing group. Data were analyzed by one-way ANOVA followed with Bonferroni's post-hoc tests. *p*-values of <0.05 were considered significant.

Results: Compared to vehicle-treated B6 mice, vehicle-treated BTBR mice displayed elevated levels $CD4^{+}$ T cells in the spleen, MLN, CLN, peripheral blood and whole brain. Treatment with 20 mg/kg CBD had no effect on the $CD4^{+}$ T cell populations in any BTBR mouse tissues. However, following 50 mg/kg CBD treatment, BTBR mice demonstrated a significant reduction of $CD4^{+}$ T cells in the MLN and CLN. Neither 20 mg/kg nor 50 mg/kg CBD treatment altered $CD4^{+}$ T cell levels in any B6 mouse tissues. There was no significant difference between vehicle-treated BTBR and B6 mouse $CD8^{+}$ T cell levels in the spleen, MLN, CLN, peripheral blood or whole brain. CBD treatment did not alter these $CD8^{+}$ T cell populations.

Conclusions: Our data demonstrate that BTBR mice exhibit elevated peripheral lymphoid $CD4^{+}$ T cells, as well as brain infiltrating $CD4^{+}$ T cells. In addition, our findings indicate that CBD treatment can attenuate the increased levels of $CD4^{+}$ T cells in both mesenteric and cervical lymph nodes of BTBR mice. The effects of CBD are both dose- and strain-dependent. Our results suggest that CBD may be a useful pharmacological agent to target the immune cell dysregulation that characterizes idiopathic ASD.

Acknowledgements: Research was supported in part by Autism Speaks Grant #11863, Norton Hospital Research Funds and University of Louisville Research Infrastructure Fund R5385

EFFECTS OF CANNABIDIOL ON MICROGLIA TO MODULATE NEUROINFLAMMATION AFTER ACUTE ISCHEMIC STROKE IN NEWBORN RATS

L. Silva[1], M. Villa[1], M. Martínez[1], A. Romero[1], M. de Hoz-Rivera[1], J. Martínez-Orgado[1]

[1]Instituto de Investigación Sanitaria San Carlos, Madrid, Spain

Background: Neonatal arterial ischemic stroke (AIS) is a prevalent condition in the perinatal period, leading to significant morbidity and long-term neurological deficits. Neuroinflammation plays a key role in perinatal AIS brain injury participating in the spreading of ischemic damage. Nevertheless, so far anti-inflammatory treatments have been ineffective or exert unacceptable side effects. Cannabidiol (CBD) has proven anti-inflammatory-based neuroprotective effects in other newborn rat models of brain damage.

Aims: to assess the role of neuroinflammation modulation in the neuroprotective effects of CBD in a neonatal rat model of AIS.

Methods: AIS was induced for 3h by introducing a filament through the left carotid artery until occluding the left medial cerebral artery (MCAO) in P7-P10 newborn *Wistar* rats. 30 min after removing it, vehicle (MCAO VEH) or CBD single dose 5 mg/kg (MCAO CBD) were administered intraperitoneally (i.p). Similarly manipulated but non-occluded rats served as controls (SHM). Seven days after the insult (P14) brain damage was assessed by magnetic resonance image (MRI) and neurobehavioral [NBS: negative geotaxis (coordination test) and grasp test (strength test)]. Inflammation was assessed by Western Blot [WB] studies to quantify proinflammatory markers (TLR-4, NFκB, TNF-α) as well as by Flow Cytometry [FC] to quantify microglia-macrophage total population (*$CD45^{+\ high\text{-}medium\text{-}low}$ in $Cd11b^{+}$*), microglia/macrophages activation ratio [*$CD45^{+high\text{-}medium}/CD45^{\ low}$ Ratio in $Cd11b^{+}$],* and M1 (proinflammatory, iNOS+)/M2(anti-inflammatory, Arginase+) microglial phenotype distribution (*Inos/Arginase Ratio in $CD45^{+high\text{-}medium}$ in $Cd11b^{+}$*).

Results: post-insult administration of CBD reduced volume of brain damage and led to some beneficial effects on coordination and strength. Those effects were associated with the modulation of neuroinflammation (Table below). CBD prevented the increase of TLR-4, NFκB and TNF-α expression after AIS. CBD prevented AIS-induced increase of microgliosis. Although CBD did not reduce microglial activation, it enhanced the polarization of activated microglia to a M2 phenotype.

		SHM	MCAO VEH	MCAO CBD
WB	TLR-4 (A.U)	109,0 (85,43-132,8)	177,1 (128,0-291,1) *	67,49 (47,81-95,77) $
	NFκ-B (A.U.)	100,6 (88,46-131,6)	138,5 (91,61-176,4) *	85,51 (79,14-108,0) $
	TNF-α (A.U.)	93,27 (91,63-101,8)	131,6 (102,5-235,1) *	83,97 (62,30-106,0) $
FC	Microglia-macrophage total (n)	128,4 (95,50-203,1)	251,5 (183,1-419,8) *	150,9 (87,39- 447,0)
	Activate/resting Microglia-macrophage Ratio	95,29 (29,34-170,7)	375,7 (169,5- 480,7) *	280,8 (167,9- 1077) *
	M1/M2 Ratio in activated Microglia-macrophage	98,48 (75,34-126,2)	229,5 (137,5- 399,1) *	88,15 (60,76- 196,5) $

Median (CI) ANOVA with Bonferroni's test or Kruskal-Wallis with Dunn's test for multiple comparisons. *N=7-8 (*) p<0,05 vs SHM; ($) p<0,05 vs MCAO+VEH*

Conclusions: Neuroprotective effects of CBD in a rat model of neonatal AIS are related to the modulation of inflammation in a manner associated to the reduction of microgliosis and the enhancement of polarization of activated microglia to an anti-inflammatory M2 phenotype.

Supported by grants from Emerald Health Pharmaceuticals and PI19/00927

ABHD6 DIFFERENTIALLY CONTROLS STIMULI-DEPENDENT INCREASES IN 2-AG LEVELS

Simar Singh[1], Dennis Sarroza[1], Anthony English[1], Dale Whittington[2], Ao Dong[3], Mario van der Stelt[4], Yulong Li[3], Larry Zweifel[1,5], Michael R. Bruchas[1,6], Benjamin B. Land[1] and Nephi Stella[1,5]

Departments of [1]Pharmacology, [2]Medicinal Chemistry, [5]Psychiatry and Behavioral Sciences, [6]Anesthesiology, University of Washington, Seattle, USA; [3]Peking-Tsinghua Center for Life Sciences, Peking University, Peking, China. [4]Leiden Institute of Chemistry, Leiden University, Leiden, Netherlands.

Introduction: 2-arachidonoyl glycerol (2-AG) is the most abundant endocannabinoid in the brain and plays key roles in multiple physiological processes, including neurotransmission and immunomodulation. 2-AG synthesis by neurons is increased by various stimuli, including the pro-inflammatory mediators ATP and bradykinin (BK). Evidence suggests that further increasing 2-AG levels in neuropathic and inflammatory pain models has an antinociceptive effect that depends on CB_1 receptor (CB_1R) activation. A strategy to increase 2-AG levels is to inhibit 2-AG hydrolysis by enzymes such as α/β-hydrolase domain containing 6 (ABHD6); however, the mechanism by which ABHD6 inhibition enhances 2-AG levels and activity at CB_1R in response to different stimuli remains unclear. Here we leveraged a genetically encoded sensor ($GRAB_{eCB2.0}$) to measure changes in endogenous 2-AG levels in response to ATP and BK and tested if and how ABHD6 inhibition modulates 2-AG levels.

Methods: We expressed $GRAB_{eCB2.0}$ in Neuro 2a mouse neuroblastoma cells, which endogenously express ABHD6 and CB_1R, and measured fluorescent changes using either live-cell confocal microscopy or a fluorescent 96-well plate reader following treatment.

Results: We characterized the pharmacological profile of $GRAB_{eCB2.0}$ by determining the potency of 2-AG (EC_{50} = 82 nM), arachidonoylethanolamine (EC_{50} = 201 nM), CP55940 (EC_{50} = 190 nM), and the CB_1R antagonist SR141716 (SR1, EC_{50}= 1.2 μM). To stimulate endogenous 2-AG production, we used BK (1 μM) and ATP (300 μM), which triggered an increase in $GRAB_{eCB2.0}$ signal that peaked at 2 and 8 min, respectively. Using LC-MS/MS, we confirmed that a treating the cells for 2 min with BK or ATP increased 2-AG by 94% and 53% respectively compared to vehicle. Using the plate reader, we found that this 2-AG production was stimulated by ATP acting at the $P2X_7$ receptor and BK acting at the B2 receptor. Further, ATP and BK-triggered $GRAB_{eCB2.0}$ signal was blocked by SR1, decreased in cells treated with the diacylglycerol lipase inhibitor D034 (10 nM), and absent in cells expressing a mutant sensor $GRAB_{eCBmut}$. The ABHD6 inhibitor KT182 (10 nM) alone had no effect on the $GRAB_{eCB2.0}$ signal; however, it enhanced the BK-triggered $GRAB_{eCB2.0}$ signal by ≈90% and, by contrast, did not affect the ATP-triggered $GRAB_{eCB2.0}$ signal.

Conclusions: This finding demonstrates that the effect of KT-182 on 2-AG levels is dependent on the mechanism of 2-AG production and suggests that ABHD6 preferentially regulates metabotropic receptor-dependent 2-AG signaling. These results will help further elucidate the role of ABHD6 in 2-AG signaling and characterize the mechanism of selective ABHD6 inhibitors with a potential for clinical use.

Acknowledgements: Funded by NIH (NS118130 to NS) and the Pharmacological Sciences Training Program of the University of Washington, Seattle.

EFFECTS OF N-OLEOYL DOPAMINE ON PORCINE RETINAL PIGMENT EPITHELIAL CELLS

Lucy Sloan[1], Shigeo Tamiya[2] and Zhao-Hui Song[1]

[1]University of Louisville, Department of Pharmacology and Toxicology, Louisville, KY, USA.
[2]The Ohio State University, Department of Ophthalmology, Columbus, OH, USA.

Introduction: Retinal fibrosis associated with conditions like ocular wounding, age-related macular degeneration (AMD), and diabetes, is detrimental to vision. Fibrosis is considered an aberrant wound healing response marked by the development of collagen-rich scars. Retinal pigment epithelial (RPE) cells contribute to several conditions resulting in retinal fibrotic scars. Upon exposure to TGF-β, a key fibrotic cytokine, RPE cells trans-differentiate to myofibroblasts marked by the integration of α-SMA fibers into F-actin stress fibers which confers strong contractility. Myofibroblasts produce and contract the collagen-rich fibrotic scar and disrupt retinal architecture. In this study, we investigated the effects of a putative endocannabinoid compound N-oleoyl dopamine (OLDA) on TGF-β2 induced porcine RPE cell contraction and α-SMA expression *in vitro*.

Methods: Porcine RPE cells were isolated as previously described. The collagen matrix contraction assay was used to assess myofibroblast function. Briefly, RPE cells (10x10^4) were plated on solidified collagen in a 24-well plate and treated with OLDA and TGF-β2 (10 ng/mL) for 72 hours. Collagen gels were released from the well, allowed to contract for 4 hours, and photographed for analysis. Collagen gels with attached cells were subsequently used for western blot analysis. For immunocytochemistry, collagen gels with cells attached were not released from the well, but rather fixed and stained for α-SMA, F-actin, and DAPI. Lastly, a bromodeoxyuridine (BrdU) incorporation assay was used to assess proliferation.

Results: Using an *in vitro* collagen matrix contraction assay, we found that OLDA inhibited TGF-β2 induced contraction of collagen matrices by porcine RPE cells. This effect was concentration-dependent, with significant inhibition of contraction at 10 μM and 3 μM. OLDA did not significantly affect the proliferation of porcine RPE cells. Immunocytochemistry showed that at 3 μM OLDA significantly decreased expression of α-SMA fibers in stress fibers of the TGF-β2 induced porcine RPE cells. Lastly, western blot analysis demonstrated that OLDA downregulated protein expression of TGF-β2 induced α-SMA *in vitro*.

Conclusions: Taken together these results indicate that OLDA has potential to inhibit TGF-β induced fibrosis in the retina. Further studies are warranted to investigate the mechanism of action, other fibrotic end points, as well as potential in *in vivo* models of retinal fibrosis.

Acknowledgements: Funding from NEI grants EY030060 and EY030186 and the Integrative Program in Biomedical Sciences at University of Louisville.

CHEMISTRY AND PHARMACOLOGY OF SYNTHETIC CANNABINOID RECEPTOR AGONISTS AB-4CN-BUTICA, MMB-4CN-BUTINACA, MDMB-4F-BUTICA, MDMB-4F-BUTINACA AND THEIR ANALOGUES

Eric Sparkes,[1,2] Rochelle Boyd,[1,2] Shuli Chen,[3] Tahira Foyzun,[4] Humayra Zaman,[4] Jia Lin Luo,[1,5] Ross Ellison,[6] Iain S. McGregor,[1,5] Roy Gerona,[6] Marina Santiago,[4] Mark Connor,[4] Michelle Glass,[3] Adam Ametovski,[1,2] Samuel D. Banister[1,2] and Elizabeth A. Cairns[1,5]*

[1]The Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Australia
[2]School of Chemistry, The University of Sydney, Australia
[3]Department of Pharmacology and Toxicology, University of Otago, New Zealand
[4]Faculty of Medicine and Health Sciences, Macquarie University, Australia
[5]School of Psychology, The University of Sydney, Australia
[6]Clinical Toxicology and Environmental Biomonitoring Laboratory, UCSF, USA

Introduction: Synthetic cannabinoid receptor agonists (SCRAs) remain one of the most prevalent classes of new psychoactive substances, with often little to no spectral or pharmacological data available at the time of detection. To facilitate proactive screening of SCRAs as they emerge, recently detected SCRAs AB-4CN-BUTICA, MMB-4CN-BUTINACA, MDMB-4F-BUTICA, and MDMB-4F-BUTINACA, and a series of 36 analogues including indole-, indazole-, and 7-azaindole-3-carboxamides have been synthesised, characterised and pharmacologically evaluated *in vitro*.

Methods: Compounds were synthesised *via* alkylation of suitable indole-, indazole-, and 7-azaindole precursors followed by trifluoroacetylation and/or hydrolysis. Finally, routine amide coupling provided the desired species, and chemical characterisation completed. Affinity for hCB_1 or hCB_2 was determined in HEK293 cell membranes using [^{3}H]-SR141716A (CB_1) or [^{3}H]-CP55,940 (CB_2). Functional activity was determined using AtT20-FlpIn cells stably transfected with hCB_1 or hCB_2, via a FLIPR Membrane Potential Assay kit (blue) and a FlexStation 3, using CP55,940 as reference.

Results: The detected compounds AB-4CN-BUTICA (-*p*Ki [hCB_1]= 6.26 M, -*p*EC_{50}[hCB_1] = 6.43 M, E_{max} = 109%), MMB-4CN-BUTINACA (-*p*Ki [hCB_1]= 7.76 M, -*p*EC_{50}[hCB_1] = 8.45 M, E_{max} = 117%), MDMB-4F-BUTICA (-*p*Ki [hCB_1]= 7.88 M, -*p*EC_{50}[hCB_1] = 8.50 M, E_{max} = 113%), and MDMB-4F-BUTINACA(-*p*Ki [hCB_1]= 8.39 M, -*p*EC_{50}[hCB_1] = 9.26 M, E_{max} = 108%) ranged from moderate to high affinity and potency at CB_1 while displaying high efficacy. Most compounds displayed similar activity at CB_1 and CB_2, ranging from subnanomolar to submicromolar affinity and potency. Structure-activity relationships observed were consistent with previous studies regarding head and core group activities at CB_1 and CB_2. Notably, the 4-fluoro derivatives displayed slightly increased potency at CB_1, whilst retaining similar activity at CB_2, compared with the 4-cyano analogues.

Conclusions: Chemical and pharmacological characterisation of recently detected SCRAs and immediate analogues revealed key structure activity relationships regarding tail group functionalisation. Such information is critical for informing the relevant health and legislative bodies on the profile of these compounds, given the high potencies and efficacies observed.

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

INFLUENCE OF CO-MORBID CANNABIS AND COCAINE USE ON IMMUNE BIOMARKERS

William W. Stoops and Joshua A. Lile

Department of Behavioral Science, University of Kentucky, Lexington, KY USA

Introduction: Cocaine and cannabis use have independently been tied to alterations in levels of immune biomarkers like interleukin-6 and TNF-alpha, with cocaine tending to increase inflammatory biomarkers and cannabis use tending to reduce inflammatory biomarkers. Cocaine and cannabis are commonly used together, making it important to understand how this pattern of use impacts immune biomarkers. Although recent research has indicated that combined cannabis and cocaine use may produce a pro-inflammatory state, more work is necessary to confirm this finding. The purpose of this study was to further assess the influence of combined cannabis and cocaine use on immune biomarkers.

Methods: Baseline data from 103 participants with cocaine use disorder who were recruited for a larger, ongoing clinical trial were included in this analysis. Blood samples were drawn during a screening period and immune biomarkers (i.e., interleukin-6, interleukin-10, TNF-alpha and C reactive protein) were determined using ELISA. Recent cannabis use was also determined (i.e., THC positive or negative urine screen) leading to two groups: combined cocaine and cannabis use (n=30; 10 women) and cocaine use only (n=73; 22 women). Groups were compared using Welch's t-tests.

Results: Individuals with combined use were significantly younger (mean age=46 years) than individuals with cocaine use only (mean age=55 years); cigarette smokers among the combined use group also had higher expired breath carbon monoxide levels than smokers among the cocaine only group (means of 23 ppm vs. 14 ppm). Despite these differences, none of the immune biomarker outcomes differed between groups, nor did the number of self-reported days of cocaine use differ between groups.

Conclusions: These data indicate that combined use of cannabis and cocaine does not produce significant changes in levels of a range of immune biomarkers, compared to cocaine use alone. These findings do not align with those from previous studies, so future research with larger sample sizes and non-drug using matched controls is needed to better determine how cannabis use impacts immune function in individuals with cocaine use disorder.

Acknowledgements: Funded by R01 DA 043938 from the National Institute on Drug Abuse.

NOT ALL CB1 ALLOSTERIC MOLECULES ARE CREATED EQUAL: EVIDENCE FOR SELECTIVE EFFECTIVENESS IN DOPAMINE-DYSREGULATED SYMPTOMS

Kim S. Sugamori[1], Catharine A. Mielnik[1], David B. Finlay[2], Mohammed Mustafa[3], Daniel J. Liput[4], Mostafa H. Abdelrahman[5], Laurent A. Trembleau[5], Ali Salahpour[1], Amy J. Ramsey[1], David Lovinger[4], Aron H. Lichtman[3], Michelle Glass[2], Iain R. Greig[5] and Ruth A. Ross[1]

[1]Department of Pharmacology & Toxicology, University of Toronto, Toronto, ON, CA
[2]Department of Pharmacology & Toxicology, University of Otago, Dunedin, NZ
[3]Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, USA
[4]Laboratory for Integrative Neuroscience, NIAAA, Bethesda, MD, USA
[5]The Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK

Introduction: The endocannabinoid system (ECS) serves as an important homeostatic filter of afferent inputs in the dopamine system. We reported that a CB_1 allosteric modulator (ABM300) ameliorated psychosis-type behaviours in two distinct genetic mouse models of hyperdopaminergia: GluN1-knockdown (GluN1KD) and dopamine transporter knockout (DATKO) (Mielnik *et al.*, 2021). We hypothesize that allosteric modulators with improved pharmacokinetic parameters may be superior candidates in treating psychoses-type symptoms stemming from dopamine dysregulation. We characterized the *in vitro* and *in vivo* profiles of a second novel CB_1 allosteric modulator (ABD1085) with the aim of assessing therapeutic efficacy in these mouse models.

Methods: *In vitro* characterization of ABD1085 was compared to the allosteric effects mediated by ABM300 on agonist binding to CB_1, eCB binding to CB_1 via an eCB biosensor and agonist-induced β-arrestin recruitment, ERK1/2 phosphorylation, and inhibition of cAMP. *In vivo*, the pharmacokinetics of ABD1085 was assessed in C57BL/6J mice and the therapeutic efficacy of ABD1085 on hyperdopaminergic phenotypes in GluN1KD and DATKO mice were evaluated. Additionally, ABM300 and ABD1085 were characterized for their effects on the CB_1-mediated classic tetrad paradigm and drug discrimination in wild-type C57BL/6J mice.

Results: *In vitro*, ABD1085 increased agonist (CP55,940) binding to CB_1 but decreased CP55,940-mediated β-arrestin recruitment, phosphorylation of ERK1/2 and cAMP inhibition with potencies comparable to ABM300. Despite similar *in vitro* pharmacological profiles, we observed striking differences *in vivo*. While ABM300 decreased hyperactive exploratory phenotypes in both genetic models with some effects in sensorimotor gating, ABD1085 had no effect on these behaviours. The effect of these compounds in the tetrad paradigm further diverged; ABM300 acted like a positive allosteric modulator (PAM) on CP55,940-induced analgesia, whereas ABD1085 behaved like a negative allosteric modulator (NAM) on CP55,940-induced catalepsy. It must be emphasized that while both compounds act as NAMs of CB_1 signalling *in vitro*, only ABM300 had therapeutic efficacy *in vivo* in the genetic mouse models.

Conclusions: We have demonstrated that two CB_1 allosterics displaying similar *in vitro* pharmacological profiles have very different *in vivo* profiles in genetic models of hyperdopaminergia. An important distinction is that ABD1085 may be defined as a NAM *in vivo*, while ABM300 may be acting as a PAM-antagonist. Perhaps this distinction allows for a more targeted therapeutic approach with the potency and efficacy of a PAM-antagonist being potentially dependent on the activation state and pathophysiological status of the ECS in the disease model under investigation.

Acknowledgements: This project was funded by CIHR grant (PJT-173364).

DAILY EXPOSURE TO Δ^9-TETRAHYDROCANNABINOL (THC) INDUCES REACTIVE GLIOSIS IN ADOLESCENT, BUT NOT ADULT NONHUMAN PRIMATE AND RAT AMYGDALA: ANTAGONISM BY CANNABIDIOL (CBD)

Yalin Sun[1], Meenalochani Sivasubramanian[1], Marija Milenkovic[1], Marie- Ève di Raddo[1], Sarah L. Withey[3], Bertha K. Madras[3] and Susan R. George[1, 2]

[1]Departments of Pharmacology, [2]Medicine, University of Toronto, Toronto, ON, Canada
[3]McLean Hospital, Department of Psychiatry, Harvard Medical School, Belmont, MA, USA

Background: Adolescent cannabis use is rising globally, despite accumulating evidence that adolescents are particularly susceptible to its harmful effects. As the endocannabinoid (eCB) system is a key contributor to brain development and maturation, cannabis exposure can alter the normal trajectory of brain development during this phase of maturation. The amgydala, a brain region regulating anxiety and fear, matures during adolescence and is implicated in psychiatric disorders associated with adolescent cannabis exposure. Of the various cell types in amygdala, astrocytes dynamically regulate neuronal and eCB synaptic function through cannabinoid receptor 1 (CB1R) and calcium (Ca^{2+}) signalling. To determine whether THC elicits different neuroadaptive changes in amygdala as a function of age, adolescent and adult nonhuman primates and rats were exposed daily to THC.

Methods: Young, male 2-2.5-year-old adolescent squirrel monkeys (*Saimiri boliviensis)* were treated once weekly with 0.1mg/kg THC or 0.1/0.3mg/kg THC/CBD at week 1, escalating to 1.0mg/kg THC or 1.0/3.0mg/kg THC/CBD by week 3, then treated daily with vehicle (VEH), 1.0mg/kg THC, or 1.0/3.0mg/kg THC/CBD for 114 or 117 days. Male adolescent (P35) and adult (P70) Sprague-Dawley rats were treated daily for 28 days with VEH, 1.0mg/kg THC, or 1.0/3.0mg/kg THC/CBD. Monkey amygdala tissues were analyzed by proteomics/immunohistochemistry (IHC) and rat amygdala tissues by IHC. Primary astrocyte cultures were isolated from P1-2 rat pups. Following 1μM rimonabant or 1μM BAPTA-AM pre-treatments prior to 1μM THC, effects in cultures were investigated by immunocytochemistry.

Results: Proteomic analysis revealed increased glial fibrillary acid protein (GFAP) expression in THC-treated adolescent monkey amygdala compared to VEH or THC+CBD. Identical results were obtained in amygdala of THC-treated adolescent rats. In contrast, amygdala GFAP did not change in THC-treated adult rats. Since increased GFAP reflects astrocyte activation, which may drive inflammatory processes, the reactive astrocyte marker complement factor B (CFB) was quantified. In adolescent monkeys and rats, THC increased astrocyte CFB expression in amygdala, but not in THC-treated adult rats. In adolescents of both species, CBD attenuated THC-mediated GFAP and CFB elevation. In primary astrocyte cultures, THC increased GFAP and CFB, an effect blocked by CB1R antagonist rimonabant or Ca^{2+} chelator BAPTA-AM, implicating CB1R and Ca^{2+} signalling-mediated mechanism.

Conclusions: Chronic THC triggered an inflammatory process in amygdala astrocytes of adolescent but not adult monkey or rat brain, through activation of CB1R and Ca^{2+} signalling. Co-administration of CBD with THC blocked this effect, revealing a uniquely detrimental response elicited by THC during the adolescent phase of brain maturation. Our findings conceivably identify one mechanism underlying heightened vulnerability of adolescent cannabis users to subsequent psychiatric disorders. By attenuating this process, CBD functioned as a neuroprotective agent, an application warranting further investigation as a candidate therapeutic.

Acknowledgements: Funded by National Institutes of Health NIDA DA042178 (BKM, SRG).

THE EFFECT OF CANNABIGEROL ON THE EXPRESSION OF PROTEINS INVOLVED IN EXTRACELLULAR MATRIX FORMATION IN PRIMARY RAT HEPATOCYTES EXPOSED TO PALMITATE AND FRUCTOSE CONDITIONS

Klaudia Sztolsztener, Katarzyna Hodun, Adrian Chabowski, Sylwia Dziemitko and Karolina Konstantynowicz-Nowicka

Department of Physiology, Medical University of Bialystok, Bialystok, Poland

Introduction: The liver failure development on a fibrotic basis is associated with increased hepatic deposition of mainly collagen fibers and other extracellular matrix (ECM) proteins such as matrix metalloproteinase (MMP) and their tissue inhibitor (TIMP). The main factor stimulating the increased production of matrix components is transforming growth factor beta (TGF-β1). Therefore, finding a promising agent to attenuate hepatic fibrosis is essentially needed. Cannabigerol (CBG) is a non-psychoactive cannabinoid component of the endocannabinoidome, which has an impact on the regulation of inflammation and processes related to lipid metabolism and overall hepatic metabolic functioning. Despite numerous studies about lipid metabolism in the primary rat hepatocytes, there is a lack of data describing the influence of cannabigerol on the extracellular matrix proteins expression. In this research, we assessed the effect of cannabigerol on ECM synthesis and degradation in hepatic cells.

Methods: Primary rat hepatocytes isolated from the liver during a two-step perfusion procedure were incubated in standard media (Control group) or incubated with 0.75 mM palmitic acid (PA) combined with 10 mM fructose (PA-F group) for 48 h. Half of the cells from the Control and PA-F groups were treated with 5 μM cannabigerol solution for 24 h. At the end of the experiment, cells were washed twice with PBS and homogenized by the ultrasonification in ice-cold RIPA buffer with protease and phosphatase inhibitors and then frozen at -80^{o}C. The Western blot technique was used to determine the expression of selected factors regulating the fibrogenic process, i.e., TGF-β1, MMP-2, MMP-9, TIMP-1, TIMP-2 as well as two types of collagen fibers (COL-1A1, COL-3A1). The results were analyzed by two-way ANOVA followed by an appropriate post-hoc test (statistically significant at $p < 0.05$).

Results: Our results have shown a significant influence PA-F on intracellular fibrosis development through reduced total expression of MMP-2 and simultaneously augmented total expression of TGF-β1 and TIMP-1. Importantly, a significant decrease in TIMP-2 and TIMP-2 expression after exposure to CBG combined with PA-F from that Control and PA-F groups was observed. CBG also decreased expression of TGF-β1 in PA-F group. PA-F-induced increase of collagen fibers (COL-1A1 and COL-3A1) expression, which was abolished by a cannabigerol exposition. Moreover, treatment with CBG provoked an impairment in TIMP-1 in standard media conditions. Compared with PA-F group, CBG elevated MMP-2 expression in lipid overload conditions.

Conclusions: Our results clearly showed that CBG may affect the expression of proteins involved in fibrogenesis. Fibrosis in hepatocytes was decreased after cannabigerol exposition by a substantially decreased regulating factor TGF-β1. We can assume the hypothesis that cannabigerol is effective in reducing fibrosis development *via* decreased extracellular matrix proteins deposition.

Acknowledgments: Funded by the Medical University of Bialystok (Grant no. SUB/1/DN/22/008/1118).

TREATING AND PREVENTING HYPERPHAGIA AND OBESITY IN PRADER-WILLI SYNDROME WITH A CANNABIDIOLIC ACID DERIVATIVE

Elad Ben-Cnaan, Liad Hinden and Joseph Tam

Obesity and Metabolism Laboratory, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel

Introduction: Individuals with Prader-Willi syndrome (PWS) present extremely increased appetite (hyperphagia), which may lead to morbid obesity if not controlled. The currently available treatments for managing PWS are very limited and result in only partial improvements of these conditions. Cannabidiolic acid (CBDA), a major constituent of *Cannabis sativa*, is decarboxylated to cannabidiol (CBD). Whereas the biological and therapeutic properties of CBD in modulating appetite and weight gain have been reasonably identified, our knowledge of the pharmacology of CBDA is much more limited due to its instability. To stabilize CBDA and increase its efficacy, a new derivative, CBDA-O-methyl ester (EPM301), was synthesized and tested pre-clinically. Previously, we have shown that EPM301 has the ability to ameliorate diet-induced obesity and its associated metabolic abnormalities; however, its efficacy in treating genetic-induced obesity, such as PWS, has never been tested.

Methods: Here, we used three different animal settings, utilizing the *Magel2*^{null} mouse model of PWS, to assess the therapeutic potential of EPM301 for appetite reduction, weight loss, and metabolic improvements related to PWS. (**i**) *Magel2*^{null} mice and their controls, fed with a high-fat diet (HFD) or a standard diet (STD) for 14-16 weeks, were treated with EPM301 (20 or 40 mg/kg/d for 28 days) or vehicle and a full metabolic assessment was done; (**ii**) *Magel2*^{null} mice and their controls, fed with a HFD for 4 weeks, were treated with EPM301 for 7 days to assess hypothalamic leptin sensitivity; and (**iii**) *Magel2*^{null} mice and their controls, fed with a STD, were treated with EPM301 for 16 weeks, and weight gain and adiposity were determined.

Results: Chronic 28-day treatment with EPM301 was found efficacious in reducing body weight and hyperphagia in HFD-fed *Magel2*^{null} mice, two of the most important features of PWS. In contrast to the effect of EPM301 in WT obese animals, the improvements in *Magel2*^{null} mice were associated with the ability of EPM301 to upregulate fatty acid oxidation in the null animals as well as to increase the obesity-induced reduction in hypothalamic STAT3 phosphorylation, attributed to leptin resistance. In addition, when given to STD-fed *Magel2*^{null} mice for 16 weeks, as a preventive treatment, EPM301 completely inhibited weight gain and adiposity in comparison with vehicle-treated mice.

Conclusions: EPM301 demonstrated impressive results in ameliorating obesity, reducing hyperphagia, and preventing fat mass in *Magel2*^{null} mice. These results support the idea to promote the development of this synthetic CBDA derivative toward clinical evaluation in PWS patients, who currently do not have any therapeutic solution.

Acknowledgment: This study was sponsored by EPM Group Inc. via a research grant to Joseph Tam. This research is part of an ongoing preclinical program to evaluate synthetic CBDA derivatives in various medical conditions.

CANNABIDIOL (CBD) INHIBITS NEUROINFLAMMATORY RESPONSES AND SYNAPTIC LOSS FOLLOWING DAMAGE TO SONGBIRD VOCAL MOTOR CORTEX

Mark A. Tripson[1], Alexis Papariello[1], Karen Litwa[2] and Ken Soderstrom[1]

Department of Pharmacology and Toxicology[1], Department of Anatomy & Cell Biology[2], The Brody School of Medicine at East Carolina University, Greenville, NC, USA

Introduction: The non-euphorigenic phytocannabinoid CBD has been used successfully to treat childhood-onset epilepsies, conditions often associated with developmental delays that includes vocal communication. Translational to human speech, zebra finch song is a complex behavior learned during a sensitive period of vocal development, and therefore is a promising model to understand mechanisms responsible for potential CBD-related improvement of vocal learning. Like language, the quality of adult zebra finch song is maintained through continuous sensorimotor maintenance and refinement involving brain regions that control vocal learning and production. One of these brain regions, HVC (proper name) is a pre-vocal motor cortical-like region that when partially lesioned temporarily disrupts vocal behavior. Recovery from HVC microlesions takes about seven days without treatment. We found previously that CBD (10 mg/kg IM once daily) both speeds recovery and reduces the acute magnitude of disruptions. Given anti-inflammatory CBD activity in seizure and other models, we suspected involvement of similar mechanisms in vocal recovery. To test this, we investigated CBD modulation of post-lesion expression of inflammatory cytokines, markers of neuronal stress, microglial migration, and changes in synaptic densities within relevant song control regions.

Methods: Groups of 3-5 adult male zebra finches were treated with vehicle or 10 mg/kg botanically derived, purified CBD (≥ 98%) delivered IM in 50 µl QD for six days pre- and two days post-HVC microlesion (partial ablation of about 8% of the region). HVC was targeted unilaterally allowing contralateral internal controls. For qRT-PCR (IL-1β & IL-6, IL-10, MSK1, Arc/arg3.1 & BDNF) brain regions were micropunched and RNA extracted, cDNA synthesized and amplified using a kit. Distribution of protein expression was studied by immunofluorescence with fixed, cryosectioned tissue (10 µm). Tissue was stained with antibodies against inflammatory (IL-1β & IL-6), and anti-inflammatory mediators (IL-10), as well as the superoxide indicator dihydroethidium (DHE). An anti-TMEM119 antibody was used to immunofluorescently (IF) label microglia. Synaptic densities were measured using colocalization of excitatory pre- and post-synaptic markers (VGLUT2 & PSD-95).

Results: Unilateral HVC microlesions produced significant vocal deficits consistent with those observed previously following bilateral methods. Results of qRT-PCR, DHE staining and IF experiments indicate CBD-improved vocal recovery is associated with reduced oxidative stress and anti-inflammatory activity. Decreased inflammation and stress marker expression was associated with reduced densities of microglia within song regions afferent to the lesion target, HVC (including learning-essential Area X [basal ganglia] and vocal motor RA [motor cortex]). As microglia are critical regulators of synaptic degeneration, we measured densities of excitatory synapses within Area X and RA, finding significant lesion-related decreases that were largely reversed by CBD. Synaptic protection was associated with BDNF/Arc/MSK1 upregulation implicating mechanisms important to homeostatic synaptic scaling.

Conclusions: The unilateral HVC microlesion model has allowed us to determine that CBD-improved vocal recovery is associated with reduced expression of neuroinflammation and metabolic stress markers, decreased microglia infiltration of the lesion target and afferent song regions, and maintenance of synaptic densities consistent with promotion of BDNF-related homeostatic processes.

Acknowledgements: This work was funded in-part by GW Pharmaceuticals and by the Department of Pharmacology and Toxicology in the Brody School of Medicine at East Carolina University

ACID CERAMIDASE AS A THIRD *N*-ACYLETHANOLAMINE HYDROLASE IN CELLS AND TISSUES

Kazuhito Tsuboi[1], Tatsuya Tai[3,4], Ryouhei Yamashita[5], Hanif Ali[5], Takashi Watanabe[2], Toru Uyama[3], Yoko Okamoto[5], Keisuke Kitakaze[1], Yasuhiro Takenouchi[1], Iffat Ara Sonia Rahman[3], Hitoshi Houchi[4,7], Tamotsu Tanaka[5,6], Akira Tokumura[5,8], Junko Matsuda[2], Yasuo Okamoto[1] and Natsuo Ueda[3]

[1]Department of Pharmacology,
[2]Department of Pathophysiology and Metabolism,
Kawasaki Medical School, Kurashiki, Okayama, Japan;
[3]Department of Biochemistry, Kagawa University School of Medicine, Miki, Kagawa, Japan;
[4]Department of Pharmacy, Kagawa University Hospital, Miki, Kagawa, Japan;
[5]Graduate School of Biomedical Sciences,
[6]Graduate School of Technology, Industrial and Social Sciences,
Tokushima University, Tokushima, Japan;
[7]Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University,
Sanuki, Kagawa, Japan;
[8]Faculty of Pharmacy, Yasuda Women's University, Hiroshima, Japan

Introduction: Acid ceramidase (AC) is a lysosomal enzyme hydrolyzing ceramide (*N*-acylsphingosine) at acidic pH. This enzyme is abundantly expressed in several human tumors, and its role in chemoresistance is suggested. It has been noted that human AC shows 33% amino acid identity with human *N*-acylethanolamine acid amidase (NAAA), another lysosomal hydrolase. We previously showed that purified AC is able to hydrolyze lauroylethanolamide and other *N*-acylethanolamines and that the overexpression of AC in cultured cells decreases *N*-acylethanolamine levels. In the present study, we examined the role of endogenously expressed AC in the degradation of *N*-acylethanolamines in cells and tissues.

Methods: Human prostate LNCaP cells were transfected with siRNA against AC, and the intracellular levels of *N*-acylethanolamine species were analyzed by LC-MS/MS. The suppression of AC was confirmed by qPCR and the fluorescence-based activity assay. In the analysis of mice lacking saposin D, a presumed activator protein of AC, the homogenates of brain, kidney, and liver were incubated with ^{14}C-labeled substrates (C12:0-ceramide or lauroylethanolamide), and the hydrolytic activities were analyzed by thin-layer chromatography. The mRNA levels of AC in the three tissues were examined by qPCR.

Results: The suppression of AC in LNCaP cells increased the levels of various *N*-acylethanolamines. As compared with wild-type mice, the tissue homogenates from saposin D$^{-/-}$ mice showed much lower hydrolysis rates for lauroylethanolamide as well as C12:0-ceramide, representing the ability of saposin D to activate AC. The levels of AC mRNA were not significantly different between wild-type and saposin D$^{-/-}$ mice.

Conclusions: These results suggest the possibility that AC is a third *N*-acylethanolamine-hydrolyzing enzyme in addition to fatty acid amide hydrolase and NAAA.

ACCUMULATION OF *N*-ACYL-PHOSPHATIDYLETHANOLAMINES IN BRAIN ISCHEMIA BY CYTOSOLIC PHOSPHOLIPASE $A_2\varepsilon$

Toru Uyama[1], Zahir Hussain[1], Katsuya Morito[5], S. M. Khaledur Rahman[1], Mohammad Mamun Sikder[1], Tamotsu Tanaka[6], Kenichi Ota[2], Masaki Ueno[3], Hiroo Takahashi[4], Tohru Yamamoto[4], Makoto Murakami[7] and Natsuo Ueda[1]

[1]Department of Biochemistry,
[2]Department of Anatomy and Neurobiology,
[3]Department of Pathology and Host Defense, and
[4]Department of Molecular Neurobiology,
Kagawa University School of Medicine, Kagawa, Japan;
[5]Department of Environmental Biochemistry, Division of Biological Sciences, Kyoto Pharmaceutical University, Kyoto, Japan;
[6]Graduate School of Technology, Industrial and Social Sciences,
Tokushima University, Tokushima, Japan;
[7]Laboratory of Microenvironmental and Metabolic Health Science, Center for Disease Biology and Integrative Medicine, Faculty of Medicine,
The University of Tokyo, Tokyo, Japan

Introduction: *N*-Acyl-phosphatidylethanolamine (NAPE) is a class of triacyl-type glycerophospholipid, which serves as a precursor for bioactive *N*-acylethanolamine (NAE). In mammals, NAPE is biosynthesized from glycerophospholipids by an *N*-acyltransferase. Cytosolic phospholipase $A_2\varepsilon$ ($cPLA_2\varepsilon$, also known as PLA2G4E) and PLAAT (phospholipase A and acyltransferase) family proteins function as Ca^{2+}-dependent or Ca^{2+}-independent *N*-acyltransferase *in vitro*, respectively. Though NAPEs and NAEs were reported to accumulate in brain ischemia, the responsible *N*-acyltransferase was not identified *in vivo*. In this study, we investigated the age-dependent expression of $cPLA_2\varepsilon$ in the brain and its possible involvement in the accumulation of NAPEs during brain ischemia using $cPLA_2\varepsilon$-deficient ($Pla2g4e^{-/-}$) mice.

Methods: $Pla2g4e^{-/-}$ mice were generated by the CRISPR/Cas9 system. Membrane fractions were prepared from brain homogenates of wild-type (WT) and $Pla2g4e^{-/-}$ mice at various ages and analyzed for *N*-acyltransferase activity. As an *ex vivo* model of brain ischemia, heads were isolated from mice, wrapped in aluminum foil, and incubated for 6 h at 37˚C. Then, brain levels of NAPE and NAE were analyzed by liquid chromatography-tandem mass spectrometry.

Results: $cPLA_2\varepsilon$ expression was the highest during the first week of postanal life. Ca^{2+}-dependent *N*-acyltransferase activity was only detected in the brain of WT mice with the highest activity at the first week of postnatal life. The activity was undetectable in $Pla2g4e^{-/-}$ mice. Lipid analysis revealed that brain NAPE level of $Pla2g4e^{-/-}$ mice at the age of day 7 was lower than that of WT mice, while those of day 30 were similar. In a model of brain ischemia, NAPE and NAE levels in WT mice increased up to 20- and 200-fold, respectively, while these accumulations were hardly detected in $Pla2g4e^{-/-}$ mice.

Conclusions: These results demonstrated that $cPLA_2\varepsilon$ is responsible for Ca^{2+}-dependent *N*-acyltransferase activity in the brain, which is potent at the early ages, as well as the accumulations of NAPEs and NAEs during brain ischemia.

A MULTI-DIMENSIONAL PROFILE OF THE ENDOCANNABINOID SYSTEM IN POLARIZED MICROGLIA

Noëlle van Egmond[1], Floor Stevens[1], Bobby Florea[2], Berend Gagestein[1], Tom van der Wel[1], Daan van der Vliet[1], Sander van Kasteren[2] and Mario van der Stelt[1]

[1]Department of Molecular Physiology, Leiden University, Netherlands
[2]Department of Bio-Organic Synthesis, Leiden University, Netherlands

Introduction: As resident immune cells of the central nervous system (CNS), microglia are first in line in harnessing healthy CNS homeostasis through rapid polarization to pro- and anti-inflammatory states in response to environmental ques. The implication of microglial dysfunction in chronic neuroinflammation underlies their indisputable contribution to various neurodegenerative diseases, such as Multiple Sclerosis. Numerous research efforts have shown that the Endocannabinoid System (ECS) holds valuable promise for the amelioration of microglial function in neurodegenerative diseases. A multi-dimensional profile of the ECS in microglial polarization, however, is lacking. In this study, we aimed to profile ECS function in pro- and anti-inflammatory polarized microglia by combining multiple techniques assessing mRNA expression, protein expression, protein activity and metabolite levels.

Methods: Murine N9 cells were used as microglial model for polarization towards pro- and anti-inflammatory states. Pro-inflammatory microglia were acquired by stimulation with LPS, Interferon-γ (IFN-γ) or a combination of both. For anti-inflammatory microglia, Interleukin-4 (IL-4) and IL-13, or Transforming Growth Factor-β (TGF-β) were used. Polarization states were validated with qPCR for marker genes. These polarized microglia were analyzed for expression and activity of the ECS using qPCR, Western Blot, Activity-Based Protein Profiling (ABPP) and targeted lipidomics.

Results: Differential protein expression and activity levels of DAGLβ showed tight transcriptional regulation throughout microglial polarization. DAGLβ was downregulated in response to all pro-inflammatory stimulants, but upregulated upon anti-inflammatory stimulantion. ABHD12 activity and expression levels were less well correlated, mainly due to disassociated mRNA and activity levels after exposure to LPS and IFN-γ. The primary microglial cannabinoid CB2 receptor followed a similar profile as found for DAGLβ, apart from the upregulated mRNA levels upon IFN-γ stimulation. The differential levels of CB2R, DAGLβ and ABHD12 combined were in general reflected by the intracellular 2-AG levels. Inhibition of DAGLβ or ABHD12 by DH376 or DO264, respectively, downregulated pro-inflammatory responses after a LPS-stimulus. Combining ABHD12 inhibition with CB2 antagonist AM630 revealed a modulatory role for CB2 in the suppression of the inflammatory response.

Conclusions: By using a multi-OMICS approach combined with pharmacological intervention in mouse N9 microglial cells, we show that the CB2-DAGLβ-ABHD12-2-AG axis is tightly controlled in the polarization state.

MICROGLIA DENSITY, DISTRIBUTION AND MORPHOLOGY FOLLOWING ACUTE VAPOURIZED CANNABINOID EXPOSURE

Haley A. Vecchiarelli[1], Hayley Thorpe[2], Sophia Loewen[1],
Jibran Y. Khokhar[2] and Marie-Ève Tremblay[1]

[1]Division of Medical Sciences, University of Victoria, Victoria, BC, Canada
[2]University of Guelph, Guelph, ON, Canada

Introduction: Microglia are the central nervous system (CNS)'s resident immune cells, and they perform a wide variety of processes necessary for the proper formation and function of the CNS, including contributing to neural cell development, synaptic remodeling and the initiation and resolution of (neuro)inflammation. To perform these tasks, microglia can proliferate, migrate, adapt their morphology, but also their transcriptome, proteome and lipidome, metabolism, phagocytic activity, and release of soluble factors. Many studies have shown that delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) injected intravenously or intraperitoneally are able to modulate inflammation mediated by microglia, as cannabinoid receptors generally down-regulate the synthesis of pro-inflammatory cytokines and stimulate the production of anti-inflammatory cytokines. However, the effects of another translationally relevant cannabinoid exposure, inhalation, on the physiological functions of microglia that could modulate inflammation, but also promote neuroprotection and synaptic integrity remain undetermined.

Methods: Young adult, male C57Bl6 mice were exposed to vehicle vapor or *cannabis* flower over 15 minutes (one 15 second puff every 5 minutes; 3 puffs total; 0.15 g flower/puff) and euthanized 30 minutes post-onset, when THC levels peak in the CNS. Three *cannabis* strains were utilized: high THC/negligible CBD; negligible THC/high CBD; balanced THC/CBD. We immunolabeled microglia using IBA1/TMEM119 double-immunostaining followed by epifluorescent and confocal imaging. We performed this investigation within key corticolimbic brain regions important for cognition, memory, and emotional regulation, specifically, in this report, in the hippocampus.

Results and Conclusions: Our preliminary analysis revealed that IBA1 levels in the hippocampus, in relation to the vehicle group, are increased in animals exposed to the balanced THC/CBD strain, while animals exposed to the high CBD strain show reduced IBA1 levels, without apparent differences in the group exposed to the high THC strain. Additionally, potentially, microglial clustering in the hippocampus observed in the vehicle group was not observed in the groups exposed to any cannabis strains. Intriguingly, while lower IBA1 levels were potentially observed in the group exposed to the high CBD strain, there was an association of IBA1-positive cells in the neurogenic niche that was potentially not observed in the other groups. Additionally, in the high THC and balanced THC/CBD strains, there was potentially an association of IBA1 levels with blood vessels. Our preliminary results suggest that acute exposure to different cannabis strains may modify microglial localization and clustering in the hippocampus. Future work will have to investigate how microglia change across specific sub-regions of the hippocampus and in other brain regions, as well as whether there are differences in morphology and function. Additionally, future analysis of ultrastructure will provide insight into how acute cannabis exposure alters microglia's relationship with neurons and synapses. This starting point will allow us in the future to compare to effects of THC and CBD alone and the outcomes of longer-term exposure, notably upon injury or infection.

CANNABIDIOL AS A TREATMENT FOR POST STROKE MOOD DISORDERS IN NEWBORN RATS

Villa M., Gallego I, Del Pozo A., Martínez M., Silva L., de Hoz-Rivera M., Romero A., Gómez Soria A*, Casarejos MJ*, Martínez-Orgado J.

NEURO-INA-IN, Hospital Clínico San Carlos-IdISSC, Madrid, (*); Servicio de Neurobiología-Investigación, Hospital Ramón y Cajal, Madrid. Spain

Introduction: Mood disorders as depression are a frequent complication of arterial ischemic stroke (AIS) in adults. However, there are no data neither about the risk of mood disturbances after perinatal AIS (PAIS) nor about the possible modulation of those disturbances by a neuroprotective treatment as cannabidiol (CBD). The aim was to assess whether AIS led to mood disturbances in newborn rats, and if those disturbances are modulated by CBD treatment.

Methods: PAIS was induced in newborn (P7-P10) Wistar rats by introducing, under sevoflurane anesthesia, a nylon filament through the left internal carotid artery until occluding medial cerebral artery for 3h. 30 minutes after reperfusion rats received i.p. vehicle (MCAO+VEH) or CBD 5mg/kg (MCAO+CBD). Similarly managed rats without MCAO served as controls (SHAM). Different functional tests were carried out at P14 (tail suspension [depressive-like behavior]) or P37 days (forced swimming test (FST) [depressive-like and/or hyperactive behavior], open field [motor activity and anxiety-like behavior]) post-insult. Dopamine (DA) levels were measured by HPLC at P14 and P37.

Results:

			SHAM	MCAO+VEH	MCAO+CBD
P14	**NBHV**	**Tail suspension (s)**	70,00 (53,00-79,00)	131,0 (92,00-146,0)*	123,0 (78,00-143,0)*
	HPLC	**DA (nmol/g)**	48,72 (42,08-57,32)	17,68 (14,47-28,79)*	17,53 (11,08-24,68)*
P37	**NBHV**	**FST-Floating (ratio)**	0,26 (0,21-0,35)	0,05 (0,00-0,13)*	0,10 (0,03-0,25)*
		FST-Swimming (ratio)	0,60 (0,53-0,65)	0,43 (0,36-0,65)	0,56 (0,51-0,71)#
		FST-Climbing (ratio)	0,13 (0,10-0,15)	0,43 (0,30-0,56)*	0,26 (0,16-0,45)*#
		Distance (cm)	1622 (1249-2080)	2784 (2275-3552)*	2291 (1412-3209)
		Entries	10,00 (5,000-15,00)	27,00 (21,00-62,00)*	18,00 (11,00-42,00)
	HPLC	**DA (nmol/g)**	5,80 (2,62-6,75)	10,21 (3,76-14,36)*	9,05 (6,58-12,29)

*Median (IC95%). Kruskal-Wallis with Dunn's test or Welch's ANOVA test with Games-Howell's test for multiple comparisons test. *p<0.05 vs SHAM. # p<0.05 vs MCAO+VEH. N=8-15.* DA: dopamine; FST: forced swimming test; NBHV: neurobehavior.

MCAO in newborn rats reproduced the typical neuroimaging and functional characteristics of PAIS. In this model, short- and long-term mood disturbances appeared, consisting in depressive-like behavior at P14, corresponding to decreased DA levels in brain and hyperactivity (manic-like) behavior at P37, corresponding to increased DA levels. CBD was neuroprotective in the short- and long-term but showed no effect on the depression-like behavior at P14. By contrast CBD administration after AIS prevented the development of the manic-like behavior at P37 preventing AIS-induced increase in DA levels.

Conclusions: In newborn rats, AIS led to mood disturbances, in particular depressive-like behavior in the short term and manic-like behavior in the long term, associated with changes in DA brain levels. Post-insult administration of CBD prevented the development of the maniac-like behavior at P37. *Supported by PI19/00927 grant*

Δ^9THC AND ITS MAJOR METABOLITES ACTIVATE GPR119 *IN VITRO* AND INDUCE WEIGHT LOSS IN DIET-INDUCED OBESE MICE

Amey S. Dhopeshwarkar, Jim Wager-Miller, Madison Walenga and Ken Mackie

Department of Psychological and Brain Sciences. Gill Center for Biomolecular Science

Introduction: The psychotropic effects of cannabis have been known for thousands of years, but its metabolic consequences are less well understood. While cannabis is known to increase the consumption of calorie-rich foods and increase the propensity for a sedentary lifestyle, recent epidemiological studies suggest that chronic smokers display better BMI, blood sugar levels, and lower waist circumferences than their non-smoking counterparts. In this work, we explore the metabolic effects of THC and CBD, the primary phytocannabinoids, on weight homeostasis in a mouse model. We focus on phytocannabinoid activation of the lipid receptor, GPR119, a GPCR highly expressed in enteroendocrine cells of the GI tract.

Methods: HEK293 cells stably expressing human GPR119 receptors were tested for THC-induced cAMP inhibition, IP1 accumulation and GPR119 internalization. C57Bl/6J wt and GPR119 KO mice were placed on a high fat rodent chow (containing sucrose and 58% of the total calories from fat) starting on postnatal day 21. Mice with weight greater than or equal to 40 grams (for females) and greater than or equal to 45 grams (for males) were considered obese and used for experiments. Mice were treated daily with THC either intraperitoneally or per os and weighed. Following treatments, tissues were harvested for RTqPCR and other analyses.

Results: Activation of GPR119 by THC is evidenced via internalization (Emax, 59+/-2.1 SEM), cAMP inhibition (Emax 13.1 +/- 2.9), and IP1 accumulation (Emax 78 +/- 6.5) assays. THC treatment of obese mice increases pancreatic and intestinal GLP1 and PYY mRNA transcripts. It also promotes weight loss in wildtype, but not GPR119 knockout, obese mice in a dose dependent manner. In contrast, CBD failed to activate GPR119 and showed none of the effects observed with THC treatment.

Conclusions: These results indicate a novel role for THC to induce weight loss via GPR119. THC displays strong functional selectivity in activating GPR119–mediated intracellular signaling pathways. Furthermore, oral or intraperitoneal THC administration promotes weight loss in C57BL/6J high fat diet-induced obese wild type (WT) mice., but not in GPR119 knockout mice. Cannabidiol, another major, but non-intoxicating component of cannabis, failed to activate GPR119 and did not cause weight loss in obese mice.

This work was supported by the NIH, DA043982 and DA046196

AHR REGULATES PULMONARY INFLAMMATION FROM CANNABIS SMOKE

Emily T. Wilson[1,3], David H. Eidelman[1,2] and Carolyn J. Baglole[1,2,3]

[1]Research Institute of the McGill University Health Centre, Montreal, QC H4A 3J1, Canada
[2]Department of Medicine, McGill University, Montreal, QC H4A 3J1, Canada
[3]Department of Pharmacology and Therapeutics, McGill University,
Montreal, QC H3G 1Y6, Canada

Introduction: Δ^9-Tetrahydrocannabinol (THC) is a well-known cannabinoid in *Cannabis sativa*. While THC may be anti-inflammatory, there is inconclusive literature on the effects of cannabis smoke on lung inflammation. This is relevant as inhaling cannabis smoke is the most common way that cannabis is consumed. Although cannabinoids classically interact with CB1 and CB2 receptors, non-G protein coupled receptors (GPCRs) can also interact with cannabinoids. This may include the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor highly expressed in the lungs that controls inflammation. We therefore hypothesize that THC exerts its possible anti-inflammatory effects through the AhR.

Methods: Age-matched (8-12 weeks) heterozygous (*Ahr*$^{+/-}$) and knock-out (*Ahr*$^{-/-}$) mice were exposed to cannabis joints containing 0.5g dried cannabis (total THC: 200 mg/g; total CBD: <1 mg/g). Nose-only inhalation exposures were performed using SCIREQ® inExpose™ system twice per day in groups of two joints at 3 puffs/minute for 3 days. Plasma, lung, spleen, and liver were collected. THC ELISA was performed on the plasma. Cytokine profiling microarray was performed on the plasma and bronchoalveolar lavage. Immune cells of the lung and spleen were assessed by flow cytometry. Inflammatory markers of the total lung tissue were analyzed by qPCR and LC/MS. Expression of cannabinoid metabolizing enzymes in the lungs and liver were measured by qPCR.

Results: Cannabis smoke activated the AhR only in *Ahr*$^{+/-}$ mice as indicated by increased *CYP1A1* mRNA expression in the lungs. Neutrophils and inflammatory monocytes were higher in the lungs of *Ahr-/-* mice exposed to cannabis smoke. CD4+ T-cells were decreased in *Ahr-/-* mice in both the lungs and spleen. Plasma cytokines for neutrophil recruitment increased in *Ahr-/-* mice exposed to cannabis smoke while those for T-cells decreased. Cytokine expression in the airway of the lungs showed increases of eotaxin, leukemia inhibitory factor, vascular endothelial growth factor, and IL-6 in both *Ahr+/-* and *Ahr-/-* mice treated with cannabis smoke, while the cytokines IP-10 and KC were increased only in *Ahr-/-* mice exposed to smoke. Cannabinoid metabolizing enzyme expression in the lungs was induced by cannabis smoke in *Ahr-/-* mice only.

Conclusions: Acute cannabis smoke exposure activates the AhR and increases inflammation in the absence of AhR expression. We further show that AhR suppresses the expression of cannabinoid metabolizing enzymes. Overall, this work investigates the consequences of cannabis smoke on lung inflammation and its therapeutic potential in lung health, pointing toward a pivotal role for the AhR.

Acknowledgments: Canadian Institutes of Health Research, Quebec Respiratory Health Research Network

CHARACTERIZATION OF A JUNIPER PHYTOEXTRACT AS A POSITIVE ALLOSTERIC MODULATOR FOR CB_1R. IMPLICATIONS FOR PSORIASIS

María E. Prados[1], Matthias Winkler[2], Juan D. Unciti[1], Francisco J, Ponce[1], Juan A. Collado[1], Isabel Lastres[1], Marcus R. Götz[2] and Eduardo Muñoz[3].

[1]VivaCell Biotechnology España, Cordoba, Spain, [2]Dr. Pfleger Arzneimittel GmbH, Bamberg, Germany, [3]Maimonides Biomedical Research Institute of Córdoba, University of Córdoba, Spain.

Introduction: Cannabinoid receptors (CB_1R and CB_2R), as part of the endocannabinoid system, play a critical role in numerous human physiological and pathological conditions. Thus, considerable efforts have been made to develop ligands for CB receptors, resulting in hundreds of phyto- and synthetic cannabinoids that have shown varying affinities relevant for the treatment of a multitude of diseases. From a natural products perspective, it is expected that, in addition to cannabinoids, other plant-derived molecules targeting CB receptors can be of special relevance.

Methods: We have screened a large collection of plant species for their ability to interact with targets of the endocannabinoids system such as CB_1R, CB_2R, FAAH and MAGL and found that a *Juniper communis* CO_2 extract showed potent and selective activity as a positive allosteric modulator (PAM) for CB_1R. This extract did not show CB_1R orthosteric activity but enhanced the levels of cAMP measured by a CRE-Luc promoter, the activation of ERK-1+2 measured by western blots and the recruitment of β-arrestin in HEK293-CB1-Nomad cells treated with CP-55,940. Moreover, the extract also induced autophagy and cell cycle arrest in the human keratinocyte cell line HaCaT and showed immunosuppressive activity in Jurkat T cells.

Results: Because of the relevant bioactivities *in vitro*, we explored the potential efficacy in an *in vivo* model of Psoriasis induced by imiquimod (IMQ). Symptom scores were significantly elevated in the IMQ group compared to the healthy group during the 5 to 7 days, and the application of Juniper 0.1% and Juniper 1% significantly decrease the pathological score at day 5 and Juniper 0.1% and Juniper 0.5% on day 7. To investigate the effects of Juniper extract on skin morphology and histological changes, dorsal skin and ear tissues were studied by H&E and toluidine blue staining. H&E staining confirmed a thicker dermis region in dorsal and ear skin in the IMQ-induced group as well as the increase of the mast cells number stained by toluidine. The application of Juniper 0.1% decreased the mast cells infiltrated and the treatment with Juniper 0.5%, Juniper 1% and a combination with CBD reduced the epidermal length and also improve the toluidine blue staining. Expression of CD3 and KT17 was elevated in the skin of the IMQ-induced group compared with the control group and the topical administration of Juniper at 0.1% and 0.5% was able to reduce the expression of KT17 and the lymphocyte infiltration.

Conclusion: The development of CB_1R allosteric modulators has become an area of immense importance within the cannabinoid field. It is clear that enormous potential exists to successfully target CB_1R through allosteric sites for the treatment of different diseases including psoriasis, where other avenues – such as agonists and inverse agonists – have been unsuccessful or have produced certain side effects.

CANNABIDIOL (CBD) MAY INFLUENCE SUBSTRATE UTILISATION DURING AND MUSCLE RECOVERY FOLLOWING DAMAGING EXERCISE

Christopher Gaffney, Jack Cunnington, Naomi Greathead, Evie Rigby, Dan Scott, Michelle Montemayor, Hannah Garvey and Karen Wright

Faculty of Health and Medicine, Lancaster University, Lancaster, UK

Introduction: Cannabidiol (CBD) has been shown to have anti-inflammatory, analgesic, anxiolytic and neuroprotective effects, which have attracted the attention of athletes. CBD is available commercially in the UK with a daily limit of 70mg assigned by the Food Standards Authority. At the time of the study design, there was limited published information about the effects of CBD on physiological performance during and after exercise. This study investigated the effects of CBD on key parameters of submaximal muscle-damaging exercise.

Methods: Thirteen recreationally active and healthy volunteers (7 males and 6 females) (age: 22±1 years; $\dot{V}O_2$ max: 43.2±5.8 mL·min-1·kg-1), participated in this randomised, double-blind, crossover trial. Participants completed two experimental sessions, separated by a washout period of ≥7 days, at which they were administered a single oral dose of CBD (70 mg in MCT oil) or placebo (MCT oil), followed by a standardized snack bar (Energy per bar: 257 kcal; Fat 13.3%; Carbohydrates 66.4%; Fibre 4.3%; Protein 5.2%; Salt 0.32%), 1 hour before undertaking a submaximal downhill run (30 minutes at 70% $\dot{V}O_2$ max and 10% decline). Heart Rate (HR) and respiratory gases were monitored continuously, with ratings of perceived exertion (RPE) checked every 5 minutes and blood glucose (BG) and lactate (BL) concentrations sampled pre- and post-run. Venous blood samples, algometer readings (perceived pain threshold) and counter-movement jumps (lower body power) were taken Pre- and Post-run, then at 24h and 48h post-run. Data were analysed using mixed-model ANOVA and significance was set at $p < 0.05$.

Results: There was evidence that CBD might reduce the Respiratory Exchange Ratio (RER; $p=0.08$), whereas oxygen consumption (VO_2) was unchanged by CBD. No changes in HR, RPEs, BG and BL were observed (CBD vs placebo). CBD reduced the time taken for perceived muscle soreness to improve (treatment*time, $p=0.03$), but this was not reflected in the CMJ heights. CBD had no significant effect on plasma IL-6, TNF-α, and CK concentrations (i.e., Pre- vs. Post-RUN, Post- vs 24 h and 48 h Post-RUN, $p > 0.05$). There were no observed changes in plasma levels of IL-10 at any of the timepoints, irrespective of treatment.

Conclusion: Overall, a single dose of 70mg CBD does not impact on many physiological variables during exercise-induced muscle damage. However, CBD may influence the substrate utilisation during exercise, potentially promoting greater fat oxidation. In addition, CBD could enhance the recovery of muscle tenderness but not muscle strength (and, by inference, performance). The study found no CBD-induced differences between males and females, but the group sizes were underpowered and further confirmation of these results is ongoing.

CANNABINOID CB1 RECEPTORS ARE EXPRESSED IN A SUBSET OF DOPAMINE NEURONS AND FUNCTIONALLY INVOLVED IN CANNABINOID ACTION IN MICE

Zheng-Xiong Xi, Briana Hempel and Chloe Jordan

Addiction Biology Unit, Molecular Targets and Medication Development Branch, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD 21224, USA

Introduction: Cannabinoids modulate dopaminergic (DA) transmission and DA-related behavior, which has long-time been thought to be mediated by activation of CB1 receptors (CB1R) on GABAergic and glutamatergic terminals, not on DA neurons, in the mesolimbic DA system. Unexpectedly, we often observed CB1R gene expression in a subset of tyrosine hydroxylase (TH)-positive DA neurons in our recent studies examining CB1R expression in the brain. This surprising observation inspired us to re-examine CB1R gene/protein expression in DA neurons.

Methods: Advanced RNAscope CB1R gene detection techniques, optogenetics, and dopaminergic CB1-knockout mice were used to systemically examine CB1 receptor expression in midbrain DA neuron.

Results: We detected CB1R mRNA expression in a subset of DA neurons located mainly in the middle, rather than the anterior or posterior, portion of the ventral tegmental area (VTA), as assessed by CB1R co-localization with dopaminergic neuronal markers including TH, DA transporter (DAT), D2 receptor, vesicular monoamine transporter 2 (VMAT2). The number of such CB1R-positive DA neurons decreased progressively from the medial to the lateral VTA. Triple-labeling straining showed CB1R mRNA co-localization with both TH and vesicular glutamate transporter 2 (VgluT2), suggesting CB1R expression in a dual phenotype of neurons that co-release DA and glutamate. We then used optogenetic approaches to examine the effects of cannabinoids on intracranial self-stimulation (ICSS) maintained by optical activation of DA neurons. We found that the cannabinoid agonists Δ^9-tetrahydrocannabinol (Δ^9-THC) or ACEA dose-dependently inhibited optical ICSS in DAT-Cre mice, but not in DA-CB1-KO mice in which CB1Rs were deleted from DA neurons. DA-CB1-KO mice also displayed an enhanced basal level of locomotion and a significant reduction in Δ^9-THC-induced hypoactivity and anxiolytic effects.

Conclusion: Together, these findings suggest that CB1Rs are expressed in a subset of DA neurons and functionally involve in cannabinoid modulation of DA transmission and DA-related behavior.

Acknowledgement: Funded by NIDA IRP (DA000633-01)

POSITIVE ALLOSTERIC MODULATOR OF CB_1 RECEPTOR: A POTENTIAL THERAPEUTIC TARGET FOR HAND

Barkha J. Yadav-Samudrala[1], Elizabeth Kim[1], Shreya Ramineni[1], Benjamin Gorman[1], Dai Lu[2], Justin Poklis[3], Aron H. Lichtman[3], Bogna M. Ignatowska-Jankowska[4] and Sylvia Fitting[1]

[1]Dept. of Psychology & Neuroscience, University of North Carolina, Chapel Hill, NC, USA
[2]Dept. of Pharmaceutical Sciences, Texas A&M University, Kingsville, TX, USA
[3]Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, VA, USA
[4]Okinawa Institute of Science and Technology, Neuronal Rhythms in Movement Unit, Japan

Introduction: The endocannabinoid (eCB) system has attracted interest as a therapeutic target for various neurodegenerative disorders due to its anti-inflammatory and neuroprotective properties. One such disease is HIV-associated neurocognitive disorders (HAND), a neurodegenerative cognitive disorder with an inflammatory component. The HIV-1 transactivator of transcription (Tat) is one of the viral proteins and a neurotoxin that plays a significant role in the pathogenesis of HAND. Due to the undesired effects of the direct CB_1 receptor agonists, the focus has been turned on targeting alternative components of the eCB system, such as enzymes responsible for eCB biosynthesis and degradation and, more recently, allosteric modulators. ZCZ011 has been shown to act as a positive allosteric modulator (PAM) of the CB_1 receptor. Here we aimed to assess whether it produces neuroprotective effects in the mouse model of HAND.

Methods: *In vitro* experiments assessed intracellular calcium ($[Ca^{2+}]_i$) in primary neuronal cultures derived from dissociated frontal cortex of embryonic day 17. Treatments included Tat (100 nM), AEA (10 nM), and/or ZCZ011 (10, 100, 1000 nM) in addition to CB_1 and CB_2 receptor antagonists (SR141716A and SR144528, respectively). Behavioral experiments were conducted with the use of 32 mice divided into four groups (n=8 per group): male Tat (+), male Tat (-), female Tat (+), and female Tat (-). Animals received chronic injections of vehicle or 10 mg/kg ZCZ011 for 21 days. After behavioral experiments, mice were sacrificed, and brains were harvested for mass spectrometry analysis.

Results: Primary neuronal culture experiments demonstrated that ZCZ011 enhanced the neuroprotective effects of AEA (10 nM) against Tat-induced increases of $[Ca^{2+}]_i$ in a concentration-dependent manner. ZCZ011 alone produced similar effects to AEA by itself which appeared to be blocked by a CB_1 but not CB_2 receptor antagonist. Behavioral experiments showed that ZCZ011 improved novel object recognition in female Tat (+) mice, whereas no effects were noted for rotarod, tail flick, hot plate, and locomotor activity. Mass spectrometry analysis showed that endocannabinoids AEA and molecule lipids PEA, OEA, and AA were significantly upregulated in the striatum of Tat (+) male mice, whereas 2-AG was upregulated in Tat (+) female mice.

Conclusion: ZCZ011 shows neuroprotective effects in the Tat-induced model of HAND. It appears that the effects of ZCZ011 are CB_1 receptor mediated. Behavioral memory experiments and mass spectrometry analysis show specific genotype, sex, and treatment effects, and further investigations are needed to decipher the mechanism and utility of CB_1 PAMs in the context of neuroHIV.

Acknowledgments: Funded by NIH R01 DA045596, R01 DA039942, and P30 DA033934; and JSPS P17388, 17F17388, and 21K06399.

TOTAL SYNTHESIS AND CHARACTERIZATION OF NOVEL *CIS*-CBD STEREOISOMERS

Yi Yang[1,2] and Lakshmi P. Kotra[1,2,3]

[1]Krembil Research Institute, Toronto Western Hospital,
University Health Network, Toronto, ON, Canada
[2]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy,
University of Toronto, Toronto, ON, Canada
[3]Centre For Cannabinoid Therapeutics, Toronto, ON, Canada

Introduction: The bicyclic cannabinoid cannabidiol (CBD) possesses two chiral centers on its terpenoid ring and exhibits *cis-trans* isomerism along the axis of its C3-C4 bond with four possible stereoisomers. The levorotatory *trans*-(3*R*,4*R*)-CBD, commonly known as (-)-CBD, is a secondary metabolite uniquely produced by *Cannabis* spp. of plants and has been shown to exhibit anti-inflammatory and anticonvulsant properties. Meanwhile, the dextrorotatory *trans*-(3*S*,4*S*)-CBD, also known as (+)-CBD, was synthesized in 1982 and exhibits anticonvulsant activity. We are interested in investigating the *cis*-isomers, specifically *cis*-(3*R*,4*S*)-CBD and *cis*-(3*S*,4*R*)-CBD, and thus embarked on the synthesis and isolation of pure *cis*-isomers for biological activity evaluations.

Methods: We undertook a total synthesis of the *cis*-CBD stereoisomers, beginning with the resorcinol derivative olivetol and constructing a terpenoid ring at the C2 position over 7 steps, including a key Diels-Alder reaction to introduce stereochemistry.

Results: The Diels-Alder reaction yielded the key intermediate 1-(2',6'-dimethoxy-5-methyl-4'-pentyl-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-yl)ethan-1-one, a ketone analogue of CBD dimethyl ether, as a mixture of all four *cis* and *trans* isomers. *Cis*-CBD isomers were isolated using semi-preparative chiral HPLC, structures were validated using LC-MS/MS, and stereochemistry was confirmed using homonuclear NOESY spectroscopy.

Conclusions: Here we present the methodology for the total synthesis, separation, and characterization of *cis*-CBD isomers. Further evaluation of these compounds to understand the role of stereochemistry in CBD pharmacology is currently underway.

Acknowledgements: This work is supported by a TC3 Fellowship (YY) from the Toronto Cannabis and Cannabinoids Research Consortium (TC3).

CANNABINOIDS SUPPRESS THE NEUROTOXIC PROPERTIES OF PRO-INFLAMMATORY MICROGLIA *IN VITRO*

Alexander P. Young[1] and Eileen M. Denovan-Wright[1]

[1]Department of Pharmacology, Dalhousie University, Halifax, NS, Canada

Introduction: Microglia take on a pro-inflammatory phenotype during neuroinflammation. This phenotype mediates host defense, but sustained activation can damage neurons. Endocannabinoids have attracted much attention for the purpose of dampening this neuroinflammation. Although endocannabinoids have shown promise in animal models, the individual contributions of microglial cannabinoid type 1 (CB_1) and type 2 (CB_2) receptors have not been established using selective synthetic agonists. In this work, we examine the pro-inflammatory activity of microglia and whether cannabinoid drugs may be effective to suppress this activity as well as secondary neuronal damage.

Methods: Cultured SIM-A9 microglia were stimulated with lipopolysaccharide (LPS) and interferon-γ (IFNγ) to induce a pro-inflammatory phenotype. A CB_1 receptor agonist (ACEA; >1400-fold selective), CB_2 receptor agonist (HU-308; >440-fold selective), and nonselective agonist (CP 55,940) were used to target the cannabinoid receptors. The Griess assay was used to measure nitric oxide (NO) release, reverse transcription quantitative PCR was used to measure mRNA abundance, impermeant dyes were used to measure the cell death of ST*Hdh*$^{Q7/Q7}$ neurons.

Results: LPS and IFNγ stimulated the release of NO and the upregulation of mRNA for several pro-inflammatory markers in a dose-dependent and synergistic manner. LPS and IFNγ also induced changes to the cellular endocannabinoid system in a dose-dependent manner, including changes in the abundance of cannabinoid receptors and endocannabinoid synthesizing and degrading enzymes. Conditioned media from these highly pro-inflammatory microglia induced cell death of neurons *in vitro*. Selective and nonselective cannabinoids differentially reduced mRNA expression of pro-inflammatory markers as well as NO release from cultured microglia in a pattern that suggested that selective cannabinoid receptor activation was more efficacious than dual activation. However, pre-treatment of pro-inflammatory microglia with either class of cannabinoid drug improved the subsequent survival of neurons.

Conclusions: LPS and IFNγ stimulated a classical pro-inflammatory microglia response which also involved substantial changes to the microglial endocannabinoid system. It appeared that selective and nonselective cannabinoid treatments dampened this pro-inflammatory activity of microglia. These effects were biologically relevant as the cannabinoid-treated microglia induced less secondary neuronal damage. This is indicative of the neuroprotective potential of drugs that interact with microglial cannabinoid receptors. Further work will expand on the signaling mechanisms that underlie the effects of cannabinoids on the inflammatory activity of microglia.

Acknowledgements: The work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to EDW (RGPIN-2019-37999) and an NSERC Alexander Graham Bell Canada Graduate Scholarship to APY.

THE ACTIVITY OF CANNABINOID BY PRODUCTS AND TERPENES ON TYPE 1 CANNABINOID RECEPTOR

Ayat Zagzoog[1], Ashley Cabecinha[2], Hanan Abramovici[2] and Robert B Laprairie[1,3]

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon SK Canada, [2]Office of Cannabis Science and Surveillance, Controlled Substances and Cannabis Branch, Health Canada, Ottawa, ON Canada,[3]Department of Pharmacology, College of Medicine, Dalhousie University, Halifax NS Canada

Introduction: *Cannabis* sativa contains more than 120 cannabinoids and 400 terpene compounds (i.e. phytomolecules) present in varying amounts. *Cannabis* is increasingly available for legal medicinal and non-medicinal use globally, and with increased access comes the need for a more comprehensive understanding of the pharmacology of phytomolecules. The main transducer of the intoxicating effects of *Cannabi*s is the type 1 cannabinoid receptor (CB1R). Δ^9-tetrahydrocannabinol (Δ^9-THC), a CB1R partial agonist, is the most-abundant cannabinoid present in many cultivars of *Cannabis*. Understanding the complex interplay of phytomolecules – often referred to as 'the entourage effect' – has become a recent and major line of inquiry in cannabinoid research. The purpose of this study was to assess 10 select phytomolecules (Δ^8-THC, $\Delta^{6a,10a}$-THC, 11-OH-Δ^9-THC, and cannabinol, curcumin, epigallocatechin gallate, olivetol, palmitoylethanolamide, piperine, and quercetin) with or without Δ^9-THC to determine how these compounds modulate ligand binding and signaling via CB1R *in vitro* and *in vivo*.

Methods: Phytomolecules (10 nM -10 μM) were screened for their binding to CB1R, inhibition of forskolin-stimulated cAMP accumulation and βarrestin2 recruitment in Chinese hamster ovary cells stably expressing human CB1R. Concentration-response curves were fit using non-linear regression and used to calculate EC_{50}, E_{min}, and E_{max}. Also, selected compounds were assessed further *in vivo* (10 mg/kg, *i.p*) for catalepsy, hypothermia, and anti-nociceptive effect in male mice where data were analyzed via a one-way ANOVA followed by Tukey's post-hoc test.

Results: *In vitro*, all phytomolecules yielded CB1R-dependent inhibition of cAMP with less potency and efficacy than Δ^9-THC, except for $\Delta^{6a,10a}$-THC, which displayed greater efficacy. $\Delta^{6a,10a}$-THC and 11-OH-Δ^9-THC produced βarrestin2 recruitment similar to Δ^9-THC. Δ^8-THC, $\Delta^{6a,10a}$-THC, and olivetol reduced Δ^9-THC binding at CB1R. *In vivo*, 11-OH-Δ^9-THC was able to reproduce or exceed the effects of Δ^9-THC, which hint at the possibility that 11-OH-Δ^9-THC specifically could yield intoxicating effects relative to Δ^9-THC via βarrestin2 effects. In contrast, curcumin and quercetin were able to diminish the intoxicating effects of Δ^9-THC via indirect inhibition of CB1R-dependent signaling.

Conclusion: The pharmacology of *Cannabis* is multi-factorial and complex beyond what traditional single compound pharmacodynamic experiments are capable of modelling. Here, we took a reductionist approach to understand how single phytomolecules behave alone and in concert with Δ^9-THC. Furthermore, the screening of isolated phytomolecules from *Cannabis* may lead to the development of novel ligands and drugs not yet characterized as we continue to advance knowledge in this area.

Acknowledgements: Funding for this project was provided by a Health Canada Research Contract.

THE PHYTOCANNABINOID CANNABINOL (CBN) ENHANCES SLEEP AND ALTERS SLEEP ARCHITECTURE IN RATS

Cassandra V. Occelli Hanbury Brown[1,2,3], Anand Gururajan[1,4], Lyndsey L. Anderson[1,2,3,], Joel S. Raymond[1,4], Adam Ametovski[1,5], Samuel D. Banister[1,5], Peter T. Doohan[1,2,3], Miguel A Bedoya-Perez[1,4], Iain S. McGregor[1,4] and Jonathon C. Arnold[1,2,3*]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Australia.
[2]Discipline of Pharmacology, The University of Sydney, Australia.
[3]School of Pharmacy, The University of Sydney, Australia.
[4]School of Psychology, Faculty of Science, The University of Sydney, Australia.
[5]School of Chemistry, Faculty of Science, The University of Sydney, Australia.

Introduction: Insomnia is highly prevalent in the community and there is a need to develop novel drugs to treat sleep disorders with reduced side-effects. The soporific properties of cannabis have long been known and the major phytocannabinoids cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) can increase non-rapid eye movement (NREM) sleep; however, they may also increase wake and suppress rapid-eye-movement (REM) sleep. Cannabis contains more than 100 phytocannabinoids that might also be explored for their hypnotic potential including cannabinol (CBN), which has long been suggested to enhance sleep. Indeed, with the proliferation of the cannabinoid isolates market there has been an increasing use of CBN products in the absence of evidence demonstrating any efficacy on sleep. Here we aimed to assess whether CBN enhances sleep and alters sleep architecture in rats.

Methods: Surgically implanted biotelemetry probes were utilised to examine the effects of acute and repeated exposure (15 days) to CBN on various polysomnographic measures of sleep. Zolpidem was used as a positive control and to provide a qualitative comparison to any effects of CBN. Drugs were administered at the onset of the dark phase and data were collected and manually scored over the subsequent 11 h recording window. We also conducted a pharmacokinetic study to examine the distribution of CBN and its major metabolites 11-OH-CBN and 11-COOH CBN in brain and plasma in rats.

Results: Acute CBN exposure (10, 30 and 100 mg/kg i.p.) did not affect latency to NREM sleep onset, whilst zolpidem (10 mg/kg) significantly reduced sleep onset latency. CBN did increase the percentage of time in NREM sleep and sleep bout duration, with the effect delayed to 4 h post-dosing. Zolpidem also increased the percentage of time in NREM sleep, but REM sleep was not affected. Acute CBN exposure (10 mg/kg i.p.) had a biphasic effect on REM sleep, initially suppressing then later increasing the percentage of time in REM sleep. CBN (100 mg/kg) increased REM sleep onset latency. The effects of CBN on sleep architecture were subject to a degree of tolerance with decreased magnitudes of effects by day 15 following repeated, daily dosing with CBN (10 mg/kg i.p.). Next we investigated the pharmacokinetic parameters of CBN and its major metabolites in plasma and brain given the delayed effect observed with acute dosing. CBN and 11-OH CBN achieved equivalently high brain concentrations (1 μM) with a t_{max} of 2 h following administration of 10 mg/kg CBN i.p. There was excellent brain penetration of both with brain-plasma ratios of 2:1 and 3:1.

Conclusion: CBN affected sleep architecture in rats and our data suggests it may have potential as a novel therapeutic for the treatment of insomnia. CBN may be better applied to treat sleep disorders associated with poor sleep maintenance rather than for sleep induction.

MINOR CANNABINOIDS AND TERPENES AMELIORATE HIV REPLICATION AND HIV PROTEIN-RELATED NEUROINFLAMMATORY PAIN

Nicole M Ashpole, Fakhri Mahdi, Mohammed Salahuddin, Emaya Moss, Alaa Qrareya, Miguel A De Leon, Matthew J Foster, Mohamed O Marzouk, Jessica P Marshall, Amira Wanas, Mohamed M Radwan, Mahmoud A ElSohly and Jason J Paris

University of Mississippi School of Pharmacy, University, MS, USA

Introduction: Approximately 40-50% of people living with HIV-1 infection suffer from intractable pain, chiefly sensory neuropathies. While combined antiretroviral therapies (cART) have dramatically improved survival, both HIV infection and cART treatment promote long-lasting neuroinflammation that contribute to neuropathic pain. Expression of the HIV-1 protein, trans-activator of transcription (Tat), in mice is sufficient to activate microglia in the dorsal spinal cord, promote loss of epidermal nerve fibers, and induce peripheral neuropathic pain. Identification of therapeutics capable of abrogating Tat-mediated inflammation may be an avenue for treating HIV-neuropathic pain. Clinical evidence suggests that the chemical constituents of cannabis may be beneficial for treating inflammatory pain and other adverse effects associated with HIV infection and cART treatment. We hypothesized that complementary to cART, several minor cannabinoids and terpenes will ameliorate the viremia, inflammation, and cytotoxicity caused by infectious HIV-1 *in vitro*, as well as the inflammatory pain promoted by HIV-1 proteins *in vivo*.

Methods: Isolated minor cannabinoids and terpenes were prepared by the UM Marijuana Research Laboratory. Changes in human microglial (HMC3) viability and activation state were observed with microscopy 24 hours following exposure to recombinant Tat_{1-86} and increasing concentrations of cannabinoids/terpenes. HIV-BaL-infected human peripheral blood mononuclear cells were assessed following treatment with cannabinoids/terpenes as potential regulators of HIV replication in the absence and presence of cART. For *in vivo* efficacy testing, compounds were diluted in EtOH:cremaphor:saline (1:1:8) and administered to adult Tat+ transgenic mice and Tat− control mice. Doses of 10mg/kg were administered via intraperitoneal injection 30 minutes prior to assessment of pain/nociception in the abdominal writhing assay or von Frey mechanical allodynia test. Experiments were assessed with inferential statistics and group differences were determined in a manner controlled for familywise error. All treatments were compared to vehicle control, and sex was considered an independent biological variable.

Results: Over 60 minor cannabinoids/terpenes were screened for their capacity to attenuate Tat-mediated microglial activation, with at least 7 compounds including CBD, CBG, and myrcene, resulting in significant reductions in activated cell morphology. In HIV-infected human PBMCs, several compounds including CBC, CBD, CBN, THC, and β-CP attenuated HIV replication. Lead compounds have moved to *in vivo* assays to assess their effects against Tat-mediated pain/allodynia. Multiple cannabinoids, including CBGA and CBN, reduced visceral pain and mechanical allodynia in Tat+ mice. Interestingly, sex-specific differences were observed with several compounds, such as β-CP, significantly attenuating visceral pain in Tat+ male but not female mice, and CBG attenuating visceral pain in female but not male mice.

Conclusion: Together, our studies highlight that numerous cannabis constituents exert anti-viremic, anti-inflammatory, and anti-nociceptive effects in models of HIV-associated neuroinflammation.

ORALLY ADMINISTERED *N*-OLEOYL ALANINE BLOCKS ACUTE MORPHINE WITHDRAWAL-INDUCED CONDITIONED PLACE AVERSION AND ATTENUATES SOMATIC WITHDRAWAL FOLLOWING CHRONIC OPIATE EXPOSURE IN RATS

Samantha M Ayoub[1], Erin M. Rock[1], Reem Smoum[2], Cheryl L. Limebeer[1], Marieka DeVeuno[1], Raphael Mechoulam[2] and Linda A. Parker[2]

[1.] Department of Psychology and Collaborative Neuroscience Program, University of Guelph, Guelph, ON Canada.
[2.] Institute of Drug Research, Medical Faculty, Hebrew University of Jerusalem, Jerusalem, Israel

Introduction: The endogenous *N*-acyl amino acid *N*-Oleoyl glycine (OlGly) reduces affective and somatic behaviors produced by withdrawal from acutely administered morphine in rats when delivered by intraperitoneal (i.p.) injection. However, OlGly is rapidly inactivated by amidases, limiting its therapeutic potential in clinical models. A methylated version of OlGly, named *N*-Oleoyl alanine (OlAla; HU595), was synthesized to increase the stability of this compound. Like OlGly, i.p. OlAla reduces naloxone-precipitated acute morphine withdrawal behavioral responses through similar neural mechanisms (CB_1R and PPARα), but can do so over a longer duration of time. Additionally, OlAla reduces several signs of somatic withdrawal behavior following treatment with *chronic* opiates, whereas OlGly is ineffective. Treatment with OlAla also blocks naloxone-precipitated elevations of heroin self-administration, and reverses some of the changes in brain and gut endocannabinoidome and gut microbiota induced by naloxone. Together, these data are consistent with the hypothesis that OlAla is a more stable compound and suggests OlAla may be a more effective therapeutic option for treatment. To increase the translational appeal of using OlAla in clinical drug applications, the current experiments aimed to test whether oral OlAla pretreatment could also alter the behavioral effects of opiate withdrawal in rats.

Methods: In Experiment 1, place conditioning was used to determine if orally (intragastric gavage, i.g.) administered OlAla (5 mg/kg and 20 mg/kg) or its vehicle would reduce the establishment of an acute naloxone-precipitated morphine withdrawal induced conditioned place aversion. In Experiment 2, somatic withdrawal observations were conducted following chronic steady state heroin exposure (via minipumps) in rats pretreated with oral OlAla (5 mg/kg – 80 mg/kg, i.g.)

Results: In Experiment 1, pretreatment with oral 20 mg/kg OlAla, but not 5 mg/kg OlAla, blocked the establishment of a morphine withdrawal-induced conditioned place aversion. In Experiment 2, pretreatment with oral 5 mg/kg OlAla, but not other doses tested, reduced heroin withdrawal-induced abdominal contractions ($p < 0.01$) and diarrhea ($p < 0.001$).

Conclusions: This study demonstrated a dose-specific reduction of withdrawal responses from chronic and acutely administered opiates by orally-administered OlAla, and suggests that oral OlAla could be effective in reducing opiate withdrawal in clinical populations. Given the dose-specific observations, we can speculate the loci of OlAla's effect on withdrawal behavior to be central for affective withdrawal, and peripheral for somatic withdrawal.

THE CANNABIDIOL TOPICAL FOR HAND OSTEOARTHRITIS PILOT (CATCH-OP): A SINGLE CENTRE OPEN LABEL PILOT TRIAL

Zeeta Bawa[1,2], Daniel Lewis[3], Paul Gavin[4], Roksan Libinaki[4], Lida Joubran[4], Mahmoud El-Tamimy[4], Greg Taylor[5], Ryan Meltzer[5] and Iain S. McGregor[1,2]

[1]The Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Sydney, New South Wales, Australia
[2]School of Psychology, The University of Sydney, Sydney, New South Wales, Australia
[3]The Daniel Lewis Rheumatology Centre, Victoria, Australia
[4]Avecho Biotechnology, Clayton, Victoria, Australia and
[5] The NTF Group, Sydney, New South Wales, Australia

Introduction: Hand osteoarthritis (OA) is a common, painful, progressive, and irreversible condition that significantly impairs hand strength and function and reduces quality of life. There are limited treatments options and therapy primarily involves symptom relief and preservation of function. Systemic administration of cannabidiol (CBD) has shown some promise in measures of pain and inflammation in animal models of OA, however, topical therapeutics are also of particular interest due to specificity of action and avoidance of first-pass metabolism. The aim of the current pilot study was to assess whether topical application of a CBD gel containing novel transdermal delivery technology would modulate measures of pain and improve functionality in patients suffering from hand OA.

Methods: A single-centre observational trial investigated the effects of a topical 4% w/w CBD gel on symptomatic painful hand osteoarthritis (OA) affecting the fingers and/or thumb over a 6-week period (1-week baseline; 4 weeks treatment with CBD topical gel applied 3 times a day; and 1-week washout). The CBD gel was formulated with TPM, a mixture of phosphorylated forms of Vitamin E that facilitates transdermal drug penetration. Participants with hand OA (n=14) meeting specific inclusion criteria were recruited from a rheumatology clinic in Victoria, Australia. The primary outcome measure was the change in daily hand pain (average and maximum) categorised by a Numeric Pain Rating Scale (NPRS). The secondary objectives involved hand functionality including 1) grip strength measured daily via a squeeze ball dynamometer connected via Bluetooth to a smartphone-based recording app, and 2) the Functional Index for Hand Osteoarthritis (FIHOA) score, completed at baseline and weekly until study exit. All outcome measures were collected in real time using the 10tiv smartphone app (https://10tiv.com).

Results: Participant retention and adherence was excellent with a 96% completion rate for outcome measures. Using a panel regression model, there was a significant reduction in average NPRS pain scores (1.32-point difference, baseline = 4.38, $p<.001$) and maximum NPRS pain scores (1.62-point difference, baseline = 5.57, $p<.001$) over the 4-week treatment period. Grip strength significantly increased over time ($p<.001$) and most of the 15 participants also saw improvements in FIHOA functionality scores with treatment. Weekly declines in self-reported fatigue, stiffness, and anxiety were also noted.

Conclusion: When used for symptomatic painful hand osteoarthritis (OA), a novel topical 4% w/w CBD gel applied 3 times a day over 4 weeks significantly improved NPRS pain scores and grip strength in this pilot trial. These promising initial results give impetus to plans for a larger randomised placebo-controlled trial.

Funding: Study supported by Avecho Ltd and The Lambert Initiative for Cannabinoid Therapeutics.

COMBINATION OF CANNABIDIOL AND BETA-CARYOPHYLLENE SYNERGISTICALLY MITIGATE INFLAMMATORY PAIN

[1]Henry Blanton, [2]Linda Yin, [1]Joseph Duong and [1]Khalid Benamar

[1]Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, School of Medicine Lubbock, Texas 79430.
[2] Garrison Institute on Aging, Texas Tech University Health Sciences Center, Lubbock, TX, 79430, USA

Introduction. Pain, one of the most common reasons adults seek medical care. Current pain treatment options are limited, often ineffective, and have associated side effects. Cannabis contains a high number of potentially therapeutic phytochemicals including cannabinoids and terpenes. While cannabinoids and terpenes have been investigated in pain models in isolation, there has been very little research investigating the potential analgesic effects of cannabinoids and terpenes in combination. The aim of the present study was to determine if a more effective analgesic effect could be obtained through the combination of two major constituents of the cannabis plant, the phytocannabinoid cannabidiol (CBD) and the sesquiterpene beta-caryophyllene (BCP) without producing the adverse effects associated with CNS cannabinoid receptor-1 (CB_1) activation, and to characterize the nature of the potential interaction between these two compounds.

Methods: Experiments were performed using sixteen-week-old male C57BL6/J mice purchased from Jackson Laboratories (Bar Harbor, Maine, USA). First, we determined the analgesic potency and efficacy of CBD and BCP individually in the formalin inflammatory pain model through dose-response studies. After determining the ED_{50} for each compound, we tested the analgesic effect of several fixed-ratio combinations of CBD and BCP and monitored any adverse effects on body temperature, motor impairment, and locomotor activity. We also determined the effect of this combination on inflammation. Analysis of cytokines from plasma was performed using a Mouse XL Cytokine Array Kit (R&D Systems; Minneapolis, MN, USA). This kit allows simultaneous measurement of 111 mediators/markers in a single sample. Statistics were performed in Graph Pad Prism 9 (GraphPad Software; San Diego, USA). Two and One-Way ANOVA with Dunnett's Post Hoc Tests. Significance was set at $p < 0.05$.

Results: CBD was administered at doses of 1, 2.5, 10, 25, and 50 mg/kg. BCP was administered at doses of 1, 3, 10, and 30 mg/kg. Administration of CBD and BCP resulted in dose-dependent reductions in pain behaviors in the inflammatory phase of the formalin test from minutes 20 to 40 post-formalin injection. We tested several fixed ratio combinations (based on the ED_{50} of each compound), and using isobolographic analysis, it was found that the analgesic effect of CBD and BCP in combination is synergistic. We also tested this combination for common CB_1-associated side effects. We found that CBD and BCP in combination did not produce, hypothermia, sedation and motor incoordination. Finally, the synergistic analgesic effect of the CBD and BCP in combination involves an anti-inflammatory mechanism.

Conclusions: This study has demonstrated that a minor cannabinoid and terpene found in the cannabis plant can be used as a pharmacological tool to produce an enhanced analgesic effect without side effects commonly seen with CB_1 acting cannabinoids. The idea that CBD and BCP in combination can produce a synergistic analgesic effect set the stage for future studies that will test if the beneficial effect of this combination can be reproduced in other inflammatory/neuropathic pain models.

CBD INTERACTIONS WITH PROPOFOL IN MICE

Chris Breivogel, Samantha Harrell, Linda Nguyen and Hunnain Siddiqui

Dept. of Pharmaceutical & Clinical Sciences,
Campbell University, Buies Creek, NC, USA

Introduction: Previous studies found that greater doses of midazolam, fentanyl and propofol were required for adequate sedation for endoscopic procedures in cannabis users versus non-users.[1] It is not known which constituent(s) of cannabis might have caused this effect.

Methods: C57BL/6 male and female mice were administered 50 mg/kg THC, 200 mg/kg CBD or vehicle (20% sesame oil: 8% Tween-80: 82% ddH_2O) i.p. at 0.01 ml/g. In some experiments, mice received THC or CBD daily on days 1-3 then propofol (in 13% Tween-80, 86% ddH_2O) at 125 mg/kg on day 5. In others experiments CBD was given only 1 hour before propofol. Change in body temperature was assessed with an IR thermometer and total time in which mice exhibited loss or righting reflex (LORR) was recorded with stopwatches. All data were analyzed by 2-way ANOVA (sex vs treatment) at $\alpha = 0.05$.

Results: Propofol caused changes in body temperature of -1.2°C females and -3.2°C in males and LORR for 726 s in females and 2379 s in males. Three-day pre-treatment with THC had no significant effect on propofol's effects. Pre-treatment with CBD had no significant effect on propofol-induce hypothermia in either sex or LORR in female mice (634 s, $p = 0.9984$), but virtually eliminated the effect of propofol on LORR in male mice (80.5 s, $p = 0.0019$). CBD one hour prior to propofol, showed significant enhancement of the hypothermic effect in females (-2.0 vs -6.3°C, $p = 0.003$) and males (-3.6 vs -8.4°C, $p = 0.0010$) and the LORR effect in females (1241 vs 13371 s, $p = 0.0289$) and males (2872 vs 24472 s, $p < 0.0001$).

Conclusions: CBD (but not THC) given for 3 days prior to propofol appeared to cause desensitization to some of the effects of propofol, while CBD concurrent with propofol synergistically enhanced the hypothermic and sedative effects of propofol.

Acknowledgements: Funded by the Campbell University Department of Pharmaceutical & Clinical Sciences

[1]Twardowski, M. A., Link, M. M. and Twardowski, N. M. (2019), *J Am Osteopath Assoc*. 19(5): 307-311.

VAPORIZED CANNABIS EXTRACT-INDUCED ANTINOCICEPTION IN MALE VS. FEMALE RATS

Rebecca M. Craft[1], Hannah Y. Gogulski[1], Timothy G. Freels[2], Nicholas C. Glodosky[1] and Ryan J. McLaughlin[2]

[1]Department of Psychology and [2]Department of Integrative Physiology and Neuroscience, Washington State University, Pullman, WA, USA

Introduction: Animal studies indicate that injected delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) may each produce antinociception. However, human use of these cannabinoids for pain occurs primarily via inhalation of cannabis smoke or vaporized cannabis extracts, and the inhalation vs. injection routes are known to result in different absorption and metabolism of cannabinoids. The goal of this study was to use the most common human route of administration, inhalation, to characterize the antinociceptive effects of cannabis extract in rats. Secondary goals were to determine whether THC- and CBD-predominant extracts were equally effective against persistent inflammatory pain, and equally effective in males and females.

Methods: 60-90 day old SD rats were used. On Day 1 (am), baseline mechanical threshold, weight-bearing, locomotion, and hindpaw thickness measures were obtained; immediately thereafter, CFA was injected into one hindpaw to induce pain and inflammation. Rats were exposed for 1 h to vaporized vehicle (grapeseed oil) or cannabis extract that was THC-predominant (28.4% THC, 1.2% CBD) or CBD-predominant (59.3% CBD, 1.9% THC) at concentrations of 100, 200, or 400 mg/ml, on the following schedule: Day 1 at 5 pm, Day 2 at 8 am and 5 pm, Day 3 at 8 am and 5 pm. On Day 4 at 8 am, rats were exposed again to vaporized vehicle or cannabis extract, and then re-tested on all behavioral tests at 15-120 min post-exposure. Paw thickness was measured once, after the last behavioral test.

Results: Vaporized THC-predominant extract produced dose- and time-dependent anti-allodynia (Dose x Time, $p=.038$), with maximal effect at 200 mg/ml in females and 400 mg/ml in males (Dose x Sex, $p=.031$). Vaporized THC-predominant extract did not significantly increase weight-bearing on the inflamed paw in either sex, or significantly alter locomotion or hindpaw edema. In males, vaporized CBD-predominant extract was anti-allodynic (Dose, $p=.05$), produced non-significant increases in hindpaw weight-bearing and locomotion, and non-significant decreases in hindpaw edema. Vaporized CBD-predominant extract produced no effects in females. Comparison of rats treated acutely (Day 4 only) vs. repeatedly (Days 1-4) with vaporized THC-predominant extract suggested that females developed tolerance to the high concentration of this extract, whereas males did not.

Conclusions: Passive exposure to vaporized cannabis extract is anti-allodynic in rats with persistent inflammatory pain, but is generally more effective in males than females: CBD-predominant extract produced antinociception only in males, and THC-predominant extract, although acutely effective in both sexes, lost effect with repeated exposure in females when a high concentration was used.

Acknowledgement: Supported by a Herbert L. Eastlick Professorship to R.C.

INVOLVEMENT OF CB_2 RECEPTORS IN CANNABIDIOL NEUROPROTECTION AFTER INTRAVENTRICULAR HEMORRHAGE IN IMMATURE RATS

M. de Hoz-Rivera[1], A. Del Pozo[1], A. Romero[1], L. Silva[1], M. Martínez-Vega[1], M. Villa[1] and J. Martínez-Orgado[1]

(1) Hospital Clínico San Carlos-IdISSC, Madrid

Background: Although cannabidiol (CBD) is not considered a CB_2 receptor (CB_2R) agonist, previous studies demonstrated that CB_2R antagonism abolishes CBD neuroprotection after hypoxic-ischemic brain damage in newborn rats and piglets. The present work has been designed to test if CB_2R are involved in CBD neuroprotection in a different model of acute acquired brain damage in immature brain, in this case using a rat model of intraventricular hemorrhage (IVH), a frequent complication in extremely low gestational age newborns increasing the risk of Cerebral palsy development.

Aim: to study the effects of CB_2R antagonism on CBD neuroprotection after IVH insult in immature rat brain

Methods: IVH was induced in 1-day-old Wistar rats by paraventricular injection of 0.2 U of *Chlostridium collagenase* into the left Germinal Matrix. This led to motor and cognitive sequelae in the middle (PND 14) and long term (PND 45). Animals exposed to anaesthesia but not to surgery remained as controls (SHAM). Vehicle (VEH) or CBD or were administered prenatally (10 mg/kg i. p. to pregnant rats at E21) and post-insult (5 mg/kg i. p. 6, 24 and 48h post-insult). Some rats received AM630 (5 mg/kg i. p. 1h pre-insult and 1h before VEH or CBD post-insult administration). Brain injury was assessed at PND6 using Magnetic Resonance Image (MRI) and at PND14 exploring motor coordination (negative geotaxis). Besides, participation of inflammation was analysed at PND14 using Western Blot studies to determine the expression of: TLR4, as a representative of the main biochemical pathway of neuroinflammation in immature brain IVH-induced brain damage; and MMP9, an enzyme activated by several biochemical pathways and relate to IVH-induced blood-brain barrier disruption.

Results: IVH led to brain damage as observed in MRI studies, as well as to functional impairment with worse performance in the geotaxis test. Those effects were associated to neuroinflammation, observed by increased TLR4 and MMP9 expression. Both anatomical and functional brain damage was reduced by CBD treatment in a manner linked to the modulation of inflammation. Co-administration of CBD with the CB_2R antagonist resulted in the loss of CBD protective effects. CB_2R antagonist abolished CBD effects on MMP9 but not on TLR4 expression.

Conclusions: CBD treatment reduced IVH-induced brain damage in immature rats in a manner linked to the modulation of inflammation. CB_2R antagonism blunted CBD neuroprotection, preventing CBD modulatory effects on MMP9 expression by mechanisms not dependent on TLR4 activation.

Supported by PI19/00927 grant

CANNABICHROMENE EFFECTIVELY REDUCES CISPLATIN-INDUCED NEUROPATHIC PAIN

Miguel A. De Leon, Waseem Gul, Vishvesh Raje, Mahmoud ElSohly, Nicole M Ashpole and Hannah M. Harris

University of Mississippi School of Pharmacy

Introduction: Chemotherapy-induced peripheral neuropathy is a debilitating adverse effect experienced by roughly 30-40% of patients receiving chemotherapy treatment. Unfortunately, current therapeutic strategies require long-term treatment, with limited efficacy that often requires the use of opioid-based medications. Studies have suggested potential utility of cannabis-based medicines to alleviate neuroinflammation and subsequent pain. While multiple studies have explored the effects of delta^9-tetrahydrocannabinol and synthetic cannabinoids, a cannabinoid devoid of psychoactive effects would likely be a stronger candidate for drug development. Our current study evaluated the effectiveness of cannabichromene (CBC) and related derivatives against chemotherapy-induced neuropathic pain, as evidence indicates CBC does have anti-inflammatory activity while being devoid of psychoactive effects.

Methods: Using a mouse model, we assessed whether CBC and CBC derivatives were able to attenuate or prevent cisplatin-induced neuropathic pain. Following previously established protocols, cisplatin was administered to mice over a 12-day period (alternating days of Ringers vehicle vs cisplatin injections). Increasing doses of CBC were administered one day following the final cisplatin treatment or co-administered with cisplatin throughout the treatment regimen. The withdrawal response from electronic Von Frey stimulation was used as a measure of tactile allodynia in the days following final cisplatin administration.

Results: Acute administration of CBC ablated the allodynia associated with cisplatin-induced neuropathic pain in a dose-dependent manner. Similar to oxycodone-treated controls, mice that received greater than 10mg/kg CBC or CBC derivative showed withdrawal responses at the level of non-cisplatin treated control mice. Moreover, co-administration of 25mg/kg CBC during the cisplatin regimen, significantly reduced tactile allodynia several days following the final cisplatin treatment.

Conclusion: These data indicate that CBC not only exerts acute effects against cisplatin-induced neuropathic pain, but it can also prevent or delay the onset of cisplatin-induced neuropathic pain. Further studies regarding the effects of CBC on the underlying pathophysiological response are ongoing. Together, these data suggest CBC may have utility as a potential therapy for preventing or alleviating neuropathic pain.

Acknowledgements: P30GM122733

SGIP1 MODULATES ACUTE AND CHRONIC NOCICEPTION IN A SEX-SPECIFIC FASHION

Oleh Durydivka[1], Agnieszka Kubik-Zahorodna[1], Michaela Dvorakova[1], Matej Gazdarica[1], Ken Mackie[2] and Jaroslav Blahos[1]

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Psychological and Brain Sciences, Gill Center for Molecular Bioscience, Indiana University, Bloomington, Indiana, USA

Introduction: The SH3-containing GRB2-like protein 3-interacting protein 1 (SGIP1) interaction with cannabinoid receptor 1 (CB1R) results in the inhibition of the receptor agonist-promoted endocytosis. As a consequence, following CB1R activation, the transient association of the receptor with GRK3 and β-arrestin 2 is enhanced and prolonged, while the CB1R-mediated ERK1/2 signaling is decreased (Hajkova et al., 2016, Neuropharmacology; Gazdarica et al., 2021, Journal of Neurochemistry). Using SGIP1 knock-out (SGIP1 KO) mice, we showed that sensorimotor gating, exploratory levels, and working memory are unaltered in the mice with SGIP1 absence. However, SGIP1 deletion modifies exploratory and anxiety-related behaviors and other physiological processes controlled by the endocannabinoid system. Furthermore, SGIP1 KO males have augmented responses to Δ9-THC in the cannabinoid tetrad tests (Dvorakova et al., 2021, British Journal of Pharmacology). Therefore, we asked whether SGIP1 influences both acute and chronic nociception.

Methods: We analyzed wild-type and SGIP1 KO mice in acute and chronic pain models. For acute pain experiments, the tail immersion test was implemented. Inflammatory peripheral chronic pain was induced by the intraplantar injection of 1% carrageenan. The paw withdrawal threshold was measured 2, 24, and 48 h after the carrageenan injection using an electronic von Frey aesthesiometer.

Results: In the tail immersion test, drug-naïve SGIP1 KO mice reacted more slowly to the stimulus than the wild-type mice. Δ9-THC and WIN treatments led to more pronounced anti-nociceptive effects in the SGIP1 KO males than in the wild-type males. Acute nociception was also decreased in the SGIP1 KO females after WIN treatment but not after Δ9-THC treatment. In the inflammatory pain model, the SGIP1 KO males were more sensitive to mechanical stimuli compared to the wild-type males. Δ9-THC treatment decreased nociceptive responses in the SGIP1 KO and control males, but Δ9-THC treatment in the SGIP1 KO males was less effective than in the wild-type males. On the contrary, Δ9-THC treatment in the SGIP1 KO females produced a larger analgesic effect than in the wild-type females.

Conclusions: Genetic deletion of SGIP1 alters acute and chronic inflammatory pain processing. Acute pain nociception is reduced in the SGIP1 KO mice. In the inflammatory pain model, SGIP1 deletion results in hyperalgesia in the males. The anti-nociceptive properties of CB1R agonist treatments in the chronic pain model are more pronounced in the SGIP1 KO females.

Acknowledgements: This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic project entitled "International mobility of IMG researchers II, No. CZ.02.2.69/0.0/0.0/18_053/0016981 Support of Professional Development" and the Czech Science Foundation (GACR) 21-02371S.

THC ENHANCES 2-AG SIGNALING IN MOUSE PFC AT LOCOMOTION INITIATION

Anthony English[1], David Marcus[1], Fleur Uittenbogaard[1], Yulong Li[2], Larry Zweifel[1,5], Benjamin B. Land[1,5], Nephi Stella[1,4,5] and Michael R. Bruchas[1,3,5]

Departments of [1]Pharmacology, [3]Anesthesiology [4]Psychiatry and Behavioral Sciences, [5]Center for Neurobiology of Addiction, Pain, and Emotion, University of Washington, Seattle, USA; [2]Peking-Tsinghua Center for Life Sciences, Peking University, Peking, China.

Introduction: *Cannabis* intoxication in humans impairs locomotor behavior and is associated with a 15% increase in fatal motor vehicle accidents associated with recreational legalization. Similarly, THC impairs locomotor behavior (e.g., reduced spontaneous locomotion) and is a standard cannabimimetic response in rodents; however, how THC modifies this behavior remains unknown. THC triggers a dose-dependent reduction in spontaneous locomotion in mice that is characterized by general immobility (lying prone with flattened bodies) interrupted by brief and stochastic locomotion events. Here, we leveraged fiber photometry to elucidate the neural basis of how THC reduces spontaneous locomotion by measuring neuronal activity and dynamics changes in 2-AG levels within the prelimbic cortex (PrL) in vehicle (VEH) and THC-treated mice.

Methods: WT mice were injected with AAV-hSyn-eCB2.0, while VGAT-Cre and VGLUT1-Cre mice were injected with AAV5-DIO-GCaMP6f and implanted with an optic fiber into the PrL. Mice were placed in a behavioral chamber for 10 min before receiving an i.p. treatment of either VEH or THC (10 mg/kg) and left to roam the chamber for 90 min. Locomotor behavior and fluorescent activity were time locked to one another and measured for the entire duration of the session. To validate dynamic changes in 2-AG levels, WT-$GRAB_{eCB2.0}$ mice were pretreated with the DAGL inhibitor DO34 (pretreatment, 30 mg/kg, i.p.) prior to VEH or THC treatment.

Results: PrL glutamatergic and GABAergic activities increased concomitantly to the initiation of locomotion as indicated by increased GCaMP6f signaling in both VGLUT1-Cre (Z-Score: 0.12 ± 0.07) and VGAT-Cre (Z-Score: 0.95 ± 0.16) mice treated with VEH. THC treatment moderately increased VGLUT1-Cre transient activity (Z-Score: 1.97 ± 0.24) and dramatically increased VGAT-Cre transient activity (Z-Score: 8.64 ± 0.48) associated with the initiation of locomotion. We detected two distinct $GRAB_{eCB2.0}$ responses present in THC-treated mice: (1) a gradual increase in activity over the 90 min post-THC and (2) transient increases in activity (Z-Score: 2.78 ± 0.27) associated with initiation of locomotion that was much greater than VEH-treated mice (Z-Score: 0.36 ± 0.14). DO34 did not affect the gradual increase in $GRAB_{eCB2.0}$ activities in THC-treated WT-mice and eliminated all transient activity in VEH- and THC-treated WT-$GRAB_{eCB2.0}$ mice detected at the initiation of locomotion, suggesting increased 2-AG signaling.

Conclusions: These findings suggest that THC shifts the activity of PrL glutamatergic and GABAergic neurons to increased GABAergic depolarization during initiation of locomotion compared to baseline activity in VEH-treated mice. Inhibition of DAGL with DO34 eliminates the transient increases of $GRAB_{eCB2.0}$ signal (2) in VEH- and THC-treated mice, confirming the involvement of 2-AG. The retention of the gradual increase (1), unaffected by DAGL blockade, show detection of increased THC in the PFC.

Acknowledgements: Funded by F31 DA055448-01, R37DA033396 to MRB and DA047626 to NS, and the U. Washington Alcohol and Drug Abuse Institute (ADAI, to MRB, DM).

COMPARISON OF CLINICAL OUTCOMES OF CHRONIC PAIN PATIENTS WITH CO-MORBID ANXIETY SYMPTOMS TREATED WITH MEDICAL CANNABIS: A COHORT STUDY

Lara Bapir[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Mohammed Sajad[2], Azfer Usmani[2], Mona Mubarak[2], Wendy Holden[2], Jonathan Hoare[1,2], Shaheen Khan[2,4], Mark W Weatherall[2,5], James J Rucker[2,6,7], Michael Platt[1,2] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Guy's & St. Thomas' NHS Foundation Trust, London, UK
5. Buckinghamshire Healthcare NHS Trust, Amersham, UK
6. Department of Psychological Medicine, Kings College London, London, UK
7. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Cannabis-based medicinal products (CBMPs) have been identified as novel therapeutic agents for chronic pain. The major active pharmaceutical ingredients, cannabidiol and Δ9-tetrahydrocannabinol, have demonstrated anti-nociceptive effects via peripheral pain receptors. In addition, cannabinoid receptors are implicated in central sensitisation and emotional regulation, which contribute to the pathogenesis of chronic pain. There is a paucity of evidence to identify which patients with chronic pain are most likely to respond to CBMPs. The primary aim of this study is to compare the changes in patient-reported outcomes following treatment of chronic pain patients with co-morbid anxiety symptoms against those without anxiety symptoms.

Methods: A prospective cohort study was performed of patients with chronic pain disorders enrolled on the UK Medical Cannabis Registry. Individual patient cohorts were defined according to baseline General Anxiety Disorder Scale (GAD-7) score as either having no anxiety symptoms (0-4), or anxiety symptoms (5-21). Primary outcomes were changes in Short-form McGill Pain Questionnaire-2 (SF-MPQ-2), Brief Pain Inventory short-form (BPI), Pain Visual Analogue Scale (P-VAS), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L at 1, 3, and 6 months compared to baseline. Statistical significance was defined as $p<0.050$.

Results: 1,254 patients with chronic pain were included in the analysis with no anxiety (n=543; 44.3%), and anxiety (n=711; 56.7%) symptoms respectively. Improvements were observed in both cohorts in SF-MPQ-2, BPI severity scores, BPI interference scores, P-VAS, SQS, and EQ-5D-5L index values at 1-, 3-, and 6-months following initiation of CBMP treatment ($p<0.001$). Patients with anxiety symptoms at baseline experienced larger improvements in BPI pain interference scores, SF-MPQ-2, SQS, and EQ-5D-5L index values compared to patients without anxiety symptoms at each time point ($p<0.050$). The were no significant differences between either cohort with respect to changes in P-VAS and BPI pain severity scores ($p>0.050$).

Conclusion: This study identified a significant improvement in pain-specific and general health related quality of life outcomes in patients with chronic pain treated with CBMPs. Patients with co-morbid anxiety symptoms demonstrated greater improvements in outcomes related to interference of pain on daily living, general quality of life, and sleep. This suggests that CBMPs may provide axillary effects beyond effects on peripheral pain receptors through interrupting the cyclical relationship between anxiety and chronic pain.

AN UPDATED ANALYSIS OF CLINICAL OUTCOME MEASURES FOR PALLIATIVE CARE FROM THE UK MEDICAL CANNABIS REGISTRY

Joaquin Bello[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Michael W Platt[1,2], James J Rucker[2,4,5], Shaheen Khan[2,6] and Mikael H Sodergren[1,2]

[1] Imperial College Medical Cannabis Research Group, Dept of Surgery and Cancer, Imperial College London, UK
[2] Sapphire Medical Clinics, London, UK
[3] St. George's Hospital NHS Trust, London, UK
[4] Department of Psychological Medicine, Kings College London, London, UK
[5] South London & Maudsley NHS Foundation Trust, London, UK
[6] Guy's & St. Thomas' NHS Foundation Trust, London, UK

Introduction: A growing body of literature suggests that cannabinoids, terpenes, and flavonoids have potent effects on neurotransmission, neuroendocrine signalling, and inflammatory processes. This potentially makes cannabis-based medicinal products (CBMPs) an emerging multi-faceted therapeutic option in managing primary chronic pain, cancer pain and neuropathic pain. Possible benefits have also been shown in anxiety-predominant disorders. Combined, these effects make the use of CBMPs as part of end-of-life care for patients a promising yet relatively unexplored topic. There is subsequently a paucity of evidence to guide best prescribing practices and optimal therapeutic regimes. The aim of this study is to assess changes in patient reported outcome measures and incidence of adverse events in patients treated with CBMPs in the setting of palliative care in the UK.

Methods: An observational case series of data from the UK Medical Cannabis Registry, the largest CBMP-specific registry in the UK, was analysed. The primary outcome was changes in EQ-5D-5L, General Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), Pain Visual Analog Scale (P-VAS), the Australia-Modified Karnofsky Performance Scale (AKPS), and the Palliative Care Outcome Scale (POS), at 1 and 3 months compared to baseline. Secondary outcomes included the incidence and characteristics of adverse events (CTCAE v4.0). Statistical significance was defined by p-value<0.050.

Results: 70 patients were included in the analysis with a mean age of 57.7 (±14.9) years. There were 35 (50.0%) male and female participants respectively. The median Charlson Comorbidity Score of the patients was 8.0 (IQR: 5.0-11.0). There were improvements in both GAD7 and SQS scales at 1- and 3-months following initiation of treatment (p<0.050). There was a positive change in the POS at 1-month only (p=0.011). Finally, there was a reduction in pain severity as measured by the P-VAS at 3-months only (p<0.001). There were no changes in AKPS or EQ-5D-5L values at either follow-up. Seven (10%) participants reported 48 (68.6%) adverse events. These were mostly mild (n=21; 30%) and moderate (n=20; 28.6%).

Conclusion: This small study describes the outcomes of the first cohort of patients prescribed CBMPs in the context of palliative care. Improvements were noted in anxiety and sleep-specific outcomes. However, there were no consistent improvements in palliative care specific outcomes and the EQ-5D-5L, a measure of general health-related quality of life. Further long-term safety and efficacy studies evaluating specific symptoms and involving larger cohorts, with comparisons to placebo and standard treatments are required to further understand the role of CBMPs across this heterogenous population.

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

Nish Dalavaye[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], James J Rucker[2,4,5], Jonathan Hoare[1,2,*] and Mikael H Sodergren[1,2,*]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Department of Psychological Medicine, Kings College London, London, UK
5. South London & Maudsley NHS Foundation Trust, London, UK
* joint senior authors

Introduction: Cannabis-based medicinal products (CBMPs) have been touted as a potential class of medical therapies for inflammatory bowel disease (IBD). Preclinical evaluation of their effects has demonstrated a potential role in inflammatory modulation, gut secretions, colonic motility, visceral nociception, and the gut microbiome. However, to date, clinical trials have failed to demonstrate an effect on active inflammation as determined during endoscopy, or ability to induce clinical remission, however these have identified improvements in self-reported pain severity as secondary outcomes. This study aims to analyse the outcomes of patients prescribed CBMPs in the setting of IBD to identify its effects on health-related quality of life and safety of its use.

Methods: This is a case series of patients treated with CBMPs in the setting of IBD for symptom control. Patients were identified from the UK Medical Cannabis Registry, the largest real-world evidence platform of CBMP therapies in the UK. Primary outcomes included change from baseline in the following patient reported outcome measures at 1 and 3 months: short inflammatory bowel disease questionnaire (S-IBDQ), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L. Secondary outcomes were incidence of reported adverse events (CTCAE v4.0). $p<0.050$ determined statistical significance.

Results: 76 patients with Crohn's disease (n=51; 67.1%) and ulcerative colitis (n=25; 32.9%) were included in the analysis. The mean age of patients was 38.5 (±9.3) years. Most patients were male (n=63; 13%). The median baseline S-IBDQ score was 40.0 (IQR: 32.0-48.0). The severity reduced at 1-month (median: 34.5; IQR: 24.0-44.75; $p<0.001$) and 3-months (median: 33.0; IQR: 23.0-42.0; $p<0.001$). Improvements were also demonstrated in the EQ-5D-5L index values, GAD-7 and SQS at 1- and 3-months ($p<0.050$). 123 (161.8%) adverse events were reported by 16 (21.1%) patients. The adverse events were categorised as mild (n=54; 71.1%), moderate (n=55; 72.4%), and severe (n=14; 18.4%). There were no life-threatening adverse events.

Conclusion: In summary, patients treated with CBMPs for refractory symptoms of Crohn's disease and ulcerative colitis demonstrated an improvement in IBD-specific quality of life following initiation of treatment in the short-term. In addition, general health related quality of metrics were improved at 1 and 3 months. Whilst more randomised controlled trials and long-term evaluations of efficacy and safety are required, these results provide promising indication for the use of CBMPs as an adjunct in managing symptoms of IBD.

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

Claire Wang[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Azfer Usmani[2], Mohammed Sajad[2], Mona Mubarak[2], Wendy Holden[2], James J Rucker[2,4,5], Michael W Platt[1,2] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Department of Psychological Medicine, Kings College London, London, UK
5. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Current challenges in the management of fibromyalgia are due to heterogeneity in clinical presentation and multiplicity of core symptoms. Targeting core symptoms (including pain, fatigue, unrefreshing sleep, and mood disorders) are a key component of management, but currently individual treatments seldom address all symptoms. Cannabis-based medicinal products (CBMPs), which have demonstrated early evidence of effects across each of these domains, have been proposed as a potential pharmacological management option for fibromyalgia. However, there is limited evidence from randomised controlled trials of their effects in fibromyalgia. The aim of this registry study is to assess effects of CBMPs on health-related quality of life and safety of its use in the setting of fibromyalgia.

Methods: Patients treated with CBMPs for fibromyalgia for a minimum of one month were identified from the UK Medical Cannabis Registry, the most extensive CBMP-bespoke real-world evidence platform in the UK. Demographic details of patients were extracted. Primary outcomes were changes in the Fibromyalgia Symptom Severity Scale, pain visual analogue scale (P-VAS), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L. Adverse events were recorded and analysed according to CTCAE v.4.0. Significance was defined by p-value<0.050.

Results: 306 patients treated with CBMPs for fibromyalgia were included in this case series. The mean age was 44.7 (±12.3) years. 163 (53.3%) participants were unemployed at the time of starting treatment. The median lifetime cannabis exposure was 5.0 (IQR: 1.0-12.63). There was a decrease in the Fibromyalgia Symptom Severity Scale at 1, 3, and 6 months (p<0.001). In addition, there were improvements in the P-VAS, GAD-7, SQS and EQ-5D-5L index value at each follow-up (p<0.010). There were 979 (319.9%) recorded adverse events by 72 (23.5%) patients. The most frequently reported adverse events were fatigue (n=75; 24.5%), dry mouth (n=69; 22.5%), impairment of concentration (n=66; 21.6%), and lethargy (n=65; 21.2%).

Conclusion: Patients with fibromyalgia treated with CBMPs in the UK experienced a significant reduction in symptom severity following initiation of treatment, alongside adjunctive benefits in recorded pain, sleep, anxiety, and general health-related quality of life metrics. The incidence of adverse events for those with fibromyalgia is greater than other conditions assessed within the UK Medical Cannabis Registry, suggesting despite associated improvements they are also more likely to experience adverse events during treatment. The limitations of study design mean that one cannot determine causality for either positive or negative effects of CBMP therapy. However, this provides promising signals for future evaluation in clinical trials.

A SYSTEMATIC REVIEW AND META-ANALYSIS OF CANNABIDIOL FOR PAIN AND RELATED OUTCOMES

Joel Gagnier[1-2], Daniel Nechi[3] and Kevin Boehnke[4]

1. Department of Epidemiology & Biostatistics, Schulich School of Medicine and Dentistry, London, ON, Canada
2. Department of Surgery, Schulich School of Medicine and Dentistry, London, ON, Canada
3. Department of Medicine, University of Michigan, Ann Arbor, MI, USA
4. Department of Anaesthesiology, University of Michigan, Ann Arbor, MI, USA

Introduction: Acute and chronic pain are common in those with musculoskeletal (MSK) pathologies or injuries. Many MSK conditions require pain medications (e.g. opioids, NSAIDs) or surgical interventions, the latter which have a protracted recovery period with increased acute pain. Opioids carry significant side effects, and their use in surgical settings has contributed to the US and Canadian opioid crisis that have claimed many lives. Therefore, developing alternative opioid sparing analgesics is critical for preventing opioid dependence following surgery. Cannabidiol (CBD) shows great promise, as it is non-intoxicating, exerts anti-inflammatory, analgesic, and anxiolytic effects in preclinical studies, and is well tolerated across numerous medical conditions. Taken together, this evidence suggests that CBD may be opioid sparing in surgical settings. To inform the development of a clinical trial of CBD for opioid sparing following surgery, we performed a rigorous, up to date systematic review and meta-analysis of CBD for opioid sparing and the treatment of pain and related outcomes.

Methods: We performed electronic searches in PubMed, EMBASE, Cochrane, CINAHL, SportDiscus, Scopus, and reviewed relevant conference abstracts up to December of 2021. We included only randomized, cohort, case control, or cross-sectional studies including humans and using CBD only, investigating its effects on opioid use or pain (as our primary outcomes) and anxiety, depression, sleep, physical function (as secondary outcomes). We assessed the risk of bias of all included studies, extracted data, performed fixed and random effects meta-analyses, meta-regression, subgroup analyses, explored publication bias, and performed a GRADE assessment on all outcome categories across studies.

Results: We reviewed over 859 non-duplicate abstracts for inclusion, resulting in a total of 26 included studies. A total of 21 were randomized studies and 5 cohort studies, several being open label, two cross-over studies and one 2x2 factorial design. A total of 23 studies included CBD alone and 3 included very small quantities of THC, and most were two group designs. The studies were overall of low risk of bias, with 21 fulfilling more than 70% on the risk of bias criteria. Only a single study looked at the opioid sparing effects of CBD with 53% having reduced or eliminated opioids and decreased pain; this study also showed that 94% had improved quality of life. For our secondary outcomes, studies showed consistent results for CBD compared to placebo controls: improved sleep, pain intensity & interference, decreased anxiety, decreased depression, improved emotional and behavioural dyscontrol, and improved psychotic symptoms. There was little evidence of publication bias on visual inspection of funnel plots, for studies with all available data (n=28). Overall GRADE score for opioid sparing and pain reduction was moderate, for sleep, anxiety and depression was also moderate. All other outcomes had low GRADE evidence. We could not complete the formal meta-analyses as we are still awaiting data from the contact authors on 6 of the included papers.

Conclusions: Overall the evidence if minimal for opioid sparing effects of CBD, but the evidence appears good for other secondary outcomes such as pain, anxiety, and depression. Additional rigorous research should be done in pain conditions, preferably perioperatively, of CBD to mitigate opioid use.

ADVERSE PSYCHIATRIC SIDE EFFECTS OF THE CANNABINOID CB1 INVERSE AGONIST SR141716 (RIMONABANT) WERE LIKELY PREDICTABLE FROM PRECLINICAL ANIMAL MODELS

Eliot L. Gardner[1,2] and Zheng-Xiong Xi[3]

[1]Departments of Neuroscience and Psychiatry,
Albert Einstein College of Medicine, New York, NY, USA
[2]Neuropsychopharmacology Section and [3]Addiction Biology Unit, Intramural Research Program, U.S. National Institute on Drug Abuse, Baltimore, MD, USA

Introduction: SR141716 (also known as rimonabant; trade names Acomplia, Zimulti) is an inverse agonist at the cannabinoid CB1 receptor, with anorectic anti-obesity properties. It was approved in Europe for the clinical management of obesity in 2006, but withdrawn worldwide in 2008 due to serious psychiatric side effects. Those side effects were likely predictable from preclinical animal models.

Methods: Three different preclinical animal models were used in laboratory rats – electrical brain-stimulation reward, *in vivo* brain microdialysis, and conditioned place preference/aversion.

Results: SR141716 – at moderately-high to high doses – produced a right-shift in electrical brain-reward rate-frequency functions, produced inhibition of extracellular dopamine in the nucleus accumbens of the limbic forebrain, and produced conditioned place aversion.

Conclusions: We conclude that the adverse psychiatric side effects of SR141716 in humans were likely predictable from preclinical animal models, and that preclinical animal models are useful in predicting adverse effects as well as potentially beneficial effects of cannabinoids and drugs acting on the endocannabinoid system.

Acknowledgements: Supported by the extramural research grant program of the U.S. National Institutes of Health (Grant R01DA03622), the Intramural Research Program of the U.S. National Institute on Drug Abuse (Research Projects Z01DA000478 and Z01DA000513), the New York State Office of Alcoholism and Substance Abuse Services, the Aaron Diamond Foundation, the Julia Sullivan Medical Research Fund, and the Old Stones Foundation.

CB2 RECEPTORS IN PRIMARY SENSORY NEURONS MEDIATE ANTI-ALLODYNIC EFFICACY OF THE CB2 AGONIST LY2828360 IN A MOUSE MODEL OF INFLAMMATORY PAIN

Kelsey G. Guenther[1,2], Julian Romero[3], Cecilia Hillard[4], Ken Mackie[1,2,5], Zhili Xu[1,2] and Andrea G. Hohmann[1,2,5]

[1]Program in Neuroscience, Indiana University, Bloomington, IN; [2]Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN; [3]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, Pozuelo de Alarcón, Madrid, Spain; [4]Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI; [5]Gill Center for Biomolecular Science, Indiana University, Bloomington, IN

Introduction: Cannabinoid CB_2 agonists show promise as therapeutic agents because they lack unwanted side effects commonly associated with direct activation of CB_1 receptors and activation suppresses pathological pain in animal models, however, the types of pain that best respond to CB_2 agonists and the cell types which underlie CB_2-mediated anti-allodynic efficacy are incompletely understood. The G protein-biased CB_2 receptor agonist LY2828360 attenuated the maintenance of neuropathic pain induced by the chemotherapy agent paclitaxel, but whether this finding generalizes to models of inflammatory pain is not known.

Methods: Paw withdrawal thresholds were measured in mice before and after unilateral injection of the inflammatory agent carrageenan in the hindpaw. CB_1 global KO, CB_2 global KO and CB_2 conditional KO mice were used to investigate the underlying mechanism of LY2828360 antinociceptive actions. qRT-PCR was used to examine the ability of LY2828360 to modulate cytokines and chemokines involved in the inflammatory response of carrageenan.

Results: LY2828360 (10 mg/kg i.p.) reversed carrageenan-induced mechanical allodynia, whereas lower doses were ineffective (1-3 mg/kg i.p.). Anti-allodynic efficacy was fully preserved in CB_1 knock out (KO) mice but absent in CB_2 KO mice. Additionally, anti-allodynic efficacy of LY2828360 was absent in conditional KO mice lacking CB_2 receptors in many excitatory neurons ($NEX^{CRE/+}$; $CB_2^{f/f}$) and mice primarily lacking CB_2 receptors in primary sensory neurons ($Advillin^{CRE/+}$; $CB_2^{f/f}$). Thus, CB_2 receptors in excitatory neurons and more specifically primary sensory neurons are required for anti-allodynic effects of LY2828360 in the carrageenan model of inflammatory pain. Local administration of LY2828360 directly into the inflamed paw also reduced mechanical allodynia, an effect that was absent in $Advillin^{CRE/+}$; $CB^{2f/f}$ mice. Finally, LY2828360 reduced IL-1β and IL-10 mRNAs in paw skin of animals subjected to carrageenan-induced inflammation.

Conclusions: Our results provide the first evidence that LY2828360 suppresses inflammatory nociception through a CB_2-dependent mechanism in mice and suggest that the clinical applications of LY2828360 as an anti-hyperalgesic agent should be re-evaluated.

Supported by DA047858 and DA041229 (to AGH and KM), the Research and Education Component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin (C.J.H) and the Ministerio I Ciencia e Innovación (PID2019-108992RB-I00 to JR)

MODULATION OF ACUTE LUNG INJURY-INDUCED SYSTEMIC INFLAMMATION VIA CANNABINOID TYPE 2 RECEPTOR ACTIVATION

Stefan Hall[1], Sufyan Faridi[2], Irene Euodia[2], Juan Zhou[2],
Melanie Kelly[2,3] and Christian Lehmann[1,2,3]

[1]Department of Physiology and Biophysics, Dalhousie University, Halifax, NS
[2]Department of Anesthesia, Dalhousie University, Halifax, NS
[3]Department of Pharmacology, Dalhousie University, Halifax, NS

Background: The COVID-19 pandemic has highlighted the risk of immune dysregulation in severe cases of lung infection. These cases are characterized by life-threatening complications including pneumonia, acute respiratory distress syndrome, and multi organ failure. Elevated pro-inflammatory cytokine levels are described as contributing to disease progression. Activation of the cannabinoid type 2 receptor (CB2R) exerts immunomodulatory effects in numerous pre-clinical models, including protective effects in lung inflammation. Thus, we hypothesize that experimental CB2R activation can lead to reductions in systemic inflammation in a murine model of acute lung injury (ALI).

Methods: 12-week-old C57BL/6 mice were challenged with intranasal *Pseudomonas aeruginosa* (5 mg/kg) or vehicle, followed by intravenous administration of experimental CB2R agonist, onternabez, vehicle, and/or dexamethasone (0.1 mg/kg). At t = 6 hours, lung tissue was scored for histological changes and plasma cytokines were analyzed with Luminex™ technology. In separate groups, intravital microscopy (IVM) of the intestinal or pulmonary microcirculation, respectively, was performed. IVM recordings were analyzed to quantify leukocyte adhesion, leukocyte rolling, and functional capillary density (FCD).

Results: Vehicle-treated LPS mice displayed a marked increase in lung histopathology scores consistent with ALI and significant increases in plasma CXCL2, IL-6, IL-10, and TNF-Alpha. This effect was recapitulated by intestinal IVM results which demonstrated significant increases in leukocyte adhesion in V1 and V3 venules and a significant reduction in muscularis FCD. Treatment with onternabez significantly reduced leukocyte adhesion in V1 venules. Dexamethasone treatment abolished leukocyte adhesion in V1 venules, significantly reduced leukocyte adhesion in V3 venules and significantly increased muscularis FCD. It also abolished lung histopathology and significantly reduced plasma levels of TNF-alpha. Combination treatment displayed similar effects to dexamethasone-only treatment, as well as significant decreases in CXCL2 and IL-6. Lung IVM showed similar trends of leukocyte adhesion and capillary perfusion (FCD) in selected micro-vessels.

Conclusions: Intranasal administration of LPS from *P. aeruginosa* induced ALI and a systemic inflammatory response evidenced by increased plasma cytokine levels and leukocyte activation in intestinal and pulmonary microvasculature. Dexamethasone and combination treatment exhibited immunosuppressive action, abolishing immune responses in most, but not all cases. Conversely, onternabez treatment exhibited milder immunomodulatory effects, with significant reductions in leukocyte adhesion and trends toward baseline across all other parameters. Further studies will aid in establishing its utility in treating ALI-induced systemic inflammation.

MODELING FATTY ACID AMIDE HYDROLASE FUNCTION WITH ZEBRAFISH

Robin Heider, Izzabella Green, Han Lee, Randall Krug II and Karl J. Clark

Clinical and Translational Sciences, Mayo Clinic
Graduate School of Biomedical Sciences, Rochester, MN, USA

Introduction: In humans, fatty acid amide hydrolase (FAAH) & fatty acid amide hydrolase 2 (FAAH2) are distinct enzymes that regulate endocannabinoid (eCB) levels, serving a key role in eCB-induced behaviors. Overall, the eCB system is highly conserved between humans & zebrafish, *Danio rerio*, with the latter possessing two *FAAH2* orthologs (*faah2a* & *faah2b*). Animal models commonly used to study eCB signaling, including murine species, do not carry a gene for *Faah2*. The conservation of *faah2* orthologs makes zebrafish a unique model organism to study eCB signaling. We leverage this feature of zebrafish to study the differential roles of FAAH & FAAH2 in HPA axis activation including locomotor response to acute stressors.

Methods: We have produced frame-shift alleles of *cnr1* & *faah2a* and demonstrated that fish carrying these alleles have altered locomotor responses to an acute stressor. To better understand the potential differential roles of fatty acid amid hydrolases we have designed new, conditional mutagenic alleles. We have injected zebrafish embryos at the one cell stage with guides & identified guides that efficiently produce double-strand breaks in *faah*, *faah2a*, & *faah2b*. We have produced mutagenic donor cassettes flanked by short homology domains that enable targeted integration into intronic sequences within the zebrafish genome to generate six Cre-conditional mutant zebrafish lines with two distinct donors into three genes: *faah*, *faah2a* & *faah2b*. The cassette can be integrated in both passive & active orientations, allowing for temporal control of gene silencing or activation with the introduction of Cre-recombinase. Once stable mutant lines are identified, we can assess the role of these enzymes, beginning with their role in HPA axis activation using validated behavioral assays.

Results: We have shown that loss of *cnr1* or *faah2a* in zebrafish impacts locomotor responses to an acute stressor. We have designed cassettes targeting *faah*, *faah2a* & *faah2b* to produce Cre-conditional alleles, which will provide high quality expression data & demonstrate potential alterations in behavioral responses to stress.

Conclusions: With relatively fast generation time, tractable genetics, & established eCB mutant lines, the zebrafish is a feasible vertebrate model to evaluate the roles of *faah* & *faah2*.We found that eCB genes can impact rapid stress responses in zebrafish, & this enables better understanding of the differential roles of *faah* and *faah2* genes.

SEXUALLY DIMORPHIC ENDOCANNABINOID CHANGES IN A RAT MODEL OF CHRONIC LOW BACK PAIN ASSOCIATED WITH INTERVERTEBRAL DISC INJURY

Mary A. Hopkins, Maria C Redmond, Mehnaz I Ferdousi, Stephanie Bourke, Catherine Healy, Álvaro Llorente-Berzal and David P. Finn

Pharmacology and Therapeutics, School of Medicine, Galway Neuroscience Centre and Centre for Pain Research, NCBES, National University of Ireland Galway, Galway, Ireland.

Introduction: Chronic low back pain (CLBP) is a major unmet clinical need with significant socioeconomic impact, and a large contributor to disability. Like other chronic pain conditions, low back pain is more prevalent in females than males, and mechanical sensitivity is higher in female CLBP patients. Research has revealed significant alterations in the endocannabinoid system, at a genetic level, in patients with CLBP. We have developed a novel rodent model of pain following intervertebral disc injury (IVDI) in the rat tail. Here, we investigated alterations in levels of endocannabinoids and *N*-acylethanolamines in key brain regions in this model.

Methods: 50 male and female 12-week old Sprague-Dawley rats (175-300g, n=12-13 per group) underwent either IVDI at the base of the tail (1mm diameter punch of the nucleus pulposus of the IVD at Co4-Co5 and Co5-Co6) or sham surgery. Base of the tail mechanical hypersensitivity (electronic Von Frey-eVF) was assessed at 48 hours, and weekly intervals up to 30 days post-surgery (PSD). Data was analysed by two-way repeated measures (RM) ANOVA, followed by post hoc Student-Newman-Keuls (SNK) test, while Area Under the Curve (AUC) was assessed weekly from PSD 7 to PSD 29 for eVF via 2-way ANOVA and post-hoc SNK. Following euthanasia on PSD 31, tissue levels of endocannabinoids [anandamide (AEA) and 2-arachidonylglycerol (2-AG)] and N-acylethanolamines [N-palmitoylethanolamide (PEA) and N-oleoylethanolamide (OEA)] were analysed in the prefrontal cortex (PFC) and periaqueductal grey (PAG), via LC-MS/MS.

Results: Mechanical hypersensitivity thresholds as measured with eVF were significantly lower in Male IVDI rats versus their sham counterparts at PSD21 (2-Way ANOVA: Between-subject effect Sex*Surgery $F(1,46)=6.8$, $p<0.05$, SNK $p<0.05$). From PSD7 onwards, male IVDI rats had significantly reduced mechanical withdrawal thresholds compared to male shams (AUC 2-Way ANOVA; Between-subject effect of Sex* Surgery $F(1,46)=0.582$, $p<0.01$, SNK $p<0.05$). Conversely, in females, IVD injury had no effects on mechanical hypersensitivity thresholds, compared to sham counterparts. A between-subject effect of sex was seen on levels of three analytes in the PAG (2 Way-ANOVA effect of Sex: OEA $F(1,43)=17.8$, $p<0.001$, PEA $F(1,44)=10.6$, $p<0.01$, AEA $F(1,43)=8.6$, $p<0.001$). In the PFC, only AEA showed a between-group effect of sex ($F(1,43=14.9$, $p<0.001$). Post-hoc analysis did not reveal any pairwise group differences. Increased PEA and OEA levels in the PFC of male shams correlated with lower mechanical sensitivity thresholds from PSD 21-29 (PEA: $r=-0.739$, $p<0.01$, OEA $r=-0.641$, $p<0.05$,), which was not seen in IVDI males. Increased AEA levels in PFC of female sham rats were correlated with lower mechanical sensitivity from PSD 7 onwards ($r_s=-0.832$, $p<0.001$), which was not seen in IVDI females.

Conclusions: This study revealed sexually-dimorphic alterations in brain regional endocannabinoid and N-acylethanolamine levels, and their correlation with mechanical sensitivity thresholds in an IVDI-dependent manner.

Acknowledgements: Funding provided by the Irish Research Council Postgraduate Scholarships (GOIPG/2020/1496).

MODULATION OF CANNABINOID RECEPTOR 2 TO AMELIORATE NEUROINFLAMMATION AND ALTER FORMATION OF ALPHA-SYNUCLEIN AGGREGATES IN A RAT MODEL OF PARKINSON'S DISEASE

Valerie Joers[1], Sean Kelly[2], Fredric P Manfredsson[3], Bob M Moore II[4] and Malú G. Tansey[1]

[1]Department of Neuroscience and Center for Translational Research in Neurodegenerative Disease, University of Florida College of Medicine, Gainesville, FL
[2]Emory University, Atlanta, GA
[3]Barrow Neurological Institute, Phoenix, AZ
[4]University of Tennessee Health Sciences Center, Memphis, TN

Introduction: There is a growing appreciation that a dysregulated immune system plays a role in the development of neurodegenerative disorders such as Parkinson's disease (PD), suggesting that immunomodulatory therapies to restore immune function may mitigate or slow the progression of disease. Cannabinoid type 2 receptor (CB2) are located on immune cells and upregulated in activated microglia under conditions of disease, and evidence from other labs has demonstrated that pharmacologically targeting CB2 in toxic animal models of PD results in dampened inflammation and in some cases neuroprotection. Yet, it has not been investigated if stimulating CB2 will reduce alpha-synuclein (Asyn) burden. We hypothesize that targeting CB2 will promote clearance of Asyn by altering the central inflammatory environment.

Methods: To test this, we unilaterally injected AAV2/5-human-wild-type-Asyn into the substantia nigra of rats. One week later, animals were randomly selected to receive daily injections of a novel CB2 inverse agonist, SMM-189 (6 mg/kg, ip), or vehicle, and followed for 7 weeks. Blood was collected at baseline, 4 and 8 weeks after rAAV injections and analyzed for circulating levels of SMM-189 and peripheral blood mononuclear cells isolated and flow cytometry analyses performed. At the end of the study animals were perfused with ice-cold saline and brain collected for postmortem analyses.

Results: Our results demonstrate significant reductions in pser129Asyn in the striatum ($t(15)=3.71$, $p=0.0021$) and in the substantia nigral ($t(7)=5.172$, $p=0.0013$) in rats treated with SMM-189 compared to vehicle. IBA1 count and size did not differ between groups, however significant CD68-immunoreactivity was identified in the nigra of SMM-189-treated rats ($p=0.0129$). Evaluation of PBMCs at 8 weeks, indicate SMM-189 increases non-classical monocytes and reduces pro-inflammatory classical monocytes as determined by flow cytometry and shifts PBMC gene expression towards a wound healing phenotype as determined by qPCR. Similarly, anti-inflammatory markers, YM1 and CD206, were found increased in the cortex of rats treated with SMM-189 compared to vehicle.

Conclusions: These results suggest that SMM-189 is modulating immune phenotypes and function to reduce Asyn aggregation. Future analyses will evaluate the mechanisms of SMM-189 or similar CB2 compounds on organotypic brain slice cultures using a real-time phagocytosis assay and multiplexed immunoassays of the conditioned media.

Acknowledgments: Funding by Michael J Fox Foundation For Parkinson's Research

OLEOYL GLYCINE REDUCES NICOTINE SEEKING AND REINSTATEMENT IN RATS

Kimberly N. Karin[1], Erica N. Golden[1], Raphael Mechoulam[2], Linda Parker[3], M. Imad Damaj[1], Aron H. Lichtman[1] and Joel E. Schlosburg[1]

[1]Dept of Pharmacology and Toxicology,
Virginia Commonwealth University, Richmond, VA, USA
[2]Institute of Drug Research, Medical Faculty,
Hebrew University of Jerusalem, Jerusalem, Israel
[3]Dept of Psychology and Collaborative Neuroscience Program,
University of Guelph, Guelph,2ON, N1G 2W1, Canada

Introduction: A recent study highlighted an important role for a novel endogenous lipid in murine models of nicotine reward and withdrawal. The novel *n*-acyl amino acid, *N*-oleoyl glycine (OlGly) blocked the rewarding effects of nicotine in a conditioned place paradigm (CPP) through a peroxisome proliferator activated receptor-alpha (PPAR-α) dependent mechanism, as well reversed mecamylamine-precipitated nicotine withdrawal signs. While OlGly blocked nicotine-induced CPP, OlGly has yet to be examined in models of active nicotine seeking. In the present study we investigated the effect of OlGly on nicotine seeking in rat models of nicotine self-administration and cue/prime-induced reinstatement.

Methods: Male Wistar rats implanted with jugular catheters were trained to self-administer 0.03 mg/kg infusions of nicotine on a fixed-ratio (FR) 2 schedule during daily 2 hr sessions. OlGly (5, 10, 20 mg/kg) or vehicle (1:1:8 ethanol: emulphor: saline) was administered by intraperitoneal (ip) injection 15 mins prior to the sessions. Potential underlying mechanisms of the effects of OlGly in the self-administration studies were assessed by pretreatment with PPAR-α antagonist GW6471 (2 mg/kg), PPAR-γ antagonist GW9662 (2 mg/kg), and rimonabant (0.3 mg/kg). For reinstatement studies, lever responding behavior was extinguished by removing paired cues and nicotine infusions. A subcutaneous priming dose of 0.3 mg/kg nicotine in combination with cue presentation produced robust reinstatement. During reinstatement testing OlGly (5, 10, 15 mg/kg) was administered 10 mins prior to administration of the nicotine priming dose.

Results: OlGly (10 and 20 mg/kg) significantly reduced lever presses to 76% and 39% of vehicle pretreatment responding during the 2 hr test session nicotine self-administration test session. GW6471 (2 mg/kg) prevented the reduction in nicotine self-administration produced by OlGly, while neither GW9662 nor rimonabant blocked the reduction by OlGly. In addition to reducing nicotine self-administration, OlGly (10 and 15 mg/kg) significantly reduced cue/prime-induced reinstatement to 49% and 33% of vehicle responding respectively.

Conclusions: OlGly reduced nicotine seeking in both rat self-administration and cue/prime-induced reinstatement paradigms. The observation that GW6471 prevented OlGly-induced reduction in nicotine self-administration aligns with previous antagonism studies in the mouse model of nicotine-induced CPP. Collectively, these findings indicate a necessary role of PPAR-α in OlGly-induced reductions in nicotine seeking behavior. More generally, OlGly holds promise as a potential nicotine cessation pharmacotherapy.

Acknowledgements: Funded in part by National Institute of Health (P30DA033934 and T32DA007027) and PlantEXT Ltd.

PLASMA ENDOCANNABINOID LEVELS AS NOVEL BLOOD-BASED MARKERS FOR MILD COGNITIVE IMPAIRMENT AND DEMENTIA

Daman P. Kaur[1], Stephanie Bourke[2], Magda Bucholc[3], Stephen Todd[4], KongFatt Wong-Lin[3], Paula L. McClean[1]* and David P. Finn[2]*
*Joint lead/corresponding authors

[1] Northern Ireland Centre for Stratified Medicine, Biomedical Sciences Research Institute, Ulster University, Clinical Translational Research and Innovation Centre (C-TRIC), , Northern Ireland, UK.
[2] Pharmacology and Therapeutics, School of Medicine, Galway Neuroscience Centre, Human Biology Building, National University of Ireland Galway, Galway, Ireland
[3] ISRC, School of Computing, Engineering and Intelligent Systems, Ulster University, Northern Ireland, UK.
[4] Altnagelvin Area Hospital, Western Health and Social Care Trust, Northern Ireland, UK.

Introduction: Preclinical studies have reported neuroprotective effects of endocannabinoids (eCBs) against neuroinflammation, neurotoxicity, and in particular dementia related neuropathologies. In the present study we have investigated their role as novel blood-based markers for dementia and analysed how their levels correlate with age, body mass index (BMI), and Addenbrooke's Cognitive Examination-III (ACE-III) scores.

Methods: Plasma concentrations of the eCBs anandamide (AEA) and 2-Arachidonoylglycerol (2-AG), and their lipid congeners N-palmitoylethanolamide (PEA) and N-oleoylethanolamide (OEA) were assayed using liquid chromatography with tandem mass spectrometry. Samples were collected from healthy controls (n=120), mild cognitive impairment (MCI; n=6), Alzheimer's disease (AD; n=86), and other dementia (OD; n=11) patients. Data was analysed by Ordinary One-way ANOVA (parametric data) followed by Tukey's multiple comparisons test or Kruskal-Wallis test (nonparametric data) followed by Dunn's multiple comparisons test, and for correlation analysis Pearson parametric correlation method/Spearman nonparametric correlation analysis was employed. Statistical significance was set at $p<0.05$.

Results: Significantly lower concentrations of plasma AEA ($p<0.01$), PEA ($p<0.01$), and OEA ($p<0.01$) were observed in MCI vs. OD groups. Lower OEA levels ($p<0.01$) were observed in MCI patients compared with healthy controls. Additionally, plasma PEA levels were lower in AD patients compared with healthy controls ($p<0.05$) and OD ($p<0.05$) group. Plasma 2-AG levels positively correlated with age overall ($p<0.001$), and specifically in healthy ($p<0.01$) and AD individuals ($p<0.05$). Plasma 2-AG also correlated positively with BMI overall ($p<0.001$), and specifically in healthy controls ($p<0.05$) and MCI patients ($p<0.05$). Further, plasma 2-AG correlated negatively with ACE-III scores overall ($p<0.01$), and specifically in healthy controls ($p<0.01$). Plasma AEA ($p<0.05$) and OEA ($p<0.05$) levels strongly correlated positively with BMI only in the OD group.

Conclusions: This study shows that plasma eCB and *N*-acylethanolamine levels differ significantly between healthy participants and those with MCI or dementia. Additionally, eCBs and *N*-acylethanolamine vary with age, BMI, and cognitive decline (measured by ACE-III), and these correlations can further depend on specific diagnostic groups analysed. Overall, our work suggests that plasma eCB and *N*-acylethanolamine concentrations may represent novel blood-based markers for MCI and dementia.

Acknowledgements: A project supported by the European Union's INTERREG VA Programme, managed by the Special EU Programmes Body (SEUPB).

THERAPEUTIC POTENTIAL OF CANNABIDIOL FOR THE TREATMENT OF NEUROPATHIC PAIN AND ASSOCIATED COGNITIVE IMPAIRMENT

Marta Kędziora[1], Serena Boccella[2], Jakub Mlost[1], Sabatino Maione[2] and Katarzyna Starowicz[1]

[1]Department of Neurochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland, [2]Department of Experimental Medicine, Division of Pharmacology, University of Campania "L. Vanvitelli", Naples, Italy

Introduction: Neuropathic pain (NP) is a chronic disorder that develops following nerve damage and lasts for months or years. In addition to sensory symptoms (allodynia, hyperalgesia), cognitive functioning is affected in NP patients. Because neural systems involved in cognition and pain processing are linked, they may modulate one another reciprocally. Cannabidiol (CBD) has been shown to possess considerable therapeutic potential for treating a wide range of disorders including chronic pain, psychosis and anxiety. CBD lacks psychostimulant and addictive properties, thus representing an interesting pharmacological compound to be further investigated. The aim of the research was to investigate the co-occurrence of pain and hippocampal deactivation due to NP and to identify the most accurate targets for its treatment.

Methods: Spared Nerve Injury (SNI) surgery was performed on male Wistar rats according to the method of Decosterd and Woolf. The sham procedure consisted of the same surgery without ligation and transection of the nerves. Tactile allodynia was measured with Von Frey's filaments (ascending method), anxiety-like behavior was measured with novel object recognition (NORT) and open field test 7 days post-SNI. Field excitatory post-synaptic potential (fEPSPs) responses were recorded after electrical stimulation in the perforant pathway, in contralateral dentate gyrus in hippocampus of sham and SNI animals. Finally, a potential therapeutic targets of CBD for NP and memory impairment were identified using an Open Targets Platform.

Results: After SNI induction, the mechanical withdrawal threshold decreased significantly, in contrast to sham-operated animals. Moreover, SNI rats showed anxiety-like behavior when tested in the NORT and open field test. LTP measured by both fEPSP slope and amplitude was abolished in SNI animals. An Open Targets Platform search identified 742 molecular targets associated with memory impairment and 361 associated with NP (147 targets were associated with both conditions). CBD has been reported to interact with 66 targets in the literature; however, many of them were studied in concentrations beyond physiologically relevant levels. Therefore, 30 CBD targets with Ki or EC/IC50 values < 2 μM were selected. 7 of them were associated with NP and memory impairment. The functional enrichment analysis of biological processes revealed those proteins to be involved in thermoception, fear response and stress.

Conclusions: NP is associated with anxiety-like behavior and LTP abolition in rats, suggesting a synaptic change in this condition. Novel approach to find molecular targets associated with memory impairment, NP and CBD will allow for further investigation of its role in SNI-induced NP in order to identify targets for pain and comorbid memory impairments treatment.

Acknowledgements: Funded by the Government of Quebec, collaboration with McGill University, Montrèal, Quèbec: *Pharmacological properties of CBD in neuropathic pain and associated anxiety-depressive symptoms: preclinical and clinical studies* and IP PAS statutory funds.

BRAIN REGION-SPECIFIC MOLECULAR AND BEHAVIOURAL EFFECTS OF CP55,940 THROUGHOUT THE MOUSE ESTRUS CYCLE

Hye Ji J. Kim[1], Ayat Zagzoog[1], Tallan Black[1], Sarah L. Baccetto[1], Udoka C. Ezeaka[1] and Robert B. Laprairie[1,2]

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, CA
[2]Department of Pharmacology, College of Medicine, Dalhousie University, Halifax, NS, CA

Introduction: Knowledge of sex differences in cannabinoid pharmacology have increasingly standardized equal representation of male and female animals in preclinical research. However, the fluctuations of sex hormones that occurs within female subjects is often overlooked. The mouse estrus cycle spans 4–5 days and consists of 4 phases: proestrus, estrus, metestrus, and diestrus. Relative to the latter cycle phases, proestrus and estrus are marked by increased blood and brain concentrations of 17-β estradiol (E2), which is responsible for the interactions between the endocannabinoid and estrogen neuromodulatory systems. In past behavioural studies, females in proestrus were found to be the most sensitive to the antinociceptive effects of the cannabinoid receptor type 1 (CB1R) partial agonist Δ^9-tetrahydrocannabinol. Separate molecular and electrophysiological investigations have determined that heterodimerization between estrogen and metabotropic glutamatergic receptors underlies these estrus cycle-dependent cannabinoid effects. In the current project, we intraperitoneally (i.p.) administered a high dose of the full cannabinoid receptor agonist CP55,940 at different points of the estrus cycle in order to provide a clearer picture of the relationships between the cannabinoid receptors, exogenous and endogenous cannabinoid ligands, as well as their degradation enzymes in key brain regions.

Methods: The estrus cycle phase of sexually mature or normally cycling female C57BL/6 mice aged 6–12 weeks was determined using both vaginal epithelium cytology and an enzyme-linked immunosorbent assay for plasma E2 content. Mice in either proestrus or estrus were randomly assigned to receive 1 mg/kg i.p. of CP55,940 or 200 µL i.p. of a vehicle solution. Ten min post-injections, all mice underwent the tetrad battery of *in vivo* assays: bar holding assay, internal body temperature, tail flick assay, and the open field test. Forty-five min following treatments, brain tissue from the hypothalamus, periaqueductal gray, and spine was collected for western blot analysis of CB1R and cannabinoid receptor type 2 (CB2R).

Results: Mice in proestrus were more sensitive to the antinociceptive effects of 1 mg/kg i.p. of CP55,940 as compared to metestrus mice. A similar trend was observed with regards to catalepsy; however, this difference was not statistically significant. The spine was the only region where cannabinoid-treated proestrus mice exhibited larger CB1R density or upregulation. No clear estrus cycle phase-, treatment-, nor brain region-dependent changes were detected with respect to CB2R.

Conclusions: E2 levels during proestrus enabled greater CP55,940-induced antinociception. This corresponded to CB1R upregulation that was most pronounced in the spine, a region involved in ascending and descending pain processes. We will next quantify the endocannabinoid degrading enzymes, fatty acid amide hydrolase and monoacylglycerol lipase, in different brain regions to gain perspective on endocannabinoid tone in these experimental contexts.

Acknowledgements: This work is funded by the National Sciences and Engineering Research Council (Grant no. GECR-2019-00207). AZ, TB, SLB, and ECE are supported by the University of Saskatchewan College of Pharmacy and Nutrition Graduate Scholarship.

THC PROLONGS SYNCHRONOUS BRAIN ACTIVITY

Jace B. King, Molly B. D. Prigge, Lubdha M. Shah and Jeffrey S. Anderson

Department of Radiology and Imaging Sciences,
University of Utah, Salt Lake City, UT, USA

Introduction: Interest in the use of cannabidiol (CBD) and other cannabis derivatives has increased over the last several years due to reported health benefits. Despite a growing body of literature about efficacy for medical indications, critical questions about the mechanism of action remain unanswered. For example, how cannabinoids such as Δ-9-tetrahydrocannabinol (THC) and CBD influence large-scale brain network activity is poorly understood. Traditional functional connectivity measures synchrony of intrinsic activity between brain regions and identifies resting-state functional networks. Traditional connectivity has been shown to be altered by cannabis use (with and without CBD, Wall et al. 2019); however, effect sizes are small compared to the perceived effects of THC and the temporal dynamics of that synchrony is unknown. Sustained connectivity uses the width of cross correlation curves to reflect the relative duration of synchronous activity between brain regions and may inform how the timing of brain connections is affected. Previous studies have shown a relationship between sustained connectivity and processing speed and other cognitive domains (King et al. 2018, 2019). This study examines how THC and CBD affect traditional and sustained connectivity in healthy adults.

Methods: 39 healthy adults (48.7% female, mean age 29.28±7.13) were given 10mg THC (Syndros), 600mg CBD (Epidiolex), both THC and CBD, or placebo across four study visits. At each visit, participants completed MP2RAGE (1x1x1mm, TR=5000ms, TE=2.93ms) structural scan and two 15-minute multiband resting-state fMRI scans (2x2x2mm, TR=737ms, TE=33.2ms) in a 3T Siemens Vida scanner. Image preprocessing and analyses were completed using SPM12 and MATLAB software. Preprocessing included coregistration to MP2RAGE, segmentation, motion correction, normalization to MNI space, and regression of motion parameters. A 4-way ANOVA examined the effects of THC and CBD controlling for age, sex, and head motion. A 17x17 network parcellation (Yeo, 2011) was used to evaluate traditional functional and sustained connectivity across the brain with each network treated as a single ROI. Results were corrected for multiple comparisons (FDR).

Results: When under the effects of THC, participants demonstrated increased sustained connectivity in 91 out of 153 network connections whereas traditional functional connectivity found FDR corrected findings in only 4 out of 136. No FDR corrected findings were found in either traditional or sustained connectivity in the CBD condition.

Conclusions: These findings suggest that the mechanism of action of THC on brain network function may be through alteration of the timing and stability of brain connectivity. Such a mechanism is consistent with slower cognitive and sensorimotor response times. Investigation of how THC alters the timing of synchronous brain connections may facilitate a unified explanation of perceptual and cognitive alterations of brain network function induced by THC, with greater brain network stability and impaired set shifting and traversal of distinct cognitive states.

Acknowledgements: Funded by the Wholistic Research and Education Foundation.

ENDOCANNABINOID SIGNALING REGULATES MOSSY FIBER AXON GROWTH

Alex Kuklish, Ali Sualeh, Ken Mackie and Anna Kalinovsky

The Gill Center for Biomolecular Science, Program in Neuroscience, Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA

Introduction: Cannabis use during pregnancy and lactation has been rising following its recent legalization in many states. Yet, plant-derived cannabinoids (pCBs), such as Δ9-tetrahydrocannabinol (THC), cross the placental and blood-brain barriers and are secreted in breast milk, and thus can interfere with neurodevelopment. The major barrier to evaluating clinical risks to the developing brain from exposure to pCBs is the limited understanding of the cellular and molecular cascades affected by such exposure. Thus, it is imperative to elucidate developmental mechanisms regulated by cannabinoid signaling in multiple cell types, developmental periods, and brain regions. In this work, we show that endogenous cannabinoid signaling system (ECS) is robustly expressed in the perinatal mouse cerebellum and regulates axon growth of cerebellar mossy fibers (MFs).

Methods: We assessed ECS expression in the developing cerebellum by immunohistochemistry and confocal microscopy. We characterized morphology of white matter tracts and sensorimotor behavior in mouse pups following perinatal THC exposure (0.3-10 mg/kg/day). Proteomics and phosphoproteomics analysis were performed to evaluate effects of THC exposure on effector cascades regulating axon growth. We utilized *in vitro* explant culture to investigate growth responses of isolated MF axons in the presence of ECS agonists and antagonists.

Results: We show that perinatally, cannabinoid receptor 1 (CB1) is robustly expressed in the developing MF axons, while diacylglycerol lipase α (DAGLα) expression is prominent in the Purkinje cell (PC) layer, the target region where elongating MFs stall and begin elaborating transient synaptic contacts. At lower levels, DAGLα is also expressed in MF neurons, suggesting that autocrine, as well as paracrine ECS signaling may regulate MF axon growth. Perinatal exposure to THC leads to alterations in the shapes of cerebellar white matter tracts, to shifts in expression and activation the effectors of cytoskeletal dynamics, and to behavioral alterations in mouse pups. *In vitro* experiments show that MF axon growth is directly affected by ESC activation.

Conclusions: Cannabinoid signaling regulates growth and development of cerebellar mossy fibers.

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

INVESTIGATING THE ROLE OF THE ENDOCANNABINOID SYSTEM IN INTESTINAL INFLAMMATION

Robert Leddy, Mariam Alketbi, Carol Aherne and Colm Collins

The Conway Institute, University College of Dublin, Belfield, Dublin 4

Introduction: Inflammatory Bowel Disease (IBD) is an umbrella term for relapsing and remitting disorders, including Crohn's Disease and Ulcerative Colitis, resulting in chronic inflammation of the gastrointestinal tract. Characterised by the recruitment and infiltration of immune cells into the gut, it is widely accepted that T cells and T cell gut trafficking are pivotal in disease pathogenesis. Central to T cell gut trafficking is α4β7, a heterodimeric surface integrin expressed on circulating immune cells mediating trafficking through interactions with its gut-specific ligand mucosal addressin cell adhesion molecule-1. Preliminary work is suggestive of a relationship between the endocannabinoid system and the development of α4β7-expressing, pathogenic T cells. The endocannabinoid system is a lipid-based signalling system with multiple regulatory functions, including those important in gut homeostasis such as gut motility, gastric secretions and intestinal epithelial barrier integrity. Due to its almost exclusive expression on immune cells and upregulation in both human and animal IBD models, cannabinoid receptor 2 (CB_2R) is central to these studies, where the role of CB_2R signalling on the transcriptional regulation of α4β7 on T cells was assessed.

Methods: Jurkat T cells were cultured for 6, 24 and 48hrs in the presence of 1μM retinoic acid (mimicking the requirements for endogenous α4β7 induction on T cells), the CB_2R agonist JWH-133 (1μM) and the inverse agonist GP-1a (10μM). Integrin β7 mRNA and promoter activity were assessed using both RT-PCR and luciferase reporter assays, respectively. Data was analysed by unpaired T tests ($P < 0.05$ considered significant)

Results: At 6hrs, there were no significant alterations in β7 expression at either a message or promoter level. A significant increase in integrin expression was observed at 24hrs following treatment with 1μM retinoic acid, which was expected given the role of retinoic acid in the endogenous induction of α4β7 during T cell activation. CB_2R activation with 1μM JWH-133 in combination with retinoic acid appeared to have an additive effect, further increasing β7 expression significantly across both assays after 24hrs. Increasing the treatment time to 48hrs saw identical results, with the effect of JWH-133 appearing to follow a dose-related trend. The additive effect of CB_2R activation on β7 expression was significantly inhibited at both 24 and 48hrs following receptor blockade with 10μM GP-1a, significantly decreasing β7 expression and further suggesting a role for CB_2R signalling in the transcriptional regulation of α4β7 on T cells.

Conclusions: Based on this data which clearly shows CB_2R manipulation to have a significant role in the transcriptional regulation of α4β7 expression, we propose that pharmacological CB_2R blockade may be able to attenuate IBD by suppressing intestinal homing of pathogenic human T cells through the significant downregulation of α4β7 expression, potentially preventing subsequent intestinal inflammation.

Acknowledgements: Supervised by Dr. Colm Collins. Funded by the Crohn's and Colitis Foundation.

ENDOCANNABINOIDS 2-AG AND ANANDAMIDE INDICATE CLINICAL SYMPTOMS AND DO NOT PREDICT TREATMENT OUTCOME IN VETERANS WITH PTSD

Nadia A. Leen, Antoin D. de Weijer, Sanne JH. van Rooij, Mitzy Kennis, Johanna MP. Baas and Elbert Geuze

Brain Research and Innovation Center, Ministry of Defence, Utrecht, The Netherlands

Introduction: Although current treatments for Post-Traumatic Stress Disorder (PTSD) in war veterans are effective, unfortunately 30-50% still do not benefit from these treatments. PTSD treatments, e.g. exposure therapy, are primarily based on extinction processes in which the endocannabinoid system (ECS) plays a significant role. Therefore, it is conceivable that poor treatment response on trauma-focused therapy due to extinction deficits may be associated with a poorly functioning ECS. The present study examined whether levels of the endocannabinoids anandamide (AEA) and 2-arachidonylglycerol (2-AG) are associated with post-treatment symptom reduction

Methods: Blood plasma levels of AEA and 2-AG were determined in war veterans with a PTSD diagnosis (n=54) and combat controls (n=26) before and after a 6-8 months interval. During this period veterans with PTSD received trauma-focused therapy (e.g. cognitive behavioral therapy with exposure or eye-movement desensitization and reprocessing). Clinical symptoms were assessed before and after therapy with the Clinician Administered PTSD Scale (CAPS), State-Trait Anxiety Inventory (STAI) and Mood and Anxiety Symptom Questionnaire (MASQ).

Results: Regression analysis demonstrated that pretreatment endocannabinoid levels were not predictive of PTSD symptom reduction. Additionally, baseline endocannabinoid levels did not differ between either PTSD and combat controls or between combat controls, treatment responders, and non-responders. However, endocannabinoid levels were significantly lower in individuals who reported cannabis use during their life, independent of PTSD diagnosis. Furthermore, correlational analysis revealed that pretreatment 2-AG levels in PTSD are positively correlated with anxious arousal and negatively with avoidance symptoms. Both posttreatment AEA and 2-AG were positively correlated with trait anxiety, anxious arousal and general distress depression symptoms.

Conclusions: Since endocannabinoids are mainly generated 'on demand', future work could benefit by investigating endocannabinoid circulation under both baseline and stressful conditions. In line with previous research cannabis use was associated with lower endocannabinoid levels. The correlational analysis between pre- and posttreatment endocannabinoid levels and pre- and posttreatment clinical symptomatology were exploratory analysis and should be replicated in future research.

Acknowledgement: This research was financially supported by the Dutch Ministry of Defence.

PRE-CLINICAL EVALUATION OF CANNABIDIOL TOXICITY IN RATS

Timothy W. Lefever[1], Kristen R. Trexler[1],
Rayetta G. Henderson[2] and Marcel O. Bonn-Miller[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario, Canada.

[2]ToxStrategies, Wilmington, North Carolina, USA

Introduction: Despite the myriad cannabidiol (CBD) products available, full toxicological evaluation on the isolated molecule has not been carried out or published publicly. Here we present, to our knowledge, the first complete pre-clinical data set on the toxicological profile of CBD using best practices and regulated methodologies, e.g., United States Food and Drug Administration (FDA) and Organisation for Economic Co-operation and Development (OECD). Special emphasis was placed on examination of gaps in the CBD toxicological profile noted by the FDA in communications and previous filings; reproductive toxicology and impact of metabolite 7-COOH-CBD. Purified, hemp-derived CBD isolate was evaluated across three studies in male and female rats: 14- and 90-day repeat dose oral toxicity, genotoxicity, and reproductive toxicity.

Methods: 14- and 90-day repeated oral dose study: Rats received up to 150mg/kg/day of CBD orally. Upon terminal sacrifice (14- and 90-day studies) or following a 28-day recovery (90-day recovery group), tissues were collected for analysis (OECD Guidelines 407 & 408). **Genotoxicity:** a) *in vitro* microbial reverse mutation assay up to 5,000 mg/plate in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2 *uvrA* (OECD Guideline 471), b) *in vitro* micronucleus assay in human TK6 cells up to 10–11 mg/mL with and without metabolic activation (OECD Guideline 487), and c) *in vivo* micronucleus assay in rats up to 1,000 mg/kg/day (OECD Guideline 474). **Reproductive Toxicity:** Rats were orally dosed with CBD up to 300mg/kg/day beginning 14 days prior to mating and continuing through lactation day 20 for females. Offspring were dosed from postnatal day 21 through 42 (OECD Guideline 421 with extended postnatal dosing and additional hormone analysis). All studies, except for the 14-day oral toxicity study (dose range finder), were Good Laboratory Practice (GLP) compliant.

Results and Discussion: 14- and 90-day repeated oral dose study: All doses were tolerated, and no treatment-related adverse effects were noted. Microscopic changes in liver and adrenal glands in the 90-day study resolved following the 28-day recovery period. **Genotoxicity:** All assays were negative; CBD was nonmutagenic, nonclastogenic, and nongenotoxic under the study conditions. Bioanalysis confirmed a dose-dependent increase in plasma CBD and metabolite 7-COOH-CBD in the *in vivo* micronucleus assay. **Reproductive Toxicity:** Dose-dependent effects were observed and will be discussed at length. This comprehensive examination of CBD toxicology provides new, guideline-compliant data for consideration and supports many effects others have reported previously.

DELTA-8-TETRAHYDROCANNABIVARIN (THCv), CANNABICHROMENE (CBC), AND CANNABINOL (CBN) SHOW NO TOXICITY FOLLOWING 14 DAY ORAL EXPOSURE

Kristen R. Trexler[1], Daniela Schwotzer[2], Marcel O. Bonn-Miller[1], Jacob McDonald[2] and Timothy W. Lefever[1]

[1]Canopy Growth Corporation, Smiths Falls, Ontario, Canada.

[2]Lovelace Biomedical, Albuquerque, New Mexico, USA

Introduction: As the market for recreational and medicinal cannabis products expands, a greater variety of product formats and cannabinoids have become available. Some products now showcase so called 'minor' cannabinoid ingredients that in previous years had not been widely available. While the increased interest in minor cannabinoids may present opportunities for new therapeutic applications, little is known about the safety and toxicology of many of these compounds. The present study evaluated the toxicology profiles of 3 minor cannabinoids, Delta-8-Tetrahydrocannabivarin (THCv), Cannabichromene (CBC), and Cannabinol (CBN), when administered orally over 14 days.

Methods: Male and Female Sprague Dawley rats received THCv, CBC, CBN, or medium-chain triglyceride (MCT) oil vehicle via oral gavage daily for 14 days. Each compound was administered at 6 dose levels (3.2, 10, 17, 22, 32, or 100 mg/kg). Rats were monitored daily for clinical signs and body weights, and every 3 days for food consumption. Body temperature and analgesia measures were taken on day 1 prior to and following dosing, and on day 14. Blood was collected 45 min, 90 min, 180 min and 24 h following dosing on days 1 and 14. On Day 15, rats were euthanized for gross necropsy, and blood and organ tissue were collected for clinical pathology and histopathology, respectively.

Results: No clinical signs of distress or drastic changes to body weights or food intake were observed. Similarly, no changes in body temperature or latency to tail flick were observed in any groups. Rats treated with THCv 100mg/kg showed decreased circulating compound at all time points on day 14 relative to day 1. No histopathological changes to any collected tissues were observed for any dose group.

Conclusions: Rats treated with THCv, CBC, or CBN, showed no signs of toxicity following 14 days of oral administration at a range of doses. Decreased circulating THCv (100 mg/kg) on day 14 may be indicative of the development of tolerance or of some other compensatory mechanism, which should be investigated further in future studies. While further research is needed, the present study suggests that the use of oral THCv, CBC, and CBN in MCT may present low risk of toxicological outcomes.

DESIGN OF A CLINICAL TRIAL PROTOCOL TO STUDY DAILY ORAL CANNABIS FOR CHRONIC SPINE PAIN

Alan W. J. Morris[1], Michelle Adkins[2], Jacci Bainbridge[2],
Rachael Rzasa Lynn[3*] and Emily M. Lindley[1*]

University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
1. Department of Orthopedics 2. Department of Clinical Pharmacy;
3. Department of Anesthesiology;' * Co-Senior Authors

Introduction: Back and neck pain are highly prevalent and disabling musculoskeletal conditions. Although commonly prescribed, opioids are often ineffective and can result in dependency and lethal overdose. An alternative analgesic treatment to opioids that has gained increasing recognition is cannabis. In contrast to opioids, cannabis overdoses are not fatal. Cannabinoids produce analgesic effects through both central and peripheral mechanisms via the endocannabinoid system. Although clinical data on the treatment efficacy of cannabis for chronic pain is limited, largely due to complex regulations associated with studying Schedule I drugs, research in this area is quickly accelerating. The primary aim of this trial is to compare the effects of two doses of daily oral cannabis treatment versus placebo for chronic spine pain management. Given the preclinical evidence for CBD's potential anti-inflammatory and analgesic properties, as well as it's potential to mitigate the psychoactive effects of THC, this study designed to directly compare the analgesic efficacy of THC alone to THC plus CBD in an orally delivered plant extract formulation. This abstract provides an introduction to the clinical trial protocol. It is important for cannabis researchers to be aware of other studies that are being conducted to avoid redundancy and expand on the landscape of cannabinoid research.

Methods: This within-subjects, crossover study will recruit individuals with diagnosed chronic thoracic, lumbar or cervical pain. Individuals who meet the overall inclusion and/or exclusion criteria and are taking low dose or no opioids (<45 morphine milligram equivalents daily) will be eligible. After informed consent is obtained, participants will complete three separate treatment periods of six weeks each, separated by a two-week washout period. The study treatments consist of two cannabis arms (5mg/50mg ratio THC:CBD and 5mg/0mg ratio THC:CBD) and a placebo cannabis arm. Whole plant extracts will be obtained from the NIDA source and compounded into the two oral solutions by study pharmacists. Participants will attend weekly study visits that include NRS pain scores, neurocognitive assessments, mental health questionnaires, vitals, standardized field sobriety testing, subjective ratings of drug effects, and pain thresholds measured with a computer-controlled pressure algometer. A total of 63 participants will be enrolled into this trial, with a target of 50 study completions.

Discussion: Despite the frequent use of cannabis in our chronic spine pain patient population, without high quality scientific studies, physicians cannot make clear, evidence-based recommendations to their patients regarding the clinical efficacy of cannabis. To our knowledge, this is the first randomized controlled trial designed to evaluate the analgesic effect of daily administration of a whole-plant extract oral solution for the alleviation of chronic spine pain. These findings will be the first to provide critical clinical information regarding the potential for extended use of cannabinoids to alleviate chronic spine pain and provide an alternative to opioids for pain relief.

Acknowledgements: Funded by the Colorado Department of Public Health and Environment and the University of Colorado Clinical & Translational Research Center. The CU Clinical & Translational Research Center is supported by NIH/NCATS Colorado CTSA Grant Number UL1 TR002535.

EFFECTS OF CANNABIDIOL WITH AND WITHOUT OTHER CANNABINOIDS AND TERPENES ON SHORT-TERM AND LONG-TERM STRESS-RELATED BEHAVIORS

Nicole Lookfong[1], Mariam Melkumyan[1], Wesley Raup-Konsavage[2], Kent Vrana[2] and Yuval Silberman[1]

[1]Department of Neural and Behavioral Sciences;
[2]Department of Pharmacology; Penn State College of Medicine, Hershey, PA, USA

Introduction: Stress-related disorders, such as anxiety and post-traumatic stress disorder, are a primary reason for treatment (and self-medication) with medical cannabis products. Research suggests endocannabinoids regulate neurotransmitters involved in stress but whether phytocannabinoids such as cannabidiol (CBD) reduce stress behaviors is not fully established. To that end, we sought to examine how CBD with and without other cannabinoids or terpenes might alter behavior in mouse models of short-term and long-term responses to acute stressors.

Methods: For short-term stress responses, adult male C57Bl/6J mice received a 30-60 minute restraint stress followed by testing with open field and light-dark box tests. Mice were treated with vehicle, CBD (10mg/kg), or CBD with low dose delta-9-tetrahydrocanabinol (THC, 2.5mg/kg and 7.5 mg/kg CBD for 10mg/kg total cannabinoid content) 45-60 minutes prior to stress exposure. For long-term stress behavior, mice underwent conditioned place avoidance to restraint plus predator odor contexts, with controls receiving individual stressors or no stress. Avoidance to the stress paired context was examined 1, 7, and 28 days later. Groups received vehicle, CBD (3.07 mg/ml CBD, 3mg/kg cannabigerol, low terpenes), CBD+THC$^{lo\text{-}terp}$ (3.07 mg/ml CBD, 3mg/kg cannabigerol, 0.76 mg/ml THC, low terpenes), or CBD+THC$^{hi\text{-}terp}$ (3.29 mg/ml CBD, 3mg/kg cannabigerol, 0.76 mg/ml THC, high terpenes) 30-45 minutes after stress exposure. Researchers were blinded to treatment conditions during all analyses.

Results: In the short-term experiments, mice treated with CBD trended towards an increase in the time spent and decreased latency to enter the light side of the light-dark box compared to vehicle, suggesting reduced anxiety-like behaviors. Additionally, CBD treated mice showed reductions in freezing, immobility time, and latency to enter the center of the open field compared to vehicle treated mice, with no differences in the time spent in the center of the field. CBD+THC treatment showed no significant differences compared to vehicle. In the long-term experiments, mice exposed to restraint plus predator odor showed reduced time spent in the stress paired chamber on days 1, 7, and 28 post-stress, although there did appear to be stress susceptible and resilient mice in this paradigm. Avoidance behaviors were not seen when stressors were presented individually or if no stress was used. In this paradigm, CBD+THC$^{lo\text{-}terp}$ was the only treatment to reduce avoidance behavior at the post-stress time points tested.

Conclusions: These results suggest that CBD has a differential effect on anxiety-like behaviors based on type of stress, post-stress timing of behavioral testing, and CBD/THC/cannabigerol/terpene content. Further studies are needed to uncover the effect of phytocannabinoids on short-term and long-term stress responses as well as related neurotransmitters and circuitries driving these effects.

Acknowledgements: Funding by PA Options for Wellness Graduate Student Award. Funding source had no involvement in writing or analysis of work presented

THC AND CBD IN INSOMNIA ASSOCIATED TO NEUROPATHIC PAIN: EFFECT ON SLEEP ARCHITECTURE AND FIRING ACTIVITY OF RVM NEURONS

Martha López-Canul, Anahita Oveisi, Antonio Farina, Alexandra Teggin, Justine Enns, Maria-Luisa Vigano, Luca Posa, Danilo De Gregorio, Elena He, Shelly Yin and Gabriella Gobbi

McGill University, Department of Psychiatry and Alan Edwards Centre for Research on Pain, Montréal, QC, Canada.

Introduction: Neuropathic pain (NP) is a major health problem that results in a high degree of suffering, physical and psychosocial impairments, and exorbitant health care costs. Only a restricted number of pharmacological interventions are available for treating NP associated insomnia, which has common side effects. Preclinical and clinical studies indicate that delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) possess both analgesic and hypnotic effects. In this study we examined the effects of THC and CBD in the sleep-wake cycle (EEG/EMG) in NP condition.

Method: We induced NP (spared nerve injury) in Wistar rats. 14 days later, those that developed neuropathy were treated with THC's vehicle (VEH; 5%Tween 80, 5% PEG and saline, i.p.), CBD's vehicle (VEH, 15% Ethanol, 5% Tween 80 and 80% Saline, i.p.), THC (1, 1.5, 2, 2.5 and 5 mg/kg, i.p.) or CBD (5, 10 and 20 mg/kg, i.p.) to assess mechanical allodynia. Next, NP rats 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) with a single injection of THC (5 mg/kg, i.p.) or CBD (20 mg/kg, i.p.). To assess the effect of THC (10 µg, intra-PAG microinjection) and CBD (1 µg, intra PAG microinjection) in the descending pathway of anti-nociception, in vivo single-unit electrophysiological recordings of ON and OFF cells in the RVM were performed.

Results: THC (2.5 and 5 mg/kg, i.p.) decreased mechanical allodynia in rats with NP vs VEH ($p<0.001$) 1h after administration in a dose dependent-manner, reaching an antiallodynic plateau of 6 gr. This effect lasted about 3.5 hours. THC (5 mg/kg, i.p.) was able to increase NREM ($p < 0.01$) and REM sleep ($p < 0.001$) in NP rats. On the other hand, CBD (10 and 20 mg/kg, i.p.) decreased mechanical allodynia in rats with NP vs VEH ($p<0.001$) 1h after administration, reaching an antiallodynic peak of 7 gr 3.5 h post-injection. This effect lasted about 4 hours. CBD (20 mg/kg, i.p.) was able to increase the NREM ($p < 0.01$) and REM ($p < 0.001$) sleep when compared to NP rats treated with vehicle. Microinjection of THC (10 µg) into the ventrolateral periaqueductal gray decreased the firing activity of ON cells and activated the firing of OFF cells in the rostroventral medulla. Unlike THC, CBD reduced the ongoing activity of both ON and OFF neurons in anaesthetized NP rats.

Conclusion: The findings suggest that THC and CBD has a moderate acute analgesic effect and good hypnotic effect in a NP paradigm, similar to clinical outcomes reported in humans. Therefore, THC and CBD have potential in the treatment of neuropathic pain and comorbid insomnia.

Acknowledgments: Funded by the Ministère de l'Économie, Science et Innovation (MESI) du Québec, (Grant. no. PSR-SIIRI-988), PBEEE (Postdoctoral-scholarship), Ferring postdoctoral fellowship in reproductive health, Faculty of Medicine, McGill University.

ASSESSING SUSTAINED TETRAD RESPONSIVENESS IN FEMALE RATS FOLLOWING CHRONIC CANNABIS OR CANNABINOID EXPOSURE DURING PREGNANCY

Sarah Lunardi Baccetto[1], Tallan Black[1], Ilne L. Barnard[2], Dan L. McElroy[2], John G. Howland[2] and Robert B. Laprairie[1]

[1]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada
[2]Department of Anatomy, Physiology and Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Introduction: The legalization of cannabis and cannabis derived products in Canada has led to an increase in its recreational use, despite research suggesting chronic use can lead to dependency, tolerance and withdrawal symptoms. The main psychoactive component in cannabis, Δ^9-tetrahydrocannabinol (THC), binds to cannabinoid type 1 receptors (CB1R) and mediates a variety of functions in the central nervous system. At the level of protein, chronic exposure to THC and other cannabinoids has been shown to cause downregulation of CB1R, which is reversible upon abstinence. In the brain, CB1R is widely distributed and its activation induces four characteristic behaviors: catalepsy, hypothermia, analgesia and hypolocomotion. The tetrad behavioral assay is a model used to evaluate the activation of CB1R in rodents. In this project, pregnant rats were chronically exposed to cannabis smoke or injected cannabinoids for 15 consecutive days. Then, following an abstinence period of 28 days, we assessed the behavioral and molecular consequences of acute THC administration.

Methods: Pregnant Sprague-Dawley rats were treated daily with THC 3 mg/kg i.p. (n=5), cannabidiol (CBD) 10 mg/kg i.p. (n=1), vehicle (1:1:18 Ethanol:Kolliphor:Saline) (n=3), high THC cannabis smoke (Skywalker Kush, 17.38% THC, Aphria) (n=6), high CBD cannabis smoke (Treasure Island Kush, 12.98% CBD, Aphria) (n=4) or air control (n=4) during gestational days 6 to 20. After a 28-day washout period, rats were treated once with THC 10 mg/kg i.p. Ten minutes after injections, all animals underwent the tetrad assay, consisting of the bar holding assay, internal body temperature, tail flick assay and open field test.

Results: No significant differences in catalepsy and internal body temperature were observed between treatment groups and controls. In the tail flick assay, rats exposed to Skywalker smoke showed a significant decrease in tail withdrawal latency compared to the control group. In the open field test, there was no significant difference in the total distance traveled or mean velocity. There was a decrease in the time spent in the center zone in both THC injection and Skywalker smoke exposure groups compared to controls.

Conclusion: These data provide evidence for sustained changes in CB1R-dependent nociceptive and behavioral responses due to chronic exposure to injected THC or high-THC cannabis smoke even after a 28-day abstinence period. We will next quantify CB1R in different brain regions to gain more insight in the molecular changes caused by chronic THC exposure.

Acknowledgements: This work is supported by a GlaxoSmithKline-Canadian Institutes of Health Research (CIHR) partnership grant to RBL, Brain Canada Future Leaders Award to RBL and bridge funding from the University of Saskatchewan College of Medicine to JGH. SLB is supported by Mitacs Globalink Graduate Fellowship Award.

THE ROLE OF ANANDAMIDE IN A HYPOGLUTAMATERGIC MODEL OF PSYCHOSIS-LIKE BEHAVIOURS

Claudia Lutelmowski[1], Catharine Mielnik[1], Marija Milenkovic[1], Wendy Horsfall[1], Amy J. Ramsey[1], Heather B. Bradshaw[2] and Ruth Ross[1]

[1]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, CA.
[2]Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA.

Introduction 1: The dysregulation of the endocannabinoid system (ECS) in the pathophysiology of schizophrenia is supported by multiple lines of evidence. For example, in psychosis-related disease states, higher endocannabinoid (EC) anandamide (AEA) levels are inversely correlated with symptom severity [1]. While our understanding of AEA in behaviours of psychosis is incomplete, agents that elevate AEA tone by metabolism inhibition are entering clinical trials for various indications. In this study, we address this gap by evaluating AEA's role in behaviours of psychosis, and the mechanism by which AEA exerts its effect. Here, we continue to investigate how elevations in AEA tone affect the psychosis-like phenotype of the GluN1 knockdown (GluN1KD) mouse, a genetic model of N-methyl-D-aspartate (NMDA-R) hypofunction [2].

Methods: Adult, male and female, GluN1KD and littermate wild-type mice received both acute (120 min) and chronic (over 14 days) treatment of a vehicle or PF-3845 (10 mg/kg i.p.) an inhibitor of free fatty acid amide hydrolase to increase AEA tone. Cannabimimetic behaviours (catalepsy, temperature, and locomotion), pre-pulse inhibition of the acoustic startle response (PPI), the elevated plus maze (EPM) and the light dark box test (LDB) were measured. GluN1KD and wildtype EC levels were assessed using high-performance liquid chromatography-mass spectrometry (HPCL-MS) both at baseline and following an acute dose of PF-3845 (10 mg/kg i.p.).

Results: Acute PF-3845 (10 mg/kg), was shown to ameliorate the hyper-locomotive phenotype of the GluN1KD model. Meanwhile, the effects and magnitude of changes seen in locomotion with chronic PF-3845 (10 mg/kg i.p.) were more modest. At baseline, HPLC-MS revealed that key EC lipids were altered in the GluN1KD's compared to their wild-types and following acute PF-3845 (10 mg/kg i.p.), fluctuations in EC signalling were greater in the wild-type mice. Meanwhile, little to no changes in cataleptic behaviour, PPI or ASR were observed in either acute or chronic treatment with PF-3845 (10 mg/kg i.p.).

Conclusion: The lipidomic and behaviour data suggest that NMDA-R hypofunction may be accompanied with alterations in baseline endocannabinoid tone. These differences may make model more sensitive to acute perturbations in EC signalling as was seen by changes in some psychosis-related behaviours in GluN1KD mice but not in wild-types. This study supports the need to characterize how components of the ECS correspond to psychosis-related behaviours and the considerations for the development of therapeutics that target EC enzymatic activity.

[1] Giuffrida *et al*. (2004) *Neuropsychopharmacology*. **29**(11): 2018-14
[2] Mohn *et al*. (1999) *Cell.* **98:** 427-36.

NEUROBIOLOGICAL MECHANISMS UNDERLYING VULNERABILITY AND RESILIENCE TO CANNABIS ADDICTION

Martin-Garcia E[1,2], Cajiao M[1], Casado-Anguera V[1], García-Blanco A[1], Maldonado R[1,2]

[1]Laboratory of Neuropharmacology-Neurophar, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain. [2]Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain.

Introduction: A hallmark of addiction is the loss of inhibitory control, leading to impulsive and compulsive-like behavior in addicted individuals.

Methods: We used a mouse model of drug addiction using WIN 55,512-2 intravenous self-administration (0.0125 mg/kg/infusion) in C57Bl/6J. Chemogenetic inhibition of neuronal activity in the medial prefrontal cortex (mPFC) to the nucleus accumbens (NAc) pathway was performed. In adolescent mice, we administered THC (5 mg/kg, i.p.) or vehicle during postnatal days 35 to 55, and we evaluated its impact on the vulnerability to develop drug addiction during adulthood. In addition, differential gene expression between addicted or non-addicted mice exposed to THC during adolescence was analyzed at the end of the experiment by qPCR in the main brain areas of the mesocorticolimbic system. Finally, functional validation was done with a virally-mediated overexpression of the dopamine D2 receptor, specifically in the mPFC-NAc projection.

Results: We found that the resilient or vulnerable phenotype can be obtained in the mouse model of cannabis addiction using WIN 55,512-2 intravenous self-administration. Hypoactivity of mPFC to NAc projecting neurons promoted impulsivity-like behavior in C57Bl/6J mice. Furthermore, mice previously exposed to chronic THC during adolescence showed downregulation of *Drd2* (dopamine receptor type 2) gene expression in the NAc later in adulthood. These mice showed increased impulsivity-like behavior in the model of addiction, suggesting that THC chronic administration during adolescence was a risk factor for impulsivity-like behavior in adulthood.

Conclusions: Understanding the neurobiological mechanisms underlying resilience versus vulnerability to addiction is expected to pave the way for novel and efficient interventions to battle this mental disorder.

Acknowledgments: #PNSD-2017I050 to E.M.-G.

ANANDAMIDE IN OSTEOARTHRITIS AND DEPRESSION: BEHAVIOURAL, BIOCHEMICAL AND NETWORK ANALYSIS

Jakub Mlost, Magdalena Białoń, Marta Kędziora and Katarzyna Starowicz

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

Introduction: Osteoarthritis (OA) is a chronic joint disease in which cartilage degenerates as a result of its mechanical and biochemical disturbances followed by low grade inflammatory response. OA is one of the most common disorders causing chronic pain and disability among adults and it has been recognized by the World Health Organization as a "priority disease". The association between chronic pain, depression and anxiety has gained particular attention due to high rates of co-morbidity. Recent findings imply significant role of inflammation in the development of depression. Therefore, we addressed the occurrence of depressive-like symptoms in animal model of OA with particular focus on accompanying neurochemical and neuroinflammatory changes. Moreover, we studied the role of anandamide (AEA) in reducing depression in chronic pain patients.

Methods: Behaviour was studied for 10 weeks in rats after intra-articular injection of 2 mg of sodium monoiodoacetate (MIA). Pain threshold was measured with Von Frey's test, Pressure Application Measurement test and Kinetic Weight Bearing. Anxiety-like behaviour was measured with Elevated Plus Maze (EPM) and open field (OF) tests. Molecular targets of AEA were established through literature search. Genes associated with OA and depression were obtained through Open Targets Platform. Venn analysis was performed to find common targets between AEA, depression and OA. Subsequent analysis of protein-protein interactions and functional enrichment were performed with STRING and KEGG databases. Biochemical changes in plasma were assessed using ELISA technique.

Results: MIA-treated animals were characterized by a gradual decrease in pain threshold in all behavioural tests. In addition, OA rats manifested anxiety-like behaviour in OF and EPM after 6 weeks post-MIA injection. Moreover, behavioural symptoms in OA rats were accompanied by changes in plasma levels of stress-related and proinflammatory factors. Concomitant network analysis revealed 597 shared proteins between OA and major depressive disorder. AEA could affect 128 of those proteins through CB1, 5HT3A, CB2, GlyR, Cav3.2 and nAchR, presented here in the decreasing order of their valency. Among targets modulated by AEA's, we have revealed a battery of inflammatory markers and neurotrophic factors such as IL6, TNF, CXCL8, CXCL10, CXCL12, PTGS2, CCL5, BDNF or NGF as the most influential. Enrichment analysis showed that proteins interacting with AEA molecular targets are involved in BDNF regulation of GABA signalling, serotonin and anxiety pathways, noradrenergic receptors, transcription factor regulation in adipogenesis, galanin receptor pathway and sleep regulation.

Conclusions: Our biochemical and behavioural assessment revealed depressive-like and proinflammatory phenotype accompanying development of chronic pain in animal model of OA. Bioinformatic approach allowed us to identify a significant number of proteins that are engaged in both OA and depression, which could be modulated by AEA's molecular targets, distinct from just CB1 and CB2 receptors. Of note, we revealed a number of inflammatory and neurotrophic factors that could be modulated by AEA. Further analysis suggests that AEA may impact comorbid depression by targeting GABA, noradrenaline and serotonin transmission. These results imply AEA as promising therapeutic target for OA-related mood disorders. Further experimental validation of our results is essential but if successful, it may pave a way for novel treatment strategy.

Acknowledgments: Funded by IF PAN statutory funds and by National Science Centre, Poland grant OPUS no. UMO-2019/35/B/NZ7/01176, 2020-2024.

BEHAVIORAL AND NEUROCOGNITIVE EFFECTS OF THE CANNABINOID CANNABIGEROL (CBG)

Catherine F. Moore[1], Catherine M. Davis[2], and Elise M. Weerts[1]

[1]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Introduction: The cannabinoid cannabigerol (CBG) is prevalent in retail cannabis products and is touted as having therapeutic benefits for appetite stimulation, analgesia, and mood. This study characterized the pharmacological effects of CBG on the cannabinoid tetrad of behaviors as well as, appetitive behavior, and neurobehavioral performance in rats.

Methods: Male and female Sprague-Dawley rats were orally administered CBG mixed in sesame oil (30-600 mg/kg) prior to behavioral tests. Testing with higher doses (900-1500 mg/kg) was initiated, but discontinued due to toxicity. Neurocognitive effects were measured using the rodent Psychomotor Vigilance Test (rPVT) and the Y-maze spontaneous alternation task. Appetitive effects were determined in an operant task using a progressive ratio schedule of chow pellet reinforcement. Effects in the standard cannabinoid tetrad (pain sensitivity, body temperature, catalepsy, and locomotor activity) were also determined. ANOVAs or mixed models were used to analyze effects of CBG dose, and time where appropriate, on behavioral outcomes.

Results: At doses of 30-100 mg/kg CBG did not alter neurocognitive outcomes, but 600 mg/kg CBG impaired sustained attention in the rPVT (main effect of CBG; $p=0.02$) and impaired working memory in the Y-maze (main effect of CBG; $p=0.02$). CBG (300-600 mg/kg) increased locomotor activity in the first hour of a continuous 4-hr locomotor activity assessment (CBG x time interaction; $p<0.001$). CBG did not have any effects on other outcomes in the cannabinoid tetrad (changes in body temperature, pain sensitivity, or catalepsy). CBG also did not affect motivation for food, as measured by progressive ratio breakpoints. Some sex differences in CBG effects were observed.

Conclusion: CBG did not produce effects on the cannabinoid tetrad typical of cannabinoid type-1 receptor agonists (e.g., delta-9-tetrahydrocannabinol; THC) at any of the doses tested. CBG had modest, but appreciable increases in motor activity, and impaired neurocognitive behavior. For context, in our prior work, THC produced typical cannabinoid tetrad effects as well as impairment in the rPVT when administered orally, while CBD produced little to no effects on these assessments. These data demonstrate that over a wide range of doses, CBG produced no behavioral effects on appetite or acute pain sensitivity, and at higher doses caused modest stimulatory effects and disrupted performance on cognitive tests.

Acknowledgments: Research was supported in part by Johns Hopkins University departmental funds. CBG was provided by Canopy Growth Corporation as a gift.

THE EFFECTS OF G-PROTEIN COUPLED RECEPTOR 55 (GPR55) SINGLE NUCLEOTIDE POLYMORPHISMS ON BRAIN VOLUME AND ATROPHY IN LATER LIFE

Daniel K. Mori-Fegan[1], Che-Yuan Wu[1,2], Yuen Yan Wong[1,2], Ruth A. Ross[1] and Walter Swardfager[1,2]

[1]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Canada
[2] Hurvitz Brain Sciences Program, Sunnybrook Research Institute, Toronto, Canada

Introduction: The G protein-coupled receptor 55 (GPR55) is a lysophosphatidylinositol (LPI)-sensitive receptor. Various cannabinoid molecules have been shown to modulate GPR55 signalling. mRNA for GPR55 is expressed in several brain areas such as the frontal cortex, hippocampus, hypothalamus, cerebellum and more. GPR55 signalling and single nucleotide polymorphisms (SNPs) have been indicated to be involved in the modulation of inflammation, homeostasis and as a potential therapeutic target for neurodegenerative diseases with neuroinflammatory backgrounds such as Alzheimer's disease (AD), Parkinson's disease, and multiple sclerosis. However, the foundational knowledge on GPR55's role in brain morphology, and neurodegeneration is lacking. We hypothesised that presence of *GPR55* SNPs minor alleles will show more brain volume atrophy over time and neurodegeneration as evidenced by lower brain volume over time in areas where GPR55 is expressed.

Methods: This retrospective, longitudinal, observational study used data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) composed of 750 participants with normal cognition, mild cognitive impairment (MCI) or AD diagnosis. SNPs were genotyped via the GenomeStudio v2009.1 *(Illumina*) from blood DNA. Patients of European ancestry confirmed via multidimensional scaling were included. With inclusion criteria of SNPs having a minor allele frequency (MAF) between 5-10% and those with relevant ClinVar information from dbSNP, a total of 9 *GPR55* SNPs of interest were identified. Whole-brain, hippocampal, medial temporal, fusiform and entorhinal cortex regional volumes were obtained from volumetric segmentation via FreeSurfer on MRI T1-weighted images acquired at 3.0T. ANCOVAs were employed to evaluate baseline volume difference between SNP alleles in a dominant model with respect to the minor alleles in all brain areas of interest, controlling for sex, age and baseline diagnosis for cognitive status. Effects of interactions between SNPs over time were assessed as and across timepoints (baseline, 6, 12, and 24 months) in mixed models for longitudinal associations.

Results: Preliminary results show a significant association with rs2969126G/C (MAF:6%) in whole-brain ($F(1,718)=8.49$, $p=0.004$) and hippocampal ($F(1,684)=4.70$, $p=0.03$) volumes at baseline. Furthermore, rs2969126G/C minor allele presence was associated with a trend for decrease in hippocampal volumes over time ($F(3,1443)=2.25$, $p=0.081$) compared to major allele homozygotes, however a larger sample may be required to observe significance.

Conclusions: Presence of the *GPR55* SNP rs2969126G/C minor allele showed significant associations with brain volumes and atrophy over time in a sample of older people with and without Alzheimer's disease. In addition to these preliminary results, additional analyses investigating the relationships among allele presence, neurodegeneration biomarkers and clinical diagnoses of MCI/AD, are being conducted to elaborate effects GPR55 SNPs may have. This may support suggestions of GPR55 as a potential therapeutic target for neurodegenerative diseases in aging, prompting further investigations for GPR55 involvement.

PRELIMINARY DATA COMPARING THE EFFICACY OF ACUTE VAPORIZED CANNABIS TO ORAL OXYCODONE AND PLACEBO FOR CHRONIC SPINE PAIN

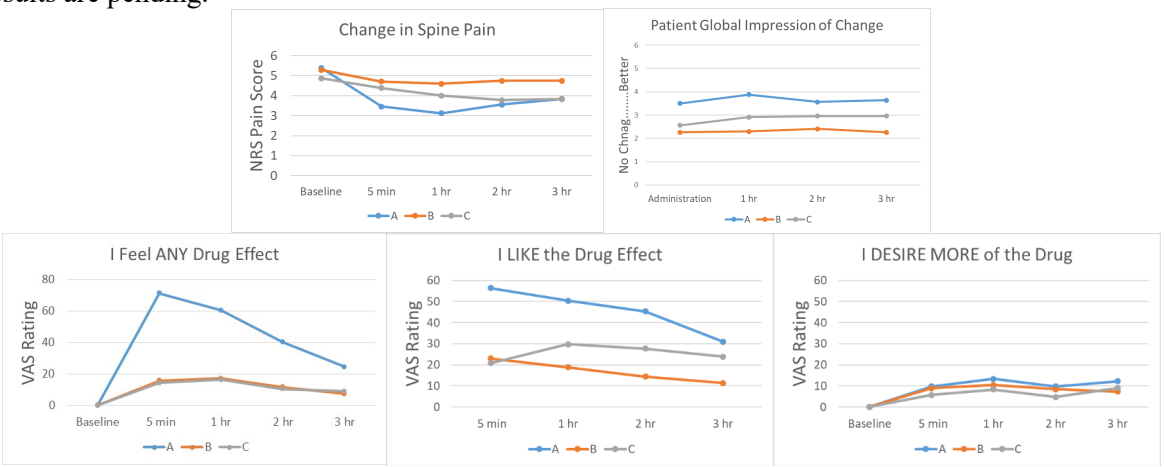
Alan W. J. Morris[1], Rahwa Netsanet[1], Jacci Bainbridge[2], Vikas V. Patel[1], Rachael Rzasa Lynn[3] and Emily M. Lindley[1]

University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
1. Department of Orthopedics 2. Department of Clinical Pharmacy;
3. Department of Anesthesiology

Introduction: Back and neck pain are highly prevalent and disabling musculoskeletal conditions. Although commonly prescribed, opioids are often ineffective and can result in dependency and lethal overdose. An alternative analgesic treatment that has gained increasing recognition is cannabis. The primary aim of this double-blind crossover trial is to compare the efficacy of acute cannabis exposure to a commonly prescribed opioid (oxycodone), as well as placebo, for spontaneous pain relief.

Methods: After informed consent and screening, participants attended 3 separate 4-hour study visits with pre- and post-drug assessments including NRS pain scores, neurocognitive assessments, subjective ratings of drug effects, and pain thresholds (PTh) measured with a computer-controlled pressure algometer. Participants received one of the following drugs across the 3 visits: active vaporized cannabis (placebo capsule), active oxycodone (placebo cannabis), and placebo/placebo. Blood samples were taken at baseline, +5 minutes, and +1 hour. Enrollment continues in this study, thus final data analyses have not been completed. Descriptive statistics were performed on a subset of data collected from participants who completed all three study visits. The data remain blinded and are shown as drugs A, B, and C (3 drug treatments: Cannabis, Oxycodone, and Placebo). Data are presented as means.

Results: To date, a total of 26 participants with chronic spine pain (12 males, 14 females) have completed the study. Select results are shown below. Serum cannabinoid levels and neurocognitive results are pending.



Conclusions: These initial data appear to show that chronic spine pain is alleviated by Drug A, and to a lesser extent, by Drug C. Subjective drug effect ratings show that Drug A has a rapid onset of effects that tapers off over time, while Drugs B and C have fewer subjective effects. All three drugs have similar, low ratings for desiring more drug. The study is still recruiting and final results are expected to be completed by mid-2023.

Acknowledgements: Funded by the Colorado Department of Public Health and Environment. The CU Clinical & Translational Research Center is supported by NIH/NCATS Colorado CTSA Grant Number UL1 TR002535.

INVESTIGATION OF CANNABIDIOL IN THE DRUG DISCRIMINATION PARADIGM

Mohammed A. Mustafa, Joseph Porter, Joel E. Schlosburg, Jayden Elmer, Justin B. Poklis and Aron H. Lichtman

Department of Pharmacology & Toxicology,
Virginia Commonwealth University, Richmond, VA

Vast public interest has grown in the phytocannabinoid cannabidiol (CBD) because of its FDA-approval as adjunct medication to treat severe forms of epilepsy, other potential therapeutic effects, and use in a wide range of products. The lack of intoxicating effects of CBD in humans is consistent with its failure to substitute for the active cannabis-derived CB1 receptor agonist delta-9-tetrahydrocannabinol as well as synthetic CB1 receptor agonists in the drug discrimination paradigm in multiple laboratory animal species. However, anecdotal reports frequently describe a "pleasant" subjective effect of CBD in humans. Thus, the primary goal of the present study was to determine whether mice would learn to discriminate CBD from vehicle in the drug discrimination paradigm. Additionally, because CBD interacts at targets within the endocannabinoid system, we evaluated whether it would alter the subjective effects of the high efficacy CB1 receptor agonist CP55,940 in this paradigm. Mice did not learn to discriminate CBD from vehicle following 74 days of training at 100 mg/kg and an additional 50 days at 200 mg/kg. These mice subsequently achieved acquisition criteria for CP55,940 discrimination. We next evaluated CBD in a second group of mice trained to discriminate CP55,940 from vehicle. Consistent with previous studies, CBD (200 mg/kg) failed to substitute for CP55,940 and did not alter response rates. Moreover, it did not alter the CP55,940 generalization dose-response curve. A single injection of 100 or 200 mg/kg CBD led to high drug levels in blood and whole brain, but did not affect brain levels of endogenous cannabinoids and related lipids. These preclinical findings demonstrating that high dose CBD does not produce an interceptive stimulus in the drug discrimination assay and neither affects the subjective effects of CP55,940 nor alters brain levels of endogenous cannabinoids support the conclusion that CBD lacks intoxicating effects and possess low abuse potential.

Acknowledgements: This work supported by NIH grant #'s R01DA039942 and P30DA033934

EFFECT OF THC AND THE ROLE OF PERIPHERAL CANNABINOID RECEPTOR (CB2R) ON RESISTANCE TO SYSTEMIC *CANDIDA ALBICANS* INFECTION IN IMMUNE SUPPRESSED AND IMMUNE COMPETENT MALE MICE

Nathalie Nadales, R. Michael Little and Nancy E. Buckley

Department of Biological Sciences,
California State Polytechnic University, Pomona, Pomona, CA, USA

Introduction: *Candida albicans* (*C. albicans*) is an opportunistic pathogen that can cause systemic candidiasis which is detrimental to immunocompromised individuals. The endocannabinoid system has immune modulatory effects which may play a role in candidiasis. The best-known cannabinoid receptors are the central cannabinoid receptor (CB1R) and the peripheral cannabinoid receptor (CB2R). In the Unites States, there has been increased use of marijuana products for recreational and medicinal purposes. One of the psychotropic components of marijuana is Delta-9-tetrahydrocannabinol (THC) which is known to suppress the immune responses to bacterial, viral, and protozoan infections, but little is known on THC's effect on fungal infections. In this study we investigate the effect of THC and the role of CB2R on resistance to a systemic C. *albicans* infection in immune competent and immune suppressed male mice.

Methods: Male CB2R wildtype (CB2R +/+), and knockout (CB2R -/-) mice were used to conduct these studies. CB2R +/+ and CB2R -/- mice were injected intravenously (IV) with either 1XPBS or 5-fluoruracil (5F), a chemotherapy drug that immunosuppresses the mice. After three days, mice were infected with $7.5x10^5$ *C. albicans* cells/mouse, IV. Mice were observed daily for morbidity and survival. Mouse tissues were collected to assessed fungal load (kidneys, brains, liver) and cytokine levels in serum, and spleens. Splenocytes were cultured, treated with lipopolysaccharide (LPS, 1μg/mL). Supernatants were collected and analyzed for secreted cytokines. In addition, male CB2R-/-mice were treated with a vehicle, or THC (8mg/kg) intraperitoneally (IP) on days -11 to -8 and -4 to -1. On day -3, the mice were injected IV with either 1XPBS or 5F. On day 0, the mice were infected with $7.5x10^5$ *C. albicans* cells/mouse, IV.

Results: CB2R +/+ and CB2R -/- 5F treated male mice lost more weight and had increased kidney and brain fungal load compared to the 1X PBS treated mice. There was an increase in IL-6 in serum, kidney and brain in CB2R +/+ and CB2R -/- 5F treated male mice compared to CB2R +/+ and CB2R -/- PBS treated mice. CB2R -/- mice treated with THC/5F trended to have greater percent weight loss compared to the PBS treated mice. There were high levels of IL-6 in serum of mice treated with THC/5F, but THC had no effect on tissue fungal burden and splenocyte cytokine secretion in PBS and 5F treated mice.

Conclusions: Immunosuppressed mice had more of an effect on weight change, tissue burden and cytokine production compared to immunocompetent mice and compared to THC effects. However, THC may exacerbate the infection by elevating serum IL-6. These effects seem to be CB2R independent.

NATURALLY PRODUCED CANNABINOIDS FOR PAIN MANAGEMENT AND NEUROPROTECTION FROM CONCUSSION DURING PARTICIPATION IN CONTACT SPORTS: NFL FUNDED STUDY PROTOCOL

[1,10]J. Patrick Neary, [2,10]Jane Alcorn, [2,10]Robert B. Laprairie, [3,6]Payam Dehghani, [4]Bruce H. Bjornson, [5]Thomas Hadjistavropoulos, [1]Kim D. Dorsch, [1]Cameron S. Mang, [1]Holly A. Bardutz, [6]Lanishen Bhagaloo, [7]Zachary Walsh, [8]Philip N. Ainslie, [9,10]Michael Szafron, [1, 3, 10]Jyotpal Singh and [2,10]Elizabeth S. Thompson.

[1]Faculty of Kinesiology & Health Studies, University of Regina, Regina, Canada
[2]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Canada
[3]Department of Cardiology, Prairie Vascular Research Inc, Regina, Canada
[4]Faculty of Medicine – Neurology, University of British Columbia, Vancouver, Canada
[5]Department of Psychology, Centre of Aging and Health, University of Regina, Regina, Canada
[6] College of Medicine, University of Saskatchewan, Saskatoon, Canada
[7]Department of Psychology, University of British Columbia, Kelowna, Canada
[8]Centre for Heart, Lung and Vascular Health, University of British Columbia, Kelowna, Canada
[9]School of Public Health - Biostatistics, University of Saskatchewan, Saskatoon, Canada
[10]Cannabinoid Research Initiative of Saskatchewan (CRIS), Saskatchewan, Canada

Introduction: Cannabinoids (CBD, THC) are neuroprotective and have been used for prescription medications to facilitate pain management. However, no research with human participants to date, has explored the potential prophylactic effects of cannabinoids on brain function in elite contact sport athletes during a competitive season or in response to post-concussion syndrome sequelae. The objective of this project, funded by the National Football League (NFL), is to investigate the potential effects of medical cannabis in contact sport athletes suffering from acute and chronic long-term concussion symptoms, and the use of high-CBD cannabis formulations as an alternative to opioids for pain management. We anticipate beneficial effects in our primary (cardiovascular metrics) and secondary (cerebral oxygenation, GABAergic activity, pain intensity/interference, reduced medication use, fMRI-Default Mode Network, and blood biomarkers) outcome measures. Our goal is to develop individualized treatment plans for contact sport athletes.

Methods: We will conduct a multi-centre, multi-study project using dose escalation (Study 1), double-blind cross-over (Study 2), and Latin-square double-blind cross-over (Study 3) studies with randomized placebo-controlled designs to systematically assess clinical, neuro-physiological, perceptual-cognitive, biopsychological and motor skill performance outcomes. We will analyse venous blood for cannabinoid levels, pharmacodynamic markers, immune biomarkers, and genetic analysis for known protein polymorphisms (single nucleotide polymorphisms, etc.) and relate our findings to the observed drug effect in the study participants. My Next Health Inc. will also analyse saliva samples for metabolic genes and relate these to phenotypic expression in our athletes.

Results: This poster presentation will highlight the proposed NFL study experimental design.

Conclusion: We anticipate that our research will have immediate clinical implications for the treatment planning of elite athletes who sustain concussion and associated chronic injuries.

Acknowledgments: NFL/NFLPA Joint Pain Management Committees, My Next Health Inc.

MAGL INHIBITION PREVENTS MEMORY DEFICIT CHEMOTOXICITY BY AROMATASE INIBITION IN MICE

Lesley D O'Brien[1], Delaney Place[1], Aidan Jones[1] and Aron H Lichtman[1]

[1]Pharmacology and Toxicology Department, Virginia Commonwealth University

Introduction: Breast cancer is the most common cancer in women, with two thirds of cases being estrogen dependent. Inhibition of aromatase, the primary biosynthetic enzyme of estrogen, leads to a reduction of estradiol production in breast and other tissues and is used as a second line treatment that effectively prevents breast cancer recurrence. Despite the efficacy of aromatase inhibitors (AIs) in preventing disease recurrence, these drugs produce a variety of side effects including learning and memory deficits for which treatment options are currently limited to AI switching or discontinuation. Here we developed a mouse model of AI-induced memory deficit using the object location (OL) task to evaluate the endocannabinoid (eCB) system as a potential target to treat AI-induced memory deficit. Specifically, given that the eCB ligand 2-arachidonoylglycerol (2-AG) plays an integral role in eCB-mediate short-term synaptic plasticity, we tested whether inhibition of its primary degradative enzyme, monoacylglycerol lipase (MAGL), prevents AI-induced memory deficit.

Methods: Female ovariectomized (OVX) C57BL/6J mice were administered the AI letrozole (0.5 mg/kg, p.o.) once daily for a week, and then on alternating days until day 14. In the first experiment, mice were co-administered the MAGL inhibitor MJN110 (0.0625, 0.125, 0.25 mg/kg) daily with letrozole, then tested on day 14 in the OL task. The second experiment evaluated if repeated administration of MJN110 doses used in Experiment 1 in combination with letrozole would produce functional CB1 receptor tolerance inferred by a rightward shift of the cumulative dose-response curve of CP55,940 in the triad assay (using the bar test to assess catalepsy, the tail immersion test to assess antinociception, and rectal temperature to assess hypothermia).

Results: The MAGL inhibitor MJN110 protected against AI-induced OL task memory deficit at 0.125 and 0.25 mg/kg. Repeated administration of MJN110 in letrozole-treated mice did not alter the dose-response curves of CP55,940 in catalepsy, antinociception, or hypothermia.

Conclusions: The present data suggest that low dose repeated MAGL inhibition represents a strategy to prevent AI-induced hippocampal-dependent memory task deficits without reducing CB1 receptor function.

Acknowledgements: Funded by NIH T32 Training Grant 5T32DA007027-45 awarded to VCU and startup funds from the VCU School of Pharmacy (AHL), and Postdoctoral Independent Research Award from VCU (LOB).

CANNABINOID RECEPTOR 2 DELETION ENHANCES RESPIRATORY CAPACITY IN NAÏVE AND TLR4-STIMULATED MICROGLIA

Bolanle F. Olabiyi, Eike Geissmar, Andreas Zimmer and Anne-Caroline Schmöle

[1]Institute of Molecular Psychiatry, Medical Faculty, University of Bonn, Germany
[2]Institute of Innate Immunity, Medical Faculty, University of Bonn, Germany

Introduction: Over the last decade, studies in the growing field of immunometabolism have sought to characterise metabolic changes that occur in immune cells after activation. It is known that activated macrophages undergo glycolytic switch from oxidative phosphorylation which impairs mitochondrial respiratory capacity following LPS stimulation (Lauterbach *et al*., 2019, O'Neill L.A.J., 2019). However, it remains unknown whether toll-like receptor 4 (TLR4) stimulation causes microglia to undergo metabolic changes mediated by CB2 receptor. To investigate this, we characterised the effects of CB2 constitutive deletion and pharmacological inhibition on microglia glucose metabolism and inflammatory profile following TLR4 stimulation.

Methods: Primary microglia cultures obtained from neonatal wildtype (WT) and constitutive CB2 knockout ($CB2^{-/-}$) mice were stimulated with TLR4 ligand (LPS/IFNγ) at 100ng/ml and 20ng/ml respectively for 16 hours. Bone marrow derived macrophages (BMDM) generated from 3-4months WT or $CB2^{-/-}$ mice were subjected to the same TLR4 stimulation. The metabolic parameters: oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were analysed using the Seahorse XF analyser. Inflammatory cytokines and transcriptional profile were assessed by ELISA and RNA sequencing respectively. Furthermore, WT microglia or BMDM pre-treated with CB2 specific antagonist SR144528 (SR) were stimulated with LPS/IFNγ for 16hours after which inflammatory and metabolic profile were assessed.

Results: Our OCR results showed that CB2 deletion not only improves the maximal respiration in primary microglia subjected to TLR4 stimulation but also in naive microglia after sequential injection of FCCP. We observed no significant difference in glycolytic switch between WT and $CB2^{-/-}$ microglia/BMDM following TLR4-stimulation. Our RNA sequencing data showed downregulated glycolytic genes in TLR4-stimulated $CB2^{-/-}$ microglia compared to the WT. CB2 inhibition with SR144528 decreases cytokine production in TLR4 stimulated microglia but did not produce any significant difference in OCR and ECAR between TLR4-stimulated microglia with or without SR.

Conclusions: To conclude, our results suggest that CB2 deletion plays a role in improving mitochondrial respiratory capacity in microglia but not in Bone marrow derived macrophages. Even though TLR4 stimulation displayed glycolytic switch in both WT and CB2 microglia, treatment with SR144528 does not seem to have a significant effect on mitochondrial function in both microglia and BMDM. Future experiments are still needed to fully understand how CB2 interferes with microglia metabolism under inflammatory settings.

ENDOCANNABINOID UPREGULATION INFLUENCES THE BEHAVIORAL AND NEUROENDOCRINE RESPONSE TO STRESS

Gavin Petrie[1,2], Georgia Balsevich[1,2], Hiulan Yau[1,2], Tamas Fuzesi[1,2], David Rosenegger[1,2] Robert Aukema[1,2], Mario Van Der Stelt[3], Jaideep Bains[1,2] and Matthew Hill[1,2]

[1]Hotchkiss Brain Institute,
[2]University of Calgary, Calgary, AB, Canada,
[3]Department of Molecular Physiology

Introduction: Endocannabinoid (eCB) signalling is known to regulate many aspects of the stress response including 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). ECB signalling mediates the glucocorticoid dependent negative feedback mechanism on CRH neurons following stress, therefore pharmacological upregulation of this system may dampen stress-induced consequences associated with CRH neuron activity. As such, using an array of cellular, endocrine and behavioral readouts associated with activation of CRH neurons in the PVN, we examined how upregulating eCB signaling ameliorates stress-induced generation of a stress response.

Methods: Prior to a foot shock stressor, endogenous Anandamide (AEA) and 2-AG levels were elevated via pharmacological inhibition of their respective hydrolytic enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Our endpoint measurements of a stress response included the examination of self-directed homecage behaviors (i.e., grooming), activation of CRH neurons in the PVN and corticosterone release. Physiological readouts were measured using respective immunohistochemical and ELISA approaches. Cannula were implanted for central delivery of the MAGL inhibitor (JZL184) prior to foot shock stress exposure.

Results: Foot shock stress elevated grooming, PVN CRH neuron activation and circulating corticosterone. While systemic FAAH inhibition had no effect, MAGL inhibition ameliorated stress-induced grooming in a temporally dependent manner. Interestingly, systemic MAGL inhibition did not influence the effects of stress on HPA measures. However, preliminary results suggest that intra-PVN, but not intra-BLA MAGL inhibition may ameliorate grooming as well as HPA measures following foot shock stress.

Conclusions: These data suggest that pharmacological upregulation of 2-AG, specifically in the PVN, can alter the behavioral and HPA response to stress. Techniques with better temporal sensitivity are currently being used to further understand the effects of systemic MAGL inhibition on CRH neuron activity in the PVN. These results lay the foundation for the development of cannabinoid-based therapies targeting symptoms of stress-related disorders.

The research reported here was funded by Canadian Institutes for Health Research (Grant. no. FDN-143329 :CIHR) to Matthew Hill.

CANNABIGEROL (CBG) ATTENUATES MECHANICAL HYPERSENSITIVITY ELICITED BY CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Diana E. Sepulveda[1,2], Daniel P. Morris[3], Wesley M. Raup-Konsavage[1], Dongxiao Sun[1], Kent E. Vrana[1] and Nicholas M. Graziane[1,2*]

[1]Department of Pharmacology, Penn State University College of Medicine, Hershey, PA 17033 [2]Department of Anesthesiology and Perioperative Medicine, Penn State University College of Medicine, Hershey, PA 17033 [3]Penn State Harrisburg, 777 W Harrisburg Pike, Middletown, PA 17057

Introduction: Cannabigerol (CBG) is a non-psychoactive phytocannabinoid produced by the plant *Cannabis sativa* with affinity to various receptors involved in nociception. As a result, CBG is marketed as an over-the-counter treatment for many forms of pain. However, there is very little research-based evidence for the efficacy of CBG as an anti-nociceptive agent.

Methods: To begin to fill this knowledge gap, we assessed the anti-nociceptive effects of CBG in C57BL/6 mice using three different models of pain; cisplatin-induced peripheral neuropathy, the formalin test, and the tail-flick assay.

Results: Using the von Frey test, we found that CBG dose dependently reversed mechanical hypersensitivity evoked by cisplatin-induced peripheral neuropathy in both male and female mice. Additionally, we observed that this CBG-induced reduction in mechanical hypersensitivity was attenuated by the α_2-adrenergic receptor antagonist atipamezole (3 mg/kg, i.p.) and the CB_1R antagonist, AM4113 (3 mg/kg, i.p.), and blocked by the CB_2R antagonist/inverse agonist, SR144528 (10 mg/kg, i.p.). We found that the TRPV1 antagonist, SB705498 (20 mg/kg, i.p.) was unable to prevent CBG actions. Furthermore, we show that CBG oil (10 mg/kg, i.p.) was more effective than pure CBG (10 mg/kg) at reducing mechanical hypersensitivity in neuropathic mice. Lastly, we show that pure CBG and CBG oil were ineffective at reducing nociception in other models of pain, including the formalin and tail flick assays.

Conclusions: Our findings support the role of CBG in alleviating mechanical hypersensitivity evoked by cisplatin-induced peripheral neuropathy, but highlight that these effects may be limited to specific types of pain.

Acknowledgements: This project is supported by the NARSAD Young Investigator Award (27364; NMG) and by the Pennsylvania Department of Health using Tobacco CURE Funds (NMG). KEV (and the Penn State College of Medicine) is the recipient of research support from PA Options for Wellness (a state-approved medical marijuana clinical registrant)

CANNABINOID AND OPIOID RECEPTOR APPROACHES TO REDUCE HISTAMINE-INDUCED PRURITUS

A. Matthew Reck[1], Frances Kim[2], and Steven G. Kinsey[1,2]

[1]Department of Psychological Sciences, University of Connecticut, Storrs, CT, USA
[2]School of Nursing, University of Connecticut, Storrs, CT, USA

Introduction: Pruritus is an unpleasant sensation that leads to scratching. This sensation can be caused by various exogenous stimuli and certain systemic diseases. Animal models of pruritus have been developed, many using mast cell degranulators such as Compound 48/80, which releases histamine and induces scratching behavior localized to the injection site. The current study aimed to reduce scratching by modulating the endocannabinoid system using the synthetic cannabinoid agonist WIN 55,212-2, the CB_1-selective positive allosteric modulator ZCZ011, or vehicle. The κ-opioid agonist Nalfurafine HCL, which has been used clinically in Japan since 2009 to treat pruritus, was used as a positive control. We hypothesized that both orthosteric and allosteric binding of CB_1 would reduce Compound 48/80-induced scratching.

Methods: Adult male and female C57BL/6J mice were administered WIN 55,212-2 (0.1-0.3-1-3mg/kg, i.p. -50 min), ZCZ011 (10-40mg/kg, i.p. -75 min) or Nalfurafine HCL (0.02mg/kg, s.c. -20 min) prior to injection of Compound 48/80 (50ug in 100uL, s.c.). The mice were immediately placed into individual sound-attenuating test chambers and video recorded for 30min. Hind paw scratching was hand-scored by observers blinded to treatment condition. In addition, spontaneous locomotor activity was assessed using ANYMaze software.

Results: As previously reported, Nalfurafine HCL (≥0.02mg/kg, s.c.) significantly reduced Compound 48/80-induced pruritus. The synthetic orthosteric cannabinoid agonist WIN 55,212-2 (≥1 mg/kg) also blocked histamine-induced pruritus. WIN 55,212-2 reduced locomotor activity at the highest dose only (i.e., 3mg/kg). The CB_1-selective positive allosteric modulator ZCZ011 had no effect on pruritus.

Conclusions: The current study revealed that orthosteric, but not allosteric, cannabinoid receptor binding produced a significant decrease in histamine-induced pruritus. The lack of an effect of ZCZ011 was unexpected and may be related to the pruritus model chosen. Thus, ongoing studies will probe additional cannabinoid targets as well as different mouse models of pruritus.

Research was supported financially by the National Institutes of Health: R01 DA048153.

BEHAVIOURAL CHARACTERISATION OF A PRECLINICAL INCISIONAL WOUND MODEL AND INVESTIGATION OF ASSOCIATED ALTERATIONS IN THE ENDOCANNABINOID SYSTEM

Maria C. Redmond[1,2,3,4], *Catherine R. Healy[1,2,3,4], Mehnaz I. Ferdousi[1,2,3], Georgina Gethin[4,5,6], Abhay Pandit[4] and David P. Finn[1,2,3,4]

[1]Pharmacology and Therapeutics, School of Medicine, National University of Ireland Galway, [2]Galway Neuroscience Centre, National University of Ireland Galway, [3]Centre for Pain Research, National University of Ireland Galway, [4]CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland Galway, [5]School of Nursing and Midwifery, National University of Ireland Galway, [6]Alliance for Research and Innovation in Wounds, National University of Ireland Galway *joint first authors

Introduction: Anxiety is a highly prevalent comorbidity in individuals with chronic wound-associated pain. The endocannabinoid system (ECS) is involved in the modulation of both pain and anxiety. This study aimed to further characterise the behavioural phenotype of the rat back hairy skin incision (BI) model and investigate potential ECS alterations in the prefrontal cortex (PFC), periaqueductal grey (PAG), left amygdala (AMY-L), and right amygdala (AMY-R).

Methods: Male and female Sprague-Dawley rats (150-200g, n=4-5/group) underwent back incision or sham surgery. Mechanical hypersensitivity was assessed at baseline and post-surgery days (PSD) 1-33 in the dorsum and paw using manual and electronic von Frey testing, respectively. Anxiety-related behaviour was assessed using the Elevated Plus Maze (EPM) test on PSD 6 and 26, the Light-dark Box (LDB) test on PSD 9 and 29, and the Open Field (OF) test on PSD 13 and 32. Following euthanasia on PSD 34, brains were gross-dissected and snap-frozen. Quantification of endocannabinoids, 2-AG and AEA, and *N*-acylethanolamines (NAEs), OEA and PEA, in the PFC, PAG, AMY-L and AMY-R was carried out by LC-MS/MS.

Results: Male BI rats showed incision-related mechanical hypersensitivity at 1cm ipsilateral to the dorsum incision on PSD 1, 3 and 7 vs male sham ($p<0.05$). Male BI rats displayed reduced paw withdrawal threshold in the ipsilateral hind paw on PSD 1, 3, 20 and 22 vs male sham ($p<0.05$). No pain-related behavioural differences were observed in female rats. Male ($p<0.01$) and female ($p<0.05$) BI rats spent less time in the open arms of the EPM vs sham counterparts on PSD 6, and this effect persisted to PSD 26 in females only ($p<0.05$). There were no observed differences between male and female BI rats vs sham counterparts in the LDB or OF tests. Levels of 2-AG were higher in the PFC ($p<0.05$) and PAG ($p<0.05$) of male BI rats compared to male sham rats. 2-AG, AEA, PEA and OEA levels in the AMY-L and AMY-R were similar between male BI rats and male shams. No differences were found in the levels of analysed endocannabinoids or NAEs in the PFC, PAG, AMY-L or AMY-R between female BI rats and female shams.

Conclusions: These results indicate that while only male rats show primary and secondary hypersensitivity following dorsum incision, both sexes exhibit anxiety-related behaviour. The findings suggest sexual dimorphism in behavioural responses following incision-related injury. Further work is required to determine the implications of increased 2-AG levels in the PFC and PAG on anxiety- and pain-related behaviours in the male BI rats.

Acknowledgements: Funding was provided by Science Foundation Ireland (SFI), B. Braun Hospicare and the Irish Research Council Postgraduate Scholarship and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073-P2.

SEX-DEPENDENT SENSORY AND AFFECTIVE INFLAMMATORY PAIN RESPONDING IN A PRECLINICAL RAT MODEL OF AUTISM AND ASSOCIATED CHANGES IN ENDOCANNABINOID GENE EXPRESSION IN THE ANTERIOR CINGULATE CORTEX

Rachel M. Humphrey[1,3,4], Andrés Illanes-Rosales[1], Danny M. Kerr[2], David. P. Finn[2,3,4] and Michelle Roche[1,3,4]

[1]Physiology, School of Medicine, [2] Pharmacology and Therapeutics, School of Medicine and [3] Galway Neuroscience Centre and [4]Centre for Pain Research, National University of Ireland Galway

Background and Aims: Up to 95% of individuals with autism exhibit sensory abnormalities, including altered pain responding, however the underlying neurobiology remains poorly understood. The endocannabinoid system is a key modulator of social, affective and nociceptive responding, known to be altered in autism. This study examined sensory and affective pain responding in a preclinical rat model of autism, the effect of sex, and associated alterations in immediate early gene and endocannabinoid gene expression in the anterior cingulate cortex (ACC).

Methods: Male and female adolescent rats, prenatally exposed to saline- or the antiepileptic valproic acid (VPA), were assessed for mechanical (von Frey) and thermal (hot plate/Hargreaves test) sensory responding prior to, and following, intraplantar administration of complete Freund's adjuvant (CFA). Social, anxiety and affective pain responding was also assessed. Expression of the immediate early gene *c-fos* and endocannabinoid related genes *cnr1, faah* and *mgll*, were assessed in the ACC using qRT-PCR.

Results: VPA-exposed male and female rats displayed tactile hyposensitivity compared to saline-treated counterparts. Intraplantar-CFA resulted in mechanical, cold and heat hypersensitivity in all animals, the time-course and magnitude of which was reduced and shortened by prenatal VPA-exposure in a sex-dependent manner. There were no changes in social or anxiety-like behaviour post-CFA. However, VPA-exposed male rats exhibited reduced negative affect in the place escape/avoidance paradigm, an effect associated with decreased *c-fos* but no change in *cnr1*, *faah* or *mgll* expression in the contralateral ACC.

Conclusions: VPA-exposed rats exhibit sex-dependant sensory and affective inflammatory pain responding, effects accompanied by reduced immediate early gene expression in ACC. Although no change was observed in endocannabinoid gene expression in the ACC, further studies are required to determine the role, if any, of endocannabinoid system in altered affective pain responding in this autism model.

Acknowledgements: Hardiman Postgraduate Scholarship and College of Medicine, Nursing and Health Sciences, NUI Galway.

MAGL INHIBITION ATTENUATES POST-SURGICAL PAIN IN MICE

Carl Erwin B. Rodriguez and Steven G. Kinsey

School of Nursing and Department of Psychological Sciences,
University of Connecticut, Storrs, CT, USA

Introduction – Although opioids and NSAIDs are the mainstay treatments for post-surgical pain, their negative side effects (e.g., respiratory depression, constipation, gastric inflammation) are placed persistent limitations on their use, especially over prolonged administration. Thus, the endocannabinoid system has garnered interest in recent years due to its analgesic therapeutic potential. For example, inhibiting monoacylglycerol lipase (MAGL) exhibits antinociceptive, anti-allodynic, and inflammatory properties in various rodent models. Thus, we hypothesized that MAGL inhibition, via JZL184, attenuates allodynia caused by hindpaw incision (HPI) a post-surgical model of pain in mice.

Methods - Under isoflurane anesthesia, equal numbers of adult male and female C57Bl/6J mice were subjected to HPI, in which a small incision was made on the plantar surface of one hind paw. The incision was closed with a single suture. Approximately 24 hours post-surgery, JZL184 (1-40 mg/kg, ip), the NSAID Diclofenac sodium (50 mg/kg, ip), or vehicle (5% ethanol, 5%, Cremophor, and 90% saline, ip) was administered. After two hours, paw withdrawal threshold is quantified using an up-down approach with von Frey filaments to measure the magnitude of mechanical allodynia.

Results – Hindpaw incision induced mechanical allodynia that persisted for up to 10 days post-surgery. Paw withdrawal threshold did not differ between paws contralateral to HPI or sham-operated mice. At 24 hours post-surgery, acute treatment with either diclofenac or JZL184 (4, 40 mg/kg, ip) attenuated mechanical allodynia induced by HPI. No sex differences were observed.

Conclusion – In the present study, inhibition of MAGL expressed anti-allodynic effects in the post-surgical model in mice. In a dose-dependent manner, JZL184 attenuated allodynia 24 hours after the hindpaw incision. Ongoing studies will probe potential cannabinoid receptor mechanism. These data support targeting the endocannabinoid system for post-operative pain treatments.

Research was supported financially by the National Institutes of Health R21 DA052690

DESIGN OF A CLINICAL TRIAL PROTOCOL OF DAILY ORAL CANNABIS TO REDUCE OPIOID INTAKE IN PATIENTS WITH CHRONIC SPINE PAIN

Alan W. J. Morris[1], Michelle Adkins[2], Jacci Bainbridge[2], Rachael Rzasa Lynn[3] and Emily M. Lindley[1*]

University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
1. Department of Orthopedics 2. Department of Clinical Pharmacy;
3. Department of Anesthesiology;' * Co-Senior Authors

University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
1. School of Pharmacy, Department of Clinical Pharmacy; 2. School of Medicine, Department of Anesthesiology; 3. School of Medicine, Department of Orthopedics

Introduction: Back and neck pain are highly prevalent and disabling musculoskeletal conditions. Patients with chronic spine pain represent one of the largest populations of opioid analgesic consumers and opioids are the most commonly prescribed class of drug for back pain. Patients with chronic spine pain are also more likely to use higher doses of opioids than patients with other chronic pain conditions and thus are at increased risk for opioid abuse and overdose. An alternative analgesic treatment that has gained increasing recognition is cannabis. In contrast to opioids, cannabis overdoses are not fatal. As more states approve recreational and medical cannabis, it has been suggested that cannabis legalization might be helping to ease the opioid epidemic. The rates of annual opioid overdose and opioid prescribing are lower in states where medical cannabis is legal than those where it is illegal. Several survey-based clinical studies and a retrospective cohort study reported that medical cannabis use is associated with reduced opioid use for chronic pain. The primary aim of this trial is to compare the effects of daily adjunct treatment with an orally delivered THC/CBD oral solution versus placebo on the ability of patients to taper high-dose opioids, as measured by percent reduction from baseline of total daily morphine milligram equivalents. This abstract provides an introduction to the clinical trial protocol. It is important for cannabis researchers to be aware of other studies that are being conducted to avoid redundancy and expand on the landscape of cannabinoid research.

Methods: This clinical trial will recruit individuals with diagnosed chronic thoracic, lumbar or cervical pain. Individuals who meet the overall inclusion and/or exclusion criteria and are taking high doses of opioids (>50 morphine milligram equivalents daily) will be eligible. After informed consent is obtained, participants will be randomized to adjunctive treatments with either oral cannabinoid (5mg/50mg ratio THC:CBD) or placebo treatment for 13 weeks. Whole plant extracts will be obtained from the NIDA source and compounded into the oral solution by study pharmacists. After stabilization on their maintenance dose of cannabis, patients will receive instruction for the CDC-recommended gradual tapering of their chronic opioid over 12 weeks, starting with a reduction of 10% (or the nearest achievable fraction available in their opioid formulation) of the total daily dose per week. Participants will attend weekly study visits that include NRS pain scores, neurocognitive assessments, mental health questionnaires, vitals, standardized field sobriety testing, subjective ratings of drug effects, and pain thresholds measured with a computer-controlled pressure algometer. Craving for opioids and study drug will also be assessed. A total of 94 participants will be enrolled into this trial, with a target of 84 study completions (42 per treatment group).

Discussion: Despite the frequent use of cannabis in our chronic spine pain patient population, without high quality scientific studies, physicians cannot make clear, evidence-based recommendations to their patients regarding the clinical efficacy of cannabis. To our knowledge, this is the first randomized controlled trial designed to evaluate daily use of a whole-plant extract oral solution as an adjunct therapy to reduce high-dose opioid use. These findings will be the first to provide critical clinical information regarding the potential for cannabinoids to alleviate chronic spine pain and reduce reliance on opioids for pain relief.

Acknowledgements: Funded by the Colorado Department of Public Health and Environment. The CU Clinical & Translational Research Center is supported by NIH/NCATS Colorado CTSA Grant Number UL1 TR002535.

EXPLORING THE EFFECTS OF EXERCISE AND CANNABIDIOL ON THE ENDOCANNABINOID SYSTEM

Ayshe Sahinovic, Peter Doohan, Richard C Kevin, Iain S McGregor and Danielle McCartney

Lambert Initiative for Cannabinoid Therapeutics, University of Sydney, Sydney, Australia

Introduction: Circulating concentrations of endocannabinoids (eCBs) are influenced by various factors such as feeding, circadian rhythms and physical activity. Studies consistently demonstrate increases in arachidonyl ethanolamide (AEA) following moderate intensity aerobic exercise (~70-80% HR_{max}). The response of other eCBs, however, is yet to be fully elucidated. Exogenous cannabinoids, such as cannabidiol (CBD), can modulate eCB concentrations and are increasingly used by athletes; thus, the interactive effects of exercise and CBD on eCBs are of relevance. Accordingly, this pilot study investigated the response of a larger panel of eCBs and related molecules to exercise, alone and in combination with CBD.

Methods: On two occasions, nine endurance-trained males (VO_{2max}: 57.4±4.0 $mL \cdot min^{-1} \cdot kg^{-1}$), who had not used cannabinoids in >3 months, ran for 60 mins at a fixed, moderate intensity (70% VO_{2max}) (MOD) before completing an incremental run to exhaustion (MAX) on a treadmill. Participants received 300mg CBD (oral) or placebo with a standardised breakfast 1.5h prior to exercise in a randomised, double-blind, crossover design. Blood was drawn at Baseline (pre-treatment), Pre- and Post-MOD, Post-MAX and 1h Post-MAX. Plasma was analysed using LC-MS/MS for arachidonic acid (ARA); the *N*-acyl ethanolamines (NAEs) AEA, oleoyl ethanolamide (OEA), linoleoyl ethanolamide (LEA), and docosahexaenoyl ethanolamide (DHEA); and the monoacylglycerols (MAGs) 2-arachidonly glycerol (2-AG), 1-AG, 2-linoleoyl glycerol (2-LG), 1-LG, 2-oleoyl glycerol (2-OG) and 1-OG. Data were analysed using repeated-measures ANOVA with paired t-tests providing *post hoc* comparisons.

Results: All analytes demonstrated an effect of Time (p's<0.05); however the responses of NAEs and MAGs differed. The NAEs (and ARA) decreased after food intake, and increased from Pre-MOD to Post-MOD, remaining elevated Post-MAX and 1h Post-MAX, irrespective of CBD treatment (p's<0.05). With the MAGs, 2-AG and 1-AG concentrations were below the lower limit of quantification (0.25 $ng \cdot mL^{-1}$) and were not subjected to further analysis; however all others were generally unaffected by feeding and MOD but were higher Post-MAX than at Baseline, irrespective of CBD treatment (p's<0.05). With CBD, two eCBs (AEA and OEA) trended towards a Treatment × Time interaction (p's<0.10) with lower concentrations in the CBD condition than placebo Post-MAX (AEA: p=0.002; OEA: p=0.045). Time to exhaustion during MAX exercise did not differ with CBD (Placebo=1246±197, CBD=1286±150 s, p=0.612). CBD concentrations were 0±0, 3±2, 77±18, 164±35 and 99±26 $ng \cdot mL^{-1}$ across timepoints.

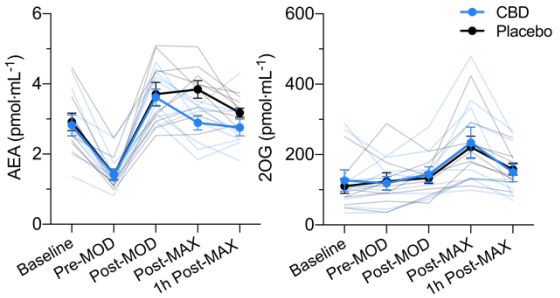


Figure 1. Plasma AEA and 2-OG concentrations

Conclusion: NAEs and MAGs exhibit distinct responses to feeding and moderate intensity aerobic exercise, with NAEs more responsive to both interventions. MAGs appear to increase with maximal intensity exercise only. CBD may subtly modulate the eCB response to maximal exercise, however, further research with a larger participant sample size and additional controls is required to confirm and better understand these preliminary findings.

Acknowledgements: Funded by the Lambert Initiative for Cannabinoid Therapeutics.

SCREENING FOR CENTRALLY-MEDIATED AVERSIVE EFFECTS OF CB1 RECEPTOR ANTAGONISTS USING INTRACRANIAL SELF-STIMULATION (ICSS)

Erica N. Golden[1], George Amato[2], Rosamond M. Goodson[1], Rangan Maitra[2] and Joel E. Schlosburg*[1]

1) Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, USA; 2) Discovery Sciences, RTI International, Research Triangle Park, NC, USA

Introduction: Preclinical studies show selective CB_1-receptor inverse agonists (e.g. rimonabant) produce suppression of reward-like effects of drugs and food, and suppress operant responding for almost all types of rewards. Clinical studies demonstrated severe risks of depression and suicidal ideation as a consequence of long-term use of CB_1 inverse agonists. Since this time, researchers have attempted to use preclinical screens to predict these untoward effects, and whether modifying the degree of inverse agonist properties or central activity can reduce the risks. He et al. (Acta Pharmacol Sin, *40*(3): 365-373) showed that the neutral antagonist AM4113 reduced heroin intake without altering ICSS responding, while rimonabant shifted the rate-frequency curve downward, suggesting a non-selective suppression. Other groups show no acute effect of rimonabant immediately after injection. Deroche-Gamonet et al. (Psychopharmacology (Berl), *157*(3):254-9) showed that rimonabant's response reduction fades after a few hours, while the aversive-like effects (threshold elevation) remain. We chose to reconcile these findings and create a rapid behavior screen for both potentially beneficial and untoward central activities of CB_1 antagonists using the reward sensitivity measurements of ICSS.

Methods: Male Wistar rats were implanted with electrodes in the medial forebrain bundle and trained on discrete-trial ICSS, which only requires turning a wheel approximately every 30s. These tests give a singular readout of reward threshold while requiring low operant responding, and are resistant to sedative and suppressive drug effects. The two-test battery used involved a time-course (0, 3, 6, 24hr) of either the test drugs alone to elucidate aversive-like activity, or as a pretreatment to cocaine (5mg/kg, ip) to examine reward-blocking activity. Our tests so far include rimonabant, and a handful of peripherally-preferential CB_1 antagonists with lower inverse agonist activity (RTI-1008348, RTI-1092661).

Results: Our tests show that rimonabant, in agreement with previous literature using the alternative rate-frequency curve ICSS, immediately and significantly reverses the reward-like threshold reductions of cocaine. However, the aversive-like properties (significant threshold elevation above vehicle control) of the same dose of rimonabant alone do not appear until 3hr post-injection. When repeating the procedures with moderately brain-permeable antagonists, we found no significant elevation of thresholds at any time, and a delayed reversal of cocaine's rewarding effects until 3hr, when brain levels peak.

Conclusions: Our results suggest that full timelines of ICSS alone can demonstrate aversive-like effects that may correlate to untoward negative mood states, while the cocaine reversal timeline can demonstrate general central activity that may apply to anti-addictive or feeding effects. To further validate and expand the model, we are expanding the study to a wider array of antagonists with diverse structure and activity, such as otenabant, taranabant, and AM4113.

DEVELOPMENTAL AND BEHAVIORAL CONSEQUENCES OF 2-AG ATTENUATION IN THE CEREBELLUM: IMPLICATIONS FOR AUTISM SPECTRUM

Gabriella R. Smith, Mayil Bhat., Blake Woods,
Max Rose, Ken Mackie and Anna Kalinovsky

The Gill Center for Biomolecular Science, Program in Neuroscience,
Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA

Introduction: Autism Spectrum Disorder (ASD) is one of the most prevalent neurodevelopmental disorders, affecting 1:44 children in the United States according to the latest CDC estimates. ASD is characterized by a wide range in prevalence and severity of cognitive, social, and emotional symptoms; however, deficits in social interactions, increased social avoidance and anxiety are at the core of this neurological condition. Despite years of intensive research, there are no effective pharmacological treatments for ASD, but following its legalization, some families have started to experiment with cannabis as means to alleviate social anxiety. Yet, neither safety, nor efficacy and specific neurological substrates of this treatment in ASD, are clearly understood. This study will investigate the role of cannabinoid signaling in cerebellar circuits regulating social approach. Since this behavior is strongly implicated in ASD pathology, understanding of these mechanisms is essential to guide the development of diagnostic and therapeutic tools.

Methods: To elucidate the role of cannabinoid signaling in the regulation of cerebellar circuits' development and activity, we generated cerebellar Purkinje cell specific conditional knockout mouse line (PC-Dagla-cKO). Anatomical and immunohistochemical staining and confocal microscopy and volumetric reconstruction were utilized to assess cellular and molecular consequences of endocannabinoid signaling attenuation in the cerebellum. Behavior of PC-Dagla-cKOs was assessed in motor and social tasks.

Results: Cannabinoid receptor 1 (CB1) is robustly expressed in the axons of basket cells (BCs), wrapping around Purkinje cell (PC) somata and axon initial segments, and providing strong inhibition, thus controlling cerebellar output. At the same time, Diacylglycerol Lipase a (Dagla), the primary synthesizing enzyme of the most abundant neuronal endocannabinoid *2*-Arachidonoylglycerol (2-AG), is primarily expressed in PCs. Thus, 2-AG is likely to serve as a paracrine retrograde signal regulating development and function of BC-PC synapses. Our results show that attenuation of cannabinoid signaling affects the size of BC-PC synapses, leads to behavioral alterations, and molecular changes in PCs. In sum, our results point to the important role of PC-derived 2-AG in regulation of cerebellar PC excitability and cerebellar-influenced behaviors.

Conclusions: Attenuation in cerebellar endocannabinoid signaling is associated with increased social anxiety, alterations in intrinsic PC excitability, and in BC-PC synapses.

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

ENDOCANNABINOID SYSTEM REACTIVITY DURING STRESS PROCESSING IN HEALTHY HUMANS

J. Spohrs[1], B. Abler [2], L. Bindila[3], M. Prost[2], P. Plener[1] and M. Ulrich[2]

[1]Dept. Child and Adolescent Psychiat., University Hospital Ulm, Ulm, Germany, [2]Dept. Psychiat., Ulm University, Ulm, Germany, [3]Medical University of Vienna, Vienna, Austria, [4]Facility of Endocannabinoids/Lipidomics, University Mainz, Mainz, Germany

Introduction: The past decades have shed insight into the mechanisms of the endocannabinoid system (ECS). Particularly its function as a contributor to stress adaptation seems of great interest due to its ubiquitous role in acute stress-related processes and anxiety disorders (Morena et al., *Neurophychopharmac.* 2019; Hill et al., *Mol. Psychiatry* 2013; Spohrs et al., *Transl. Psychiatry* 2021). Previous research has largely focused on rodent models to investigate the ECS, and more translational studies are necessary. Summarizing, the findings suggest that the ECS acts as a stress buffer and may exert a protective function against anxiety provoked by stress or aversive stimuli (Morena et al., *Neurophychopharmac.* 2019, (Mayo et al. *Biol. Psychiatry* 2019). Thus, the ECS poses a great opportunity to enhance therapeutic approaches for stress-related disorders. The two main endocannabinoids of interest are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). AEA is degraded by fatty acid amide hydrolase (FAAH) and 2-AG by monoacylglycerol lipase (MAGL). Inhibition of these enzymes seems to be a promising approach to modulate ECS-activity (Mayo et al. *Biol. Psychiatry* 2019; Dincheva et al. *Nat. commun*. 2015).

Methods: In the present study, 22 healthy men were exposed to a thermal heat stressor in a fear conditioning paradigm with geometric figures (Spohrs et al., *Transl. Psychiatry* 2021, Spohrs et al., *Europ. Arch. of Psychiatry and Neuroscience*, 2021). To elucidate the role of circulating plasma AEA and 2-AG in this processes, blood samples were taken before and after the experiment and analysed using liquid chromatography-mass spectrometry. Here, we report data related to the stress response to the thermal stimuli as measured with the skin conductance response (SCR), ECS-signalling, task-related anxiety ratings and data on critical life experiences (CLE).

Results: Interestingly, particularly pre-to-post increases in 2-AG were correlated with the other parameters investigated: smaller stress responses as measured by EDA (r (20) = -0.50, p = 0.017), by trend a higher number of critical life events (r (20) = 0.39, p = 0.077), and greater stimulus-related conditioned anxiety (r (20) = 0.50, p = 0.018). The latter parameter was also correlated with the baseline level of 2-AG *(*r (20) = 0.50, p = 0.017). Last, smaller stress responses (EDA) correlated with a higher number of critical life events (CLE) (r (20) = -0.52, p = 0.013).

Discussion: Results from our current experimental setup, designed to advance the transfer of animal research regarding stress responses and ECS-signalling, are in alignment with the hypothesis of an essential role of endocannabinoids in the modulation of stress-related responses in humans. We demonstrate a relation between endocannabinoid level changes from pre-to-post acute stress and the stress response. Our results suggest that investigating the role of 2-AG in the response to stress could be promising in finding treatments in the immediate aftermath of traumatic events. However, our results cannot disentangle the potentially different roles of 2-AG, AEA and adverse early life experiences, and future research is necessary.

EFFECT OF GAMMA IRRADIATION ON THE CANNABINOIDS AND TERPENES CONTENT OF CANNABIS BIOMASS

Chandrani Majumdar[1]; Mohamed M. Radwan[1]; Donald Stanford[1]; Suman Chandra[1]; Amira Wanas[1]; Mostafa A. Elhendawy[1], Elsayed A. Ibrahim[1] and Mahmoud A. ElSohly[1,2]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences and [2]Department of Pharmaceuticals and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677

Most of the cannabis plant material produced for research and provided to investigators through the National Institute on Drug Abuse (NIDA), Drug Supply Program (DSP) is from outdoor grown cannabis. It follows that these materials are subject to environmentally dominant and innocuous organisms. While we have been providing these materials without irradiation for the last 50 years without any issues and have encountered no problems related to microbial contamination, it was decided that decontamination of the plant biomass would be advisable given the fact that some studies might involve immune compromised subjects, hence the decision to carry out the irradiation.

This study is designed to determine the effect of irradiation on the chemical profile of the cannabis biomass, particularly the cannabinoids content, terpenes content and moisture content.

Irradiation was carried out (under proper security controls) at Sterigenics' facility (West Memphis, Arkansas), with irradiation specification range of 10-30 KGY. Dosimeters were placed in every container to determine the irradiation dose attained for each box. The results showed that the minimum dose was 15 KGY, and the maximum dose was 20.8 KGY for the 33 boxes irradiated, well within the specifications.

The plant material was analyzed for cannabinoids, terpenes, and moisture content before and after irradiation, as well as total aerobic bacteria and total yeast and mold count.

The results showed that while irradiation has little to no effect on the cannabinoid, terpene and moisture contents, it resulted in virtual sterilization of the plant material. These results are in agreement with the work previously reported. (Arno Hazekemp, Frontiers in Pharmacology, 7, article 108, 2016).

This work is Supported by the National Institute on Drug Abuse, Contract # NO1DA-15-7793

CLINICAL OUTCOME DATA OF CHRONIC PAIN PATIENTS TREATED WITH CANNABIS-BASED OILS AND DRIED FLOWER IN THE UNITED KINGDOM: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

James Tait[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Azfer Usmani[2,4], Mohammed Sajad[2,5], Jonathan Hoare[1,2], Shaheen Khan[2,6], Mark Weatherall[2,7], James J Rucker[2,8,9], Michael Platt[1,2] and Mikael H Sodergren[1,2]

1. Medical Cannabis Research Group, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Dartford and Gravesham NHS Trust, Kent, UK
5. Dudley Group of Hospitals NHS Trust, West Midlands, UK
6. Guy's and St. Thomas' NHS Foundation Trust, London, UK
7. Buckinghamshire Healthcare NHS Trust, Amersham, UK
8. Department of Psychological Medicine, Kings College London, London, UK
9. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Chronic pain is estimated to affect up to one-half of adult patients. Despite research on the therapeutic use of cannabis-based medicinal products (CBMPs), many datasets remain heterogeneous and inconclusive due to the diversity of treatment regimens. This study aims to evaluate clinical outcomes of patients in the UK Medical Cannabis Registry (UKMCR) treated exclusively with an inhaled dried flower (Adven EMT2, Curaleaf International, Guernsey,UK), a sublingual medium-chain triglyceride based oil (Adven 20 & Adven 50, Curaleaf International, Guernsey, UK), or a combination of both CBMPs for chronic pain.

Methods: A prospective cohort of patients from the UKMCR who were treated with dried flower, oils, or both CBMPs for chronic pain were analysed. Primary outcomes were changes in Short-form McGill Pain Questionnaire-2 (SF-MPQ-2), Brief Pain Inventory short-form (BPI), Pain Visual Analogue Scale (P-VAS), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L at 1, 3 and 6 months compared to baseline. Secondary outcomes included adverse event (CTCAEv4) analysis. Independent variables were examined for their association with adverse events utilising a logistic regression model. Statistical significance was defined as $p<0.050$.

Results: 761 patients were included in the final analysis. 348, 36 and 377 patients were treated with oils, dried flower, or a combination of both CBMPs respectively. For patients treated with oils or a combination of oils and dried flower, statistically significant improvements were seen within P-VAS, BPI, SQS, and SF-MPQ-2 at 1, 3 and 6 months ($p<0.050$). Improvements were also seen at 1 and 3 months for dried flower. Patients treated with both CBMPs had an improvement in GAD-7 at 1, 3 and 6 months ($p<0.050$). Oil only patients had an improvement in GAD-7 at 1 and 3 months. Significant changes in EQ-5D-5L-index were observed at 1,3 and 6 months for oils only and combination therapy, and at 1 and 3 months for dried flower patients ($p<0.050$). 1,276 (167.7%) adverse events were recorded. On multivariate analysis cannabis naïve patients were most likely to experience adverse events compared to prior consumers [Odds ratio: 3.399; 95% confidence interval: 2.022–5.715; $p<0.001$]. There was no effect according to route of administration [OR:0.869; 95% CI:0.561–1.347; $p=0.530$].

Conclusions: This study, which represents the largest cohort reported in the UK, indicates an association between treatment with dried flower and sublingual oils with health-related quality of life improvements for chronic pain patients. Adverse events were more likely to be experienced in cannabis naïve patients, and route of administration had no effect on the chances of developing an adverse event. Placebo-controlled trials are still necessary to distinguish the efficacy of CBMPs in the setting of chronic pain.

CHARACTERISATION OF DELTA-8 THC DISTILLATES USING HIGH RESOLUTION MASS SPECTROMETRY (HRMS) AND CYCLIC ION MOBILITY SPECTROMETRY COUPLED WITH HRMS

Marian Twohig[1], Andy Baker[2], Douglas Stevens[1], Andrew Aubin[2] and Christopher J. Hudalla[2]

[1]Waters Corporation, Milford, MA, USA;
[2]Waters Corporation, Pleasonton, CA, USA
[3]ProVerde Laboratories, Milford, MA, USA

Introduction: The use of delta-8 THC in consumer products has caused safety concerns in the US. Though delta-9-THC is the main pharmacologically active component in the cannabis plant, its psychoactive isomer, delta-8 THC, naturally occurs in the cannabis plant at low levels. Bulk delta 8-THC is typically produced from hemp derived CBD (<0.3% delta-9 THC) which many producers consider legal under the 2018 US Farm Bill. The regulations governing the use of synthetic components derived from hemp are not clearly addressed which has created a growing market for delta-8 THC production and use. The conversion of CBD to delta-8 THC requires harsh conditions leading to multiple reaction byproducts which need to be characterised to enhance understanding of the chemical components produced

Methods: Distillate samples were diluted with acetonitrile and analyzed using ESI-LC with a high-resolution quadrupole time-of-flight mass spectrometer (QToF) further work was performed on a quadrupole time-of-flight mass spectrometer equipped with a cyclic ion mobility separator. A C18 column, 2.1 x 100 mm, 1.6 µm maintained at 25^0 C was used to separate the cannabinoids using isocratic elution and a mobile phase consisting of 0.1% formic acid in water and acetonitrile. The mass spectrometer was operated in electrospray positive ion mode.

Results: Cannabinoid libraries were generated using available authentic standards, including accurate mass fragment databases and additional properties such as retention times, which were used to identify target components. The purity of the distillate samples measured by UV ranged from 79.0%-93.6%. Several known cannabinoids were identified in the samples based on retention time and UV spectra, including delta-8 THC, delta-9 THC and exo-THC. However, several unidentified peaks were also detected in the UV data. The UV spectra indicated that there may be structural similarities between the unknown components and the primary compound in the distillates, delta-8 THC. In the HRMS analysis, the software highlighted *m/z* 315.23186 as the base peak for several unknowns with proposed elemental compositions of $C_{21}H_{30}O_2$. The fragmentation data suggests the components share structural characteristics with the C21 neutral cannabinoids including delta-9 THC. Ion mobility spectrometry, which separates species on the basis of size, shape, and charge suggests that these species are additional isomeric forms of the C21 neutral cannabinoids. A chlorinated component with a proposed elemental composition corresponding to $C_{21}H_{31}ClO_2$ and common fragments with delta-9 THC and its isomers was also observed in a purified distillate sample.

Conclusion: Analysis of the delta-8 THC distillates revealed several components that appear to be structurally related based on the data that was available. Further work using ion mobility technology, showed evidence of co-eluting isomeric components illustrating the complexity of the distillate samples.

CANNABICHROMENE ACTIVITY AT CB2 RECEPTORS IS ENANTIOSELECTIVE

Michael Udoh[1,2], Adam Ametovski[2], Charles K. Marlowe[3], Jia Luo[2], Marina Santiago[1], Jianping Sun[3], Marika Heblinski[2], Iain McGregor[2], Philip J. Barr[3], Mark Connor[1] and Jonathon Arnold[2]

[1]Dept Biomedical Sciences, Macquarie University, Sydney, Australia,
[2]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Australia;
[3]BayMedica Inc, NV, USA.

Cannabichromene (CBC) is a phytocannabinoid that was demonstrated to potentiate the analgesic effect of Δ^9(-)-tetrahydrocannabinol in mice. It has been shown to attenuate carrageenan-induced and lipopolysaccharide-induced inflammation in rats and mice, respectively (DeLong et al., 2010; Turner & Elsohly, 1981). We recently showed that it is a CB2 receptor agonist but does not activate CB1 receptors (Udoh et al., 2019). Therefore, it is an interesting cannabinoid with significant therapeutic potential.

CBC is the only chiral phytocannabinoid with a significant amount of both enantiomers in Cannabis. These enantiomers exist as a scalemic mixture, which means they do not occur in equal proportions in the plant. We do not know the activity of each enantiomer and how they might affect CB2 receptor activation. There is also little information on the structure-activity relationship (SAR) of CBC analogues such as cannabichromenic acid (CBCA), cannabivarichromene (CBCV), cannabivarichromenic acid (CBCA) and other synthetic derivatives at both CB1 and CB2 receptors.

We examined the actions of (±)-CBC, its enantiomers and synthetic analogues in AtT20 and HEK cells stably transfected with CB1 and CB2 receptors. We used a FLIPR membrane potential assay to measure cellular hyperpolarisation in FlexStation 3 plate reader (AtT20 cells) and investigated ERK1/2 phosphorylation using pERK AlphaLISA assay (HEK cells). CBC enantiomers are provisionally labelled as 'Slow-CBC' and 'Fast-CBC', based on their mobilities on amylose column chiral HPLC and pending determination of circular dichroism.
In membrane potential assay, CP55,940 activated CB2 receptors with a pEC50 of 7.5 ± 0.04 and (±)-CBC induced a cellular hyperpolarisation that was 88 ± 8% of CP55,940 response. While slow-CBC also activated CB2 receptors with an *E*max similar to the enantiomeric mixture (87 ± 5%), fast-CBC was only capable of 37 ± 5% at 30 μM. Slow-CBC displayed about 2-fold higher potency than the enantiomeric mixture (1.3 μM vs 2.9 μM). We found that CBCV is inactive at CB1 but activates CB2 receptors with similar potency and efficacy to CBC. CBCVA and CBCA-induced response was non-specific to CB receptors.

Further SAR studies show that 1,2,3 -Triazole substitution at position 7 increased CBC efficacy to a full agonist (107% of CP55940) while side-chain 5-fluoro substitution did not substantially change the potency and efficacy. Truncation of the pentyl side chain to methyl resulted in a complete loss of activity. None of our chemical substitutions on CBC introduced activity at CB1 receptors. (±)-CBC also activated ERK phosphorylation with an *E*max similar to CP55,940 but did not significantly activate CB1 receptors.

We have identified the active enantiomer and pharmacophores of CBC. Pure CBC enantiomers may be preferred for the studies of CB2 mediated therapeutics. We provide evidence that CBCV is also a CB2 agonist without CB1 mediated activity. CBC or its derivatives could be further investigated for their therapeutic potential in conditions such as neuropathic pain, without concern for psychoactivity.

ENDOCANNABINOIDS PLASMA LEVELS IN SUBJECTS WITH SCHIZOPHRENIA WITH AND WITHOUT CANNABIS USE DISORDER

Unzueta-Larrinaga P.[1,5], Calvo A.[1], Villate A.[3], Barrena-Barbadillo R.[2], Etxebarria N.[3,6], Callado L.F.[1,4,5] and Urigüen L.[1,4,5]

Departments of [1]Pharmacology, [2]Nursing and [3]Analytical Chemistry, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain; [4]Centro de Investigación Biomédica en Red de Salud Mental CIBERSAM, Spain; [5]Biocruces Bizkaia Health Research Institute, Bizkaia, Spain and [6]PiE, University of the Basque Country UPV/EHU, Plentzia, Bizkaia, Spain.

Introduction: Schizophrenia is a mental disorder characterized by distortions in thinking, perception, emotions, language, sense of self and behaviour. About one of every four subject with schizophrenia has also a diagnosis of cannabis use disorder. Moreover, cannabis use is associated with an earlier age of onset and poor prognosis of schizophrenia. In this way, cannabis use may represent a risk factor for schizophrenia in individuals with an underlying genetic predisposition. In either case, alterations in the endocannabinoid system have been implicated in the pathophysiology of schizophrenia. Additionally, the presence of endocannabinoids in blood makes these molecules interesting for the study in schizophrenia patients by a minimally invasive technique.

Methods: The LC/MS/MS method was used for the quantification of two most common ECs, 2-AG and AEA, and five other lipid mediators (LEA, PEA, DEA, OEA, and NADA) in human plasma samples of subjects with schizophrenia (SZ), cannabis abuse disorder (CUD), dual diagnosis of schizophrenia and cannabis abuse disorder (DUAL) and their sex- and age-matched controls (C). Significant differences in ECs levels between schizophrenia and control subjects were tested simultaneously along with the effect of cannabis use by means of a two-way ANOVA.

Results: When examining differences in ECs presence in blood, a significant increase in AEA levels was found in SZ subjects compared to controls (*p<0.05), CUD (*p<0.05) and DUAL subjects (*p<0.05), while no changes were found in the levels of 2-AG or its isomer 1-AG in none of the groups evaluated. Moreover, PEA levels were also significantly increased in SZ subjects compared to controls (****p<0.0001), CUD (****p<0.0001) and DUAL subjects (****p<0.0001), while significantly decreased in CUD patients compared to controls (*p<0.05). For DEA, differences were found between SZ-CUD (*p<0.05) and SZ-DUAL (*p<0.05), although no changes were found between SZ and controls. On the contrary, OEA levels in DUAL patients significantly decreased when compared with all the others groups (***p<0.005 for DUAL vs. C; ****p<0.0001 DUAL vs. CUD; and *p<0.05 DUAL vs. SZ). Finally, a significant increase was found in NADA levels in DUAL subjects compared to controls (*p<0.05). No changes were found in LEA levels.

Conclusions: These results show the existence of different levels of circulating endocannabinoids in the blood of schizophrenia subjects with or without cannabis use disorder compared to controls. These changes could underlie the effect of cannabis abuse or been related to a genetic vulnerability to schizophrenia.

The authors declare they have no conflicts of interest. Funded by Spanish Ministry of Science and Innovation (PID2019-106404RB-I00), Spanish Ministry of Health (PNSD 2019I021) and Basque Government (2019111082).

ASSESSMENT OF ABUSE LIABILITY AND DEPENDENCE POTENTIAL OF HEMP-DERIVED Δ^8-TETRAHYDROCANNABINOL IN MICE

S. Olivia Vanegas [1,2], A. Matthew Reck[1,2], Carl E. Rodriguez[1,2], Julie Marusich[5], Omer Yassin[4], Gregory Sotzing[3,4], Jenny L. Wiley[5] and Steven G. Kinsey[1,2]

University of Connecticut [1]School of Nursing,
[2]Department of Psychological Sciences, and
[3]Department of Chemistry, Storrs, CT, USA
[4]3BC, Farmington, CT, USA
[5]Research Triangle Institute International, Research Triangle Park, NC, USA

Delta-8-tetrahydrocannabinol (Δ^8-THC) is a psychotropic cannabinoid produced in low quantities in the cannabis plant. Recent extraction and synthetic techniques, paired with the availability of inexpensive cannabidiol substrate, has resulted in Δ^8-THC being widely marketed as a quasilegal, purportedly milder alternative to Δ^9-THC. Yet, little research has probed the behavioral and physiological effects of repeated Δ^8-THC use. The present study aimed to evaluate the effects of acute and repeated exposure to Δ^8-THC. We hypothesized that Δ^8-THC produces effects similar to those of Δ^9-THC, including signs of drug tolerance and dependence, and would substitute for Δ^9-THC in a drug discrimination paradigm. Adult male and female C57BL/6J mice were treated acutely with Δ^8-THC (0-100 mg/kg, i.p.) or vehicle and tested repeatedly in the tetrad battery to quantify cannabimimetic effects (i.e., catalepsy, antinociception, hypothermia, immobility) as compared with the non-selective synthetic cannabinoid (WIN 55,212-2; 1-30 mg/kg, i.p.) and Δ^9-THC (1-50 mg/kg, i.p.). As previously reported, Δ^8-THC-treated mice ($\geq$12.5 mg/kg) displayed classic cannabinoid effects at doses comparable to Δ^9-THC. Pretreatment with the CB_1 receptor selective antagonist rimonabant (3 mg/kg, i.p.) blocked each of these effects. A separate group of male and female C57BL/6J mice were trained to discriminate the effects of Δ^9-THC (5.6 mg/kg, i.p.) from vehicle in a 2-nosepoke choice task and are currently being tested for generalization with Δ^8-THC. Results will be available at the summer meeting. In addition, repeated administration of Δ^8-THC (50 mg/kg, s.c.) produced tolerance as well as cross-tolerance to WIN 55,212-2 (10 mg/kg, s.c.) in tetrad, consistent with downregulated CB_1 receptor function. Lastly, rimonabant-precipitated withdrawal from Δ^8-THC ($\geq$10 mg/kg BID for 6 days) produced behavioral signs of physical dependence in the somatic signs, tail suspension, and marble burying assays. Together, these findings suggest that Δ^8-THC produces qualitatively similar effects to Δ^9-THC, including induction of drug dependence.

Research was supported financially by the National Institutes of Health: R01 AT010773, R01 DA048153, and R21 DA052690

OPIOID AND CANNABINOID INTERACTIONS: A STRATEGIC APPROACH TO REDUCE THE RISK OF OVERDOSE

Beth M. Wiese[1], Erika Liktor-Busa[1], Sarah A. Couture[1], Spyros P. Nikas[2], Lipin Ji[2], Yingpeng Liu[2], Alexandros Makriyannis[2], Todd W. Vanderah[1] and Tally M. Largent-Milnes[1]

[1]Department of Pharmacology, University of Arizona, 1501 N Campbell Ave., Life Sciences North Rm 621, Tucson, AZ, USA
[2]Chemistry and Chemical Biology, Bouve College Health Sciences – Center for Drug Discovery, 116 Mugar Life Sciences Building, Northeastern University, 360 Huntington Ave, Boston, MA, USA

Introduction: While opioids continue to be unrivaled in their analgesic efficacy, the number of accidental fatalities from opioids continues to climb. With estimates projected to eclipse 100,000 lives lost in one year to opioid overdoses, the necessity for novel approaches to intervene and prevent this unnecessary loss of life are needed. Recent evidence showed cannabinoid receptors 1 and 2 (CB_1R and CB_2R) in the respiratory centers of the brainstem, as well as a CB_2R agonist that prevent morphine induced respiratory depression (MIRD) in male CD1 mice. It is vital that the mechanisms by which the cannabinoid receptors mitigate MIRD be delineated. In these studies, we investigated if the attenuation of MIRD following coadministration of AM2301, a CB_2 agonist, and morphine would translate to the C57 mouse model and across sexes.

Methods: Whole-body plethysmography recordings of awake and freely moving, male and female, C57 mice were collected before and after drug administration of morphine 10mg/kg, a mu opioid receptor agonist, AM2301 10mg/kg, a cannabinoid 2 receptor (CB_2R) agonist, or both. Following completion of the experimental timeline, SR-1445628 10mg/kg, a CB_2 inverse agonist, was administered to reverse any effects of CB_2R activation and confirm receptor of action.

Results: C57 male mice show lower baseline respiratory recordings than females. Administration of AM2301 10mg/kg does not induce respiratory depression in either sex. Additionally, MIRD was mitigated when AM2301 10mg/kg and morphine 10mg/kg were coadministered. Administration of the CB2 inverse agonist, SR-144528 10mg/kg, on its own induced respiratory depression, as seen previously in CD1 male mice. Finally, SR-144528 administration following the coadministration of AM2301 and morphine reversed the mitigation of MIRD back to independent morphine administration depression levels across strain and sex in C57 mice.

Conclusions: These studies revealed attenuation of MIRD following the coadministration of a CB_2 agonist, AM2301, and morphine translates to the C57 mouse model and across sexes. These findings are critical to allow for in depth studies into the mechanisms by which CB2R agonism can block MIRD. The addition of this CB_2R agonist may be a unique strategy to attenuate the respiratory depression associated with opioid therapies, potentially saving lives.

Acknowledgements: Funded by grant R13DA016280 from the National Institute on Drug Abuse and departmental funding University of Arizona Medical Pharmacology and NIH/NIDA PO1 DA041307 (TWV).

LEI-515, A PERIPHERALLY RESTRICTED INHIBITOR OF MONOACYLGLYCEROL LIPASE, SUPPRESSES NEUROPATHIC NOCICEPTION IN A MOUSE MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

J. Wirt[1,2], M. Jiang[3], M. Huizenga[3], A. Makriyannis[4], M. van der Stelt [3] and A.G. Hohmann[1,2,5]

[1]Dept. of Psychological and Brain Sci., Indiana University, Bloomington, IN, [2]Program in Neuroscience, Indiana University, Bloomington, IN; [3]Department of Molecular Physiology, Leiden University & Oncode Institute, Netherlands, [4]Department of Pharmaceutical Sciences, Northeastern University; [5]Gill Center for Biomolecular Science, Indiana University, Bloomington, IN.

Introduction: Inhibitors of endocannabinoid deactivation show therapeutic potential for suppressing pathological pain. Monoacylglycerol lipase (MAGL) is the major enzyme that degrades the endocannabinoid 2-arachidonoylglycerol (2-AG). In these studies, we used LEI-515, a first-in-class peripherally restricted MAGL inhibitor, to validate the therapeutic potential of inhibiting MAGL selectively outside the central nervous system (CNS) as a therapeutic strategy for suppressing chemotherapy-induced peripheral neuropathy (CIPN).

Methods: We examined the impact of LEI-515 on behavioral sensitization to mechanical and cold stimulation that was induced by the taxane chemotherapeutic agent paclitaxel in mice of both sexes using both von Frey and acetone testing. Both interperitoneal (i.p.) and oral administrations of LEI-515 were implemented. AM251, AM630, AM6545, and SR144528 were used as pretreatments to assess pharmacological specificity of LEI-515 after efficacy was established, and were administered i.p.

Results: LEI-515 suppressed established allodynia following intraperitoneal and oral administration, normalizing paw withdrawal thresholds to pre-paclitaxel levels. Anti-allodynic efficacy of LEI-515 was sustained for at least 24 h following acute administration. Anti-allodynic efficacy of LEI-515, administered via intraperitoneal and oral (p.o.) routes of administration, was fully sustained following repeated dosing over 10 consecutive days. By contrast, tolerance developed to the brain penetrant MAGL inhibitor JZL184 in the same model (Slivicki et al, 2018). LEI-515 did not alter mechanical thresholds in control mice that received the cremophor vehicle in lieu of paclitaxel. LEI-515 similarly suppressed cold allodynia in paclitaxel-treated mice but not in control mice that received the cremophor-based vehicle. Pharmacological specificity of LEI-515 anti-allodynic efficacy was assessed in paclitaxel-treated mice using CB1 (AM251 and AM6545) and CB2 (AM630 and SR144528) antagonists that differ in their ability to penetrate the CNS. Anti-allodynic efficacy of LEI-515 was fully blocked by either CNS penetrant (AM630) or peripherally restricted (SR144528) CB2 antagonists but not by CNS penetrant (AM251) or peripherally restricted (AM6545) CB1 antagonists. These observations suggest that the therapeutic profile of LEI-515 is mediated by a peripheral CB2 mechanism.

Discussion: Our studies suggest that LEI-515, a peripherally restricted MAGL inhibitor, shows promise as a therapeutic strategy for suppressing CIPN without producing unwanted pharmacological effects associated with direct CB1 activation.

Supported by National Institute on Drug Abuse Grants DA047858, DA041229 and DA042584 (A.G.H. and K.M.), and the National Cancer Institute Grant CA200417 (A.G.H.).

2-ARACHIDONOYLGLYCEROL SIGNALING IN THE LATERAL HABENULA: IMPACTS ON STRESS COPING BEHAVIOR AND DOWNSTREAM IMMEDIATE EARLY GENE EXPRESSION

H.R. Wright[1]; Z.D.G. Fisher[1]; R. Urrutia-Carmago[1]; D.E. Ginder[2]; A.M. Brown[1]; H.S Arey[1]; J.L. Ritchie[1] R.A. Fuchs[1] and R.J. McLaughlin[1,2]

Departments of Integrative Physiology and Neuroscience[1] and Psychology[2], Washington State University, Pullman, WA, USA,

Introduction: Differences in stress coping strategies impact the development of stress-related disorders and are orchestrated in part by activity of dopamine (DA) neurons in the ventral tegmental area (VTA) and serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN). The lateral habenula (LHb) regulates both DA and 5-HT activity via inhibitory neurons within the rostromedial tegmental nucleus that project to the VTA and DRN. Our previous data indicate that the endocannabinoid (eCB) 2-arachidonoylglycerol (2-AG) is recruited in the LHb during stress, and intra-LHb cannabinoid type-1 receptor agonist or inverse agonist administration increases avoidant and approach behavior respectively, suggesting that manipulating the concentration of 2-AG by interfering with its synthesis or metabolism may affect LHb-mediated regulation of behavioral responses. However, the role of LHb 2-AG in the expression of stress coping strategies and the downstream mechanisms underlying these effects remain unknown. Thus, we aimed to determine if altering LHb 2-AG concentration during acute or chronic social defeat stress (SDS) affects coping behavior and downstream expression of the immediate early gene cfos.

Methods: Male Sprague Dawley rats (n=7-9/group) were implanted with bilateral cannula aimed at the LHb. Rats received a microinfusion of the 2-AG hydrolysis inhibitor MJN110 (0, 0.5, or 1 μg/side) prior to acute SDS exposure. In the chronic SDS experiment, rats underwent 7 daily SDS sessions. Rats received a microinfusion of the 2-AG synthesis inhibitor DO34 (0, 0.07, or 0.7 μg/side) prior to the last session. Sessions were video recorded for analysis with deep learning pose-estimation and predictive classifier software to track movement and identify attack, approach, investigative, and avoidance behaviors. Rats from the DO34 experiment were euthanized 2 hr after the SDS session and brain tissue encompassing the VTA, and DRN was fixed, sliced, and immunostained for fos and tyrosine hydroxylase (TH) or tryptophan hydroxylase (TPH) expression for analysis.

Results: Preliminary results indicate that rats receiving 1μg MJN110 during an acute SDS session spent less time being attacked, had a lower mean velocity, and traveled less distance than vehicle-treated rats. In the chronic SDS experiment, rats receiving 0.7 μg DO34 before the final session trended towards being attacked less and investigated more by the resident rat, exhibiting a lower mean velocity, and traveling less distance. 28% of 0.7 μg DO34 were defeated compared to 44% of 0.07μg DO34 and 71% of VEH. Furthermore, rats receiving 0.7 μg DO34 had significantly lower fos+TPH colocalization in the DRN compared to VEH, whereas no differences were seen in fos+TH colocalization in the VTA.

Conclusions: Interestingly, the manipulation of LHb 2-AG in each of our experiments resulted in a similar passive phenotype. During acute SDS, increasing LHb 2-AG promotes a passive behavioral strategy that significantly reduces attacks, with no differences in move-away behaviors. In the chronic SDS experiment, blocking LHb 2-AG synthesis for the last session appears to promote passive behavior and reduces defeats. This did not attain statistical significance, perhaps due to low sample size. Additionally, reducing LHb 2-AG synthesis was associated with a decrease in downstream DRN 5-HT activation, against our predictions. A similar trend was seen in VTA DA activation, though a floor effect due chronic stress may obfuscate an effect. These results contribute to a growing body of evidence revealing a fascinating role for eCBs in regulating LHb activity and further our understanding of brain circuits/mechanisms that give rise to divergent stress coping strategies.

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

Jacquelyn L. Bainbridge, Sarah N. Rajkovic, Maureen Leehey, Ying Liu, Mahmoud A. ElSohly, Michelle Adkins

Department of Clinical Pharmacy and Neurology, University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, Aurora, CO, USA

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

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

Results: The clinical research pharmacists at the University of Colorado Anschutz Medical Campus took NIDA crude plant extract and compounded it into a placebo product and cannabis extract oral solution with the desired ratio of 30 CBD:1 THC for use in a clinical trial. This compounding process allows for individualized final volumes based on weight of the patient to allow for accurate, weight-based dosing. At 1 month and 21 months, the accuracy of the initial compounded product was 89.75% and 57.61% for THC and 96.45% and 92.84% for CBD stability. The numbers listed below are from original samples sent to NIDA for analysis. However, investigational drug products are compounded and dispensed for patients at the time of their participation in the trial for a dosing timeline of 2 weeks, so the 1-month stability accuracy displays a safe result.

	Time 0	Week 1	Week 2	1 Mo	2 Mo	3 Mo	6 Mo	12 Mo	15 Mo	18 Mo	21 Mo
THC	100.00 %	94.83 %	89.09 %	89.75 %	88.89 %	91.73 %	92.48 %	69.55 %	60.46 %	56.45 %	57.61 %
CBD	100.00 %	98.05 %	98.95 %	96.45 %	96.68 %	98.87 %	94.63 %	95.60 %	93.33 %	92.01 %	92.84 %

Conclusion: The NIDA crude plant extract was successfully compounded into an oral solution with the desired ratio of 30 CBD:1 THC for use in a clinical trial. Preparation of the solution provides specialized pharmacists involved in clinical trials with resources to compound the crude plant extract product for use in other clinical research trials. By increasing diversity of formulations and strengths of investigational cannabis products that both meet federal requirements and more similarly mimic what is seen in the real-world use, researchers can begin to bridge the gap in knowledge.

Acknowledgements: Funded by the Colorado Department of Public Health and Environment (ClinicalTrials.gov Identifier: NCT03582137)

DAILY ASSOCIATION WITH CANNABIS USE AND SLEEP QUALITY IN ANXIOUS CANNABIS USERS

L. Cinnamon Bidwell, Renée Martin-Willett and Sharon R Sznitman

[1]University of Colorado Boulder, Boulder, Colorado, USA
[2]School of Public Health, University of Haifa, Haifa Israel

Introduction: Cannabis is increasingly used to self-treat anxiety and related-sleep problems, without clear evidence either supporting or refuting its anxiolytic or sleep aid effects. In addition, different forms of cannabis (flower vs. edible) and primary cannabinoids (THC and CBD) have differing pharmacological effects.

Methods: Thirty days of daily data on sleep quality and cannabis use were collected in individuals who use cannabis for mild to moderate anxiety (n=347; 36% male; mean age=33 years). Participants self-reported both the form (flower or edible) and the ratio of THC to CBD in the cannabis used during the observation period.

Results: Individuals who reported cannabis use on a particular day also reported better sleep quality the following night. Moderation analyses showed that better sleep after cannabis use days was stronger for respondents with higher baseline affective symptomatology.

Furthermore, respondents who used cannabis flower reported poorer quality sleep compared to those who used edibles, and this association was particularly strong among respondents using cannabis with a higher CBD concentration.

Conclusions: Among individuals with affective symptomatology, naturalistic use of cannabis was associated with better sleep quality, particularly for those using edible and CBD dominant products.

Acknowledgements: This study was funded by a grant to LCB from the National Institute on Drug Abuse (DA044131).

PHARMACOKINETIC AND MOLECULAR CHARACTERIZATION IN A RAT MODEL OF PRENATAL CANNABIS SMOKE EXPOSURE

Tallan Black[1,2], Sarah L. Baccetto[1,2], Ilne L. Barnard[2], Dan L. McElroy[2], Emma Finch[2], Faith V. L. Austin-Scott[2], Quentin Greba[2], Deborah Michel[1], Ayat Zagzoog[1], John G. Howland[2] and Robert B. Laprairie[1]

[1]College of Pharmacy and Nutrition, and [2] Department of Anatomy, Physiology, and Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon SK CAN.

Legalization of cannabis in Canada has changed the social narrative surrounding use and risk perception of cannabis across generations. *Cannabis sativa* has gained popularity as a "natural substance", leading many to falsely assume that it is not harmful. This assumption has been documented amongst pregnant mothers, many of whom consider cannabis use during pregnancy to be benign. Mirroring shifts in perception, access to and concentration of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the main psychoactive constituents of the cannabis flower in smoked products, has increased. Fetal developmental processes are sensitive to exogenous cannabinoids because the type 1 cannabinoid receptor (CB1R) regulates synaptic architecture in the developing brain, and the type 2 cannabinoid receptor (CB2R) modulates immune function throughout the reproductive system, but specifically at the maternal-fetal interface. The purpose of this study was to validate a newly developed smoke exposure method by determining plasma levels of cannabinoids and associated metabolites in pregnant rats and assess preliminary effects of exposure on maternal and offspring health and behaviour.

Methods: Pregnant Sprague-Dawley rats were treated with high THC cannabis smoke (Skywalker Kush, 17.83% THC, Aphria), high CBD cannabis smoke (Treasure Island Kush, 12.98% CBD, Aphria), *i.p.* injections of 3 mg/kg THC, 10 mg/kg CBD, vehicle (1:1:18 Ethanol: Kolliphor: Saline), or air control daily between gestational days (GD) 6 and 20. Accumulation of phytocannabinoids in maternal plasma was assessed via LC-MS/MS where samples were collected 30 mins after treatment daily GD6 through GD20. Placenta and pup brains were collected on GD20 and post-natal day 1 (PND1) for western blot of CB1R, FAAH and MAGL. Additional animals were reared for behavioural analysis starting in adolescence (PND30-40), and adulthood >PND 54 to assess social behaviours, learning and memory, attention and impulsivity, stress, and anxiety-like phenotypes.

Results: THC and CBD but not their metabolites accumulated in maternal plasma between gestational day 6 and 20, in all treatment group 30 mins post-administration. 3 mg/kg THC decreased litter size and 10 mg/kg CBD increased uterine reabsorptions where no other treatments did. Preliminary PND 1 western blot data indicate that both high THC (Skywalker) and high CBD smoke (Treasure Island) in utero significantly increase levels of CB1R in comparison to 3 mg/kg THC and Control. No differences were detected between levels of FAAH or MAGL.

Conclusion: This model is the first of its kind to validate smoke exposure in a rat model of prenatal cannabis exposure. These results support existing, but limited knowledge on how different routes of administration contribute to inconsistent pathophysiological manifestations of endocannabinoid system dysregulation in pregnancy. Smoked cannabis is still the most common means of consumption, and more preclinical investigation is needed to determine the effects of smoked cannabis on developmental trajectories.

Acknowledgements: This work is supported by a GlaxoSmithKline-Canadian Institutes of Health Research (CIHR) partnership grant and to Brain Canada Future Leaders Award RBL, and bridge funding from the University of Saskatchewan College of Medicine to JGH. TB is supported by a scholarship from The University of Saskatchewan College of Pharmacy and Nutrition and College of Medicine.

US MEDICAL CANNABIS PATIENT REGISTRATION AND REASONS FOR USE: 2016-2020

Kevin F. Boehnke[a], Owen Dean[b] and Avinash Hosanagar[c,d]

[a]Anesthesiology Department, University of Michigan Medical School, Ann Arbor, MI, USA
[b]College of Literature, Sciences, and the Arts, University of Michigan, Ann Arbor, MI, USA
[d]Veterans Affairs Ann Arbor Healthcare System, Ann Arbor, Michigan, United States
[e]Department of Psychiatry, University of Michigan Medical School, Ann Arbor, MI, USA

Introduction: Cannabis policy liberalization has resulted in widespread cannabis availability through medical and adult-use legislation in the US. Up-to-date trends in medical cannabis licensure can inform clinical policy and care. Our goal was to describe recent trends in medical cannabis licensure in the US from 2016-2020.

Methods: We collected data from state medical cannabis registries using publicly available state reports, communications with and data requests of the governmental departments overseeing medical cannabis programs, and data requests. We measured total patient volume, patients per 10,000 of total population, and patient-reported qualifying conditions (i.e., symptoms/conditions qualifying patients for licensure) – including whether these symptoms align with current therapeutic evidence of cannabis/cannabinoid efficacy.

Results: Overall, 29 states and Washington DC reported patient numbers, and 20 states reported qualifying conditions per year. Total enrolled patients increased 4.5-fold from 661,990 in 2016 to 2,974,443 in 2020. Patients per 10,000 total population generally increased from 2016-2020, most dramatically in Oklahoma (927.1 patients per 10,000 population). However, enrollment increased in states without adult-use legalization (i.e., medical only states), while enrollment decreased in 5/7 (71%) with adult-use legalization (i.e., adult-use states). In 2020, 68.2% of patients had licenses for conditions with substantial or conclusive evidence of therapeutic value, compared to 84.6% in 2016. Chronic pain was the most common patient-reported qualifying condition in 2020 (60.6%), followed by post-traumatic stress disorder (10.6%) and multiple sclerosis (4.8%). Our findings are limited by missing state data (e.g., California), which may affect reported trends.

Conclusions: Enrollment in medical cannabis programs increased 4.5-fold from 2016 to 2020, although enrollment decreased in adult-use states. Use for conditions/symptoms without a strong evidence basis increased from 15.4% (2016) to 31.4% (2020). Thoughtful regulatory and clinical strategies are needed to effectively manage this rapidly changing landscape.

Acknowledgements: Dr. Boehnke's effort on this publication was partially supported by the National Institute on Drug Abuse of the National Institutes of Health under Award Number K01DA049219. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CANNABIDIOL-DERIVED VARIANTS AS POTENTIAL NEGATIVE ALLOSTERIC MODULATORS AT THE MU OPIOID RECEPTOR

Taryn Bosquez[1], Jessie Gudorf[2], Michael VanNieuwenhze[2] and Alex Straiker[1]

[1]Gill Center for Biomolecular Science, Department of Psychological and Brain Sciences, Program in Neuroscience, Indiana University, Bloomington, IN USA 47405
[2]Department of Chemistry, Indiana University, Bloomington, IN USA 47405

Introduction: The United States has experienced one of the most severe waves of the opioid epidemic during the last decade. The drugs with the highest abuse potential are synthetic opioids, such as fentanyl. The increased potency of these synthetic opioids can reduce the therapeutic effect of Narcan (naloxone), which competitively targets the primary (orthosteric) binding site of the mu opioid receptor (μOR). As a result, these highly potent drugs are a leading cause of many recent opioid overdose deaths. In addition to an orthosteric site, receptors like the μOR, also contain a distinct secondary binding site called an allosteric site. Ligands that occupy this site are called allosteric modulators, as they modulate the structure of the receptor and can change the binding affinity of the orthosteric site. Ligands that reduce receptor activity are called negative allosteric modulators (NAMs). NAMs have the potential to reduce synthetic opioid induced signaling without competing for the orthosteric site. Cannabidiol (CBD), one of the main constituents of *Cannabis*, has been implicated as a NAM at the μOR; however, with a relatively low affinity.

Methods: After an initial screen of ~50 CBD variants, we tested and compared seven promising synthetic analogs of CBD (JGCx) to determine if these had an improved affinity and more potent NAM activity at μOR, using a reliable *in-vitro* cAMP-based assay that used a fluorescent binding protein to measure cAMP accumulation in μOR-expressing HEK293 cells. When μOR is activated, its coupling to the $Ga_{i/o}$ G protein subunit leads to an inhibition of cAMP accumulation. Each CBD analog was tested at 500nM against μOR agonist, DAMGO, and in a concentration series against μOR agonist fentanyl, to measure cAMP accumulation in μOR-HEK293 cells. Area under the curve analysis allowed us to compare the signaling characteristics of CBD and its seven analogs.

Results: CBD and the synthetic CBD analogs (JGCx) all interfered with DAMGO- and fentanyl-induced μOR singling, by increasing cAMP production following their application to HEK293-μOR cells. Further analysis revealed the synthetic analogs were more potent than the phytocannabinoid CBD (CBD IC_{50}: 1.8μM; JGC2 IC50: 90nM; JGC13 IC50: 137nM; JGC21: 499nM; JGC22: 556nM; JGC25: 556nM; JGC26: 173nM; JGC31: 242nM) in interfering with fentanyl-induced signaling.

Conclusion: These results indicate not only the *Cannabis*-derived CBD, but also seven synthetic CBD analogs, have the potential to be considered negative allosteric modulators, as they attenuate μOR activation in the presence of potent agonists. These results also suggest specific modifications to the CBD chemical structure that make it possible to enhance the potency of the root compound. Understanding the role these structural modifications play in allosteric interactions will be important for the elucidation of the allosteric site at the μOR and will pave the way for developing a novel class of potentially therapeutic compounds that noncompetitively curb opioid signaling.

SEX DIFFERENCES IN SOMATOSENSORY SENSITIVITY, ACUTE PSYCHOLOGICAL STRESS RESPONSE AND ENDOCANNABINOIDS

Stephanie Bourke[1,3,4], *Therese O'Connor[4,5], Nikita N. Burke[1,3,4], Mary Hopkins[1,3,4], Massieh Moayedi[6], Brian E. McGuire [2,3,4] and David P. Finn[1,3,4].

[1]Pharmacology and Therapeutics, School of Medicine, [2]School of Psychology, [3]Galway Neuroscience Centre and [4]Centre for Pain Research, National University of Ireland Galway, [5]Sligo University Hospital, [6]Centre for Multimodal Sensorimotor and Pain Research, Faculty of Dentistry, University of Toronto. *Joint first authors.

Introduction: The endocannabinoid system is involved in a multitude of physiological processes, including pain processing, affective state, and the stress response. Stress and affective state have a complex, modulatory influence on pain (Corcoran *et al*. 2015, *International Review of Neurobiology*, 125, 203-255). The aim of this study was to investigate the effect of acute psychological stress on somatosensory sensitivity (using quantitative sensory testing [QST]), and peripheral endocannabinoid/*N*-acylethanolamine levels, in male and female healthy human volunteers.

Methods: 26 healthy volunteers were recruited and consented to procedures approved by the NUI Galway Research Ethics Committee (14 female, 12 male; mean age ± SEM: 26 ± 0.8 years). Participants were exposed to acute psychological stress using the Montreal Imaging Stress Task (MIST) or a non-stressful control version of the MIST, followed by QST comprising a standardized battery of tests to determine thermal (cool, warm) detection and pain (cold, heat) thresholds, heat pain tolerance and conditioned pain modulation (CPM). Blood samples, heart rate (HR), and state-anxiety scores were obtained at 3 time points throughout the study: baseline, post-MIST and post-QST. Quantification of plasma 2-arachidonoylglycerol (2-AG), anandamide (AEA), *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), was carried out by LC-MS/MS. Appropriate parametric or non-parametric statistical analyses were performed, $P<0.05$ was considered significant.

Results: Self-reported state anxiety scores were significantly higher following the MIST, compared to its control. Cool detection threshold (CDT) was significantly increased (p=0.006) and warm detection threshold (WDT) was significantly decreased (p=0.015) (i.e. greater sensitivity for both) following exposure to the MIST in females but not males. CPM was less efficient following exposure to the stress version of the MIST compared to control. There were no significant stress-induced differences in plasma 2-AG, AEA, PEA and OEA concentrations in males or females. However, AEA was negatively correlated with cold pain threshold (CPT) at post-MIST and post-QST and 2-AG was negatively correlated with CPT at baseline and post-MIST control on the control day, but not on the day participants were exposed to stress in both male and female participants. On the day of the stressor, AEA was also positively correlated with WDT post-MIST and post-QST. In females, 2-AG levels were positively correlated with pressure pain threshold during CPM post-MIST, but not post-MIST control.

Conclusions: Acute psychological stress (using the MIST) resulted in sex differences in somatosensory sensitivity. Exposure to the MIST, compared to control, resulted in increased cool and warm sensitivity in females but not males. There were no effects of stress on cold or heat pain thresholds, heat pain tolerance or endocannabinoid/*N*-acylethanolamine levels. Following exposure to the MIST, participants had less efficient CPM. Circulating endocannabinoid levels were differentially correlated with somatosensory thresholds in a sex- and stress-dependant manner.

Acknowledgements: Funded by the Irish Research Council Postgraduate Scholarship. The authors wish to thank Jens Pruessner for use of the MIST software, Ruth Defrin and Lior Pinchas for providing training in the MIST software, and the staff of the HRB Clinical Research Facility Galway.

SEX DIFFERENCES IN CANNABINOID ACTIVITY IN THE SALIVARY GLAND DRIVES CHANGES IN LIPIDOMIC PROFILES: IMPLICATIONS FOR DRY MOUTH MECHANISMS

Heather B Bradshaw, Hannah R Bentz, Clare T Johnson and Alex Straiker

Psychological and Brain Science, Indiana University Bloomington IN USA

Intro: Salivation is arguably an incredibly vital aspect of mammalian physiology that is vastly underappreciated and understudied. The side effect of "dry mouth" is a common occurrence after Cannabinoid (CB) exposure; however, the mechanism of action of this relationship is unknown. Sjögren's syndrome is an autoimmune disorder with the primary symptom of dry mouth and is predominantly diagnosed in females, suggesting a significant divergence in how salivation is regulated between males and females. Unfortunately, little data about sex differences in dry mouth with CB use have been reported; however, there are many other aspects of CB use that have significant sex differences.

Methods: Using lipid extraction and partial purification via C18 SPE and HPLC/MS/MS, we examine the lipidome of the submandibular salivary gland (SMG) in 3–5-month-old female and male mice in 4 treatment groups: vehicle (1:1:18-Cremophor:ethanol:saline), 10mg/kg THC, 10mg/kg CBD, and 10mg/kg THC +10mg/kg CBD. n=8-11 per group with a total of 78. We hypothesize that there will be underlying sex differences in endogenous lipids and that CBs will be differentially regulated within the SMG.

Results: THC levels were equivalent in females and males within treatment groups; however, the levels measured in the T+C group were significantly higher than THC only. By contrast levels of CBD in females were significantly higher in the CBD only group but equivalent in the T+C group. Importantly, levels of CBD were *significantly higher* than THC in all treatment groups, suggesting either a slower rate of transport or a slower rate of metabolism of CBD than THC in the SMG.
Levels of CB metabolites 11 nor 9-COOH THC, 11-OH THC, 7-COOH CBD and 7-OH CBD were measured in all groups. 11-OH THC and 7-OH CBD levels in females in the T+C group were significantly higher than all other groups illustrating an important sex difference in metabolism of CBs but also indicating that the combination of CBs matters in how this differentiates. Levels of 11 nor 9-COOH THC were significantly higher in the THC only group compared to the T+C group overall with no sex differences measured. 7-COOH CBD levels were equivalent across all treatment groups but had much higher variability in females suggesting underlying differences rates of metabolism.
Levels of the eCB, 2-AG and its congeners (2-LG, 2-PG, and 2-OG) were significantly lower in males compared to females at baseline. This dramatic difference was eliminated in T+C treatment but was largely unaffected in THC or CBD alone. No baseline sex differences in Anandamide and its congeners (NAEs) were measured; however, all CB treatment groups reduced the levels of most NAEs in females and partially in males. This significant reduction is in contrast to increases in NAEs measured in brain and plasma previously; however, this may be due to the increase in dose at 10mg/kg, whereas previous studies used 3mg/kg dose. There are additional insights into ~50 endogenous lipids in the SMG and how the intersection of sex and CB treatment drive their signaling changes in this data set.

Conclusions: Salivation is an intricate physiological process that involves aquaporin (AQP) proteins that regulate the flow of water through specialized pores within specialized cellular structures within the SMG. How AQP proteins are regulated is unclear; however, there is a clear relationship between CB exposure and changes in salivation in a large percentage of people. Dry mouth disorders predominate in women, therefore, there are likely sex differences in how salivation is regulated. Data here provide a unique insight into both sex differences in eCB signaling as well as CB metabolism in the SMG that may shed light on salivation regulation.

CANNABINOID TOXICITY-RELATED EMERGENCY DEPARTMENT VISITS AND INPATIENT HOSPITALIZATIONS IN KENTUCKY, 2017 TO 2019

Stevie C. Britch[1,2], Sharon, L. Walsh[1,2,3,4,5], Rachel Vickers-Smith[1,6], Shanna Babalonis[1,2] and Svetla Slavova[7,8]

[1]Center on Drug and Alcohol Research; Departments of [2]Behavioral Science, [3]Pharmacology, [4]Pharmaceutical Sciences, [5]Psychiatry, [6]Epidemiology, & [7]Biostatistics; [8]Kentucky Injury Prevention and Research Center, University of Kentucky, Lexington, KY, USA

Introduction: Cannabis use has increased over the past several decades, while simultaneously cannabis product potency has increased and social perceptions of cannabis have shifted toward the assumption that cannabis is safe. However, cannabis and synthetic cannabinoids can cause physical and psychiatric side effects for which people may seek medical care. This study aimed to characterize cannabinoid toxicity-related emergency department (ED) visits and inpatient hospitalizations, toxicity due to cannabinoids in combinations with other psychoactive substances, and the occurrence of cardiac complications during a cannabinoid toxicity event, from statewide data in Kentucky.

Methods: Administrative billing data for ED visits and inpatient hospitalizations in acute care facilities with a discharge date from January 1, 2017 to December 31, 2019 were used to characterize cases of cannabinoid toxicity in Kentucky. Pearson chi-square test and ANOVAs were used to identify factor associations. A multiple logistic regression model will also be presented at the symposium.

Results: There were 1,490 encounters of care for cannabinoid toxicity; patients were primarily non-Hispanic White males, ages 15-44, who had Medicaid and lived in a metropolitan area. Nearly 18% of these encounters experienced a cardiac complication. About 31% also involved one or more psychoactive substances in addition to cannabinoids (primarily stimulants and/or opioids). Polydrug toxicity was more likely to occur in metropolitan areas (χ^2=132.99, p<0.001), and among patients who were White (χ^2=83.75, p<0.001) and had a serious mental illness (χ^2=29.028, p<0.001). Sex was *not* associated with cannabis-polydrug toxicity. Encounters with polydrug toxicity were more likely to be treated inpatient (χ^2=199.18, p<0.001) and were associated with a higher proportion of cardiac complications (χ^2=4.58, p<0.001), compared to encounters involving only cannabinoid toxicity.

Conclusions: These results are consistent with state-level data from Colorado and Nevada. In Kentucky, cannabinoid toxicity events primarily occurred in White individuals, but cannabinoid toxicity without toxicity due to another psychoactive substance disproportionally affected minorities (in comparison to statewide demographics), although, it is possible that there is racial bias in the clinical documentation of cannabinoid toxicity. Encounters of care for cannabinoid toxicity in people aged 15-24 were relatively high, in fact, people aged 15-24 accounted for nearly a third of cannabinoid toxicity encounters. Cannabinoid use in adolescents and young adults is particularly concerning given the potentially negative impact of cannabis products on the developing brain and the heightened risk of psychosis. It is critical that the health risks of cannabis and synthetic cannabinoid products use be more broadly recognized, and that timely and accurate data be shared in order to guide policies on cannabis access. Future research on cannabinoid toxicity should take into consideration the co-involvement of other drugs as polydrug cannabinoid toxicity is associated with inpatient treatment and a higher proportion of cardiac complications.

Acknowledgments: The authors thank Zhengyan Huang for data technical assistance. This project was supported by Data-Driven Responses to Prescription Drug Misuse in Kentucky (grant no. 2017-PM-BX-K026) from the Bureau of Justice Assistance. This work was also supported, in part, by National Institute on Drug Abuse grants T32 DA035200 (Dr. Britch); and R01 DA045700 and R21 DA045101 (Dr. Babalonis). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

PROFILES OF MEDICAL AND RECREATIONAL CANNABIS USERS IN A POPULATION-BASED SAMPLE DURING COVID-19

Douglas Bruce[1], Gertrude Palillo[1], Suzanne McLone[2], Meha Singh[2] and Nik Prachand[2]

[1]Department of Health Sciences, DePaul University
[2]Division of Epidemiology, Chicago Department of Public Health

Introduction: Emergent research using convenience samples has reported that cannabis use increased among groups of medical and recreational users during the COVID-19 pandemic. We examined data derived from a population-based survey among Chicago residents in Spring 2021 to estimate the cannabis using population in Chicago based on self-reported medical use, recreational use, or combination of uses. Medical cannabis dispensaries opened in Illinois in 2016. Adult recreational use dispensaries opened in January 2020 immediately prior to the onset of the COVID-19 pandemic, and cannabis dispensaries were deemed essential services and remained open during lockdowns.

Methods: The Healthy Chicago COVID-19 Social Impact Survey used an address based sampling (ABS) frame to assess prevalence of a range of health indicators, weighting respondents by age, race/ethnicity, gender, and education level. Data were collected from respondents (N=2,198) in April-May 2021, including 2,047 computer-assisted web (CAWI) surveys and 151 paper-and-pencil (PAPI) surveys. We examined differences using tests of independence for demographics and selected behavioral health indicators among persons who self-reported use of cannabis for medical reasons, recreational reasons, or both reasons.

Results: Weighted prevalence of any cannabis use in the past 30 days was 20.7%, or 419,913 persons (95% CI: 18.4%, 23.3%). Forty-seven percent of cannabis users reported that their use had increased in the past year due to COVID-19 related stress. Individuals reporting only medical use were more likely to be women (56.9%; $F=2.34$, $p<.05$), identify as Black or Latino (38.9% and 28.1%, respectively; $F=2.37$, $p<.05$), and have lower education levels compared to other groups ($F=2.24$, $p<.05$). Those persons reporting only recreational use had lower mean psychological distress scores, less likely to report severe psychological distress ($F=6.11$, $p<.01$) and less likely to report increased cannabis use due to COVID-related stress ($F=6.21$, $p<.01$). There were no significant differences among the three groups in increased alcohol use due to COVID-related stress.

Conclusions: Individuals' cannabis use at a population level in Chicago increased during the first year of the COVID-19 pandemic, a time that also coincided with the initial opening of Illinois' recreational cannabis dispensaries. However, persons reporting medical use were more likely to report severe psychological distress and increased cannabis use due to COVID-related stressors. Demographic characteristics associated with medical use (women; Latino or Black ethnic identity) were also characteristic of populations experiencing COVID-related occupational and health disparities in Chicago during this time. More robust measures of medical and recreational use are needed to disentangle these effects in population-based samples.

A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TEST OF THE EFFECTS OF CANNABIDIOL ON FEAR ELICITED BY A 10% CARBON-DIOXIDE-ENRICHED AIR BREATHING CHALLENGE

Teah-Marie Bynion[1], L. Riley Gournay[2], Graham M. L. Eglit[1], Marcel O. Bonn-Miller[1], Matthew T. Feldner[1] and Ellen Leen-Feldner[2]

[1]Canopy Growth Corporation, Smiths Falls, ON, CA
[2]University of Arkansas Fayetteville

Introduction: Fear is a discrete, systemic response to threat and is characterized by negative affect, elevated arousal, and bodily sensations. Animal model studies have demonstrated fear reducing effects from a single dose of CBD; however, the translatability of these findings to humans remains an open question. The current study extended existing research by testing the effects of CBD on human fear elicited via a controlled, well-established laboratory-based carbon dioxide (CO_2)-enriched air breathing challenge. We hypothesized that, compared to placebo, participants randomly assigned to receive a single dose of 150, 300 or 600 mg of CBD would report less self-reported fear during the air breathing challenge.

Methods: The study was conducted between Sep2020-Mar2021. Participants were randomly assigned to one of four groups: placebo, *150 mg CBD, 300 mg CBD, and 600 mg CBD*. Participation involved a single laboratory-based session. First, participants completed a baseline period. Next, participants orally consumed their assigned dose followed by watching a 60-minute, affectively neutral documentary. Next, research staff connected the CPAP mask and instructed participants to continuously rate their fear prior to, during, and after the 5-minute CO_2- air breathing challenge. Assessments included: fear ratings, heart rate, blood oxygen levels, and panic attack symptoms. Mid-point fear rating and fear rating area under the curve (AUC) were analyzed using analysis of covariance (ANCOVA). Manipulation checks were also conducted.

Results: Participants were 84 healthy adults (M_{age} = 26.46 yrs, SD = 9.07). Manipulation checks indicated there was a significant increase in self-reported fear from pre- to post-challenge (t = 10.82, p < .001). Analysis of mid-point fear ratings indicated that there was no statistical difference between placebo and 150 mg CBD (b = -.02, p = 1.00), 300 mg CBD (b = -.08, p = .95), or 600 mg CBD (b = 0.16, p = .79) groups. Similarly, for fear rating AUC, contrasts between placebo and 150 mg CBD (b = .34, p = .79), 300 mg CBD (b = .08, p > .99), and 600 mg CBD (b = .55, p = .53) groups failed to achieve statistical significance.

Conclusions: The current results indicate that CBD does not meaningfully reduce human fear reactions. However, CBD doses tested were selected based on prior acute anxiety reduction research. Therefore, it is possible that the most effective dose for fear reduction lies outside of the range tested here. Efforts to apply research on CBD to therapeutic applications may benefit from these results; the CBD doses tested here appear unlikely to ameliorate phobic reactions or panic attacks.

Acknowledgements: The authors wish to acknowledge the entire research team Rebecca Campbell, Morgan Hill, Eric Mann, Jordan Petry, Emily Severson, Abigail Vance, and Anna Nguyen.

A MULTIPLE BASELINE STUDY OF THE EFFECTS OF CANNABIDIOL ON DAILY PERCEIVED STRESS AMONG PEOPLE EXPERIENCING HIGH LEVELS OF COVID DISTRESS

Teah-Marie Bynion[1], L. Riley Gournay[2], Jordan Petry[2], Matthew T. Feldner[1], Marcel Bonn-Miller[1] and Ellen W. Leen-Feldner[2]

[1]Canopy Growth Corporation, Smiths Falls, ON, CA
[2]University of Arkansas, Fayetteville

Introduction: Several studies have documented COVID-19-related increases in stress, anxiety, and distress in countries around the world. Global stressors such as this pandemic highlight the need to continue to improve safe and effective options for people to manage daily stress. Cannabidiol (CBD) has potential to reduce daily perceived stress. A recent study found that, compared to standard care alone, standard care plus 150mg of CBD twice per day for four weeks was associated with significant decreases in emotional exhaustion, anxiety, depression, and burnout symptoms among frontline healthcare professionals working with patients with COVID-19. The current study extends these findings by prospectively evaluating the effects of 160mg of CBD twice per day on daily levels of perceived stress among a community sample experiencing high levels of distress related to the pandemic. We hypothesized that, compared to baseline, perceived stress would decrease following a week of CBD use.

Method: A nonconcurrent, multiple baseline, time-series method was employed. Participants (N = 6) were randomly assigned to a three, five, or seven-day baseline period. During baseline, participants completed a daily electronic survey that measured stress via the 4-item Perceived Stress Scale (PSS-4). The PSS-4 was selected as the measure for the primary outcome because it is a psychometrically sound measure of perceived stress that is sensitive to change across time. Once stable PSS-4 baseline scores were established, participants began the intervention phase, during which they were instructed to orally self-administer 160 mg of CBD twice daily with food for 7 consecutive days. Tau-U analyses were conducted to examine change from the baseline to the intervention phase. The Tau-U approach was chosen due to its sensitivity to baseline length and stability, ability to index degree of overlap across phases, and allowance for examining the impact of trends across phases.

Results: The mean score for the PSS-4 during the baseline phase was 10.43 (range: 8-13) whereas the mean score during the intervention phase was 7.38 (range: 4-11). Overall, results indicated a significant decrease in PSS-4 scores during the intervention phase compared to baseline for 5 of 6 participants (z ranging between 1.15 and 3.21; $p < .05$ for 5 of 6 participants).

Conclusion: The current multiple baseline design study is the first study designed to test the potential of CBD for reducing perceived stress. Results suggest that 160mg of CBD taken twice daily can reduce perceived stress among people experiencing elevated stress related to the COVID-19 pandemic.

Acknowledgements: The authors wish to acknowledge Liviu Bunaciu, Cynthia Turcott and Canopy Growth Corporation for donating the product for this study.

FIVE YEARS OF MEDICINAL CANNABIS PRESCRIBING IN AUSTRALIA: WHERE ARE WE HEADING?

Sara L. MacPhail[1], Miguel A. Bedoya-Perez[1], Rhys Cohen[2], Vicki Kotsirilos[3], Iain S. McGregor[1] and Elizabeth A. Cairns[1]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, NSW, Australia
[2]Cannabis Consulting Australia, Sydney, NSW, Australia
[3]NICM Health Research Institute, Western Sydney University, Sydney, NSW, Australia

Introduction: A regulatory framework for legal access to medicinal cannabis (MC) has been operational in Australia since November 2016. MC prescribing is primarily conducted through the Special Access Scheme - Category B (SAS-B) pathway, through which prescribers apply to the Therapeutic Goods Administration (TGA) for individual patient access to MC products. The TGA collects data from these applications, allowing a unique opportunity to explore MC prescribing trends over time in nearly an entire population. Here we present an analysis of profiles and growth trends for the first five years of MC prescribing in the SAS-B scheme.

Methods: TGA SAS-B data on prescribing over time with respect to age, gender, product type (e.g., oil, flower, spray, etc.), THC content, indication treated, and prescriber location were analysed with a best fit non-linear regression model using R. Interactions were analysed via correspondence analysis. Indications were classified with reference to the International Statistical Classification of Diseases and Related Health Problems (10th Revision).

Results: As of 31 August 2021, a total of 159,665 approvals had been given for MC products, 82.4% of which were since January 2020. The highest number of total approvals were for pain (*N*=97,400), anxiety (*N*=25,625), sleep disorders (*N*=9,072), cancer (*N*=6,958) and polyneuropathies (*N*=4,755). Oil products were the most popular product type (70.2% total) with approval for flower products increasing markedly (2.1% in Jan 2019 vs 41.3% in Aug 2021). There has also been a recent increase in approvals in younger age groups (18-31 years old, 10.5% in Jan 2019 vs 24.4% in Aug 2021), a greater proportion of males (50.7% in Jan 2019 vs 62.2% Aug 2021), and for THC-containing products (62.6% Jan 2019 vs 79.3% Aug 2021). Associations between patient gender and age with product type were found. For example, approvals for oil products were associated with approvals for pain, while flower product approvals were associated with approvals for sleep disorders, PTSD, and anxiety.

Conclusions: After a slow start, MC prescribing has increased dramatically in the last two years. However, rates of approvals are beginning to stabilise for some indications, such as pain. It is notable that current prescribing practices do not necessarily reflect governmental guidance. For example, anxiety and sleep disorders are not covered in the current provided guidance documents, even though these make up a significant proportion of total approvals. While acknowledging some limitations in accuracy and detail of the SAS-B dataset, overall, this provides a unique resource with which to better understand current prescribing and utilisation of MC in Australia.

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

5-HT2AR AND AKT PROTEIN EXPRESSION IS DIFFERENTLY REGULATED BY CANNABIS ABUSE IN PLATELETS OF SCHIZOPHRENIA SUBJECTS

Callado L.F.[1,3,4], Ibarra-Lecue I.[1], Unzueta-Larrinaga P.[1,4], Barrena-Barbadillo R.[2], Mendivil B.[5], Landabaso M.A.[5] and Urigüen L.[1,3,4]

Departments of [1]Pharmacology and [2]Nursing, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain; [3]Centro de Investigación Biomédica en Red de Salud Mental CIBERSAM, Spain; [4]Biocruces Bizkaia Health Research Institute, Bizkaia, Spain. [5]Uribe Mental Health Center, Osakidetza, Bizkaia, Spain.

Introduction: Cannabis is one of the most widely consumed illegal substance worldwide, especially during adolescence. Many studies suggest a relationship between long-term cannabis use and schizophrenia. Moreover, cannabis use disorder is highly prevalent (~26%) among schizophrenia patients and it is associated with an earlier age of onset and a poor schizophrenia prognosis.
Serotonin 2A receptors (5-HT2AR) are involved in psychotic symptoms and, like Akt kinase, they are known to be modulated by THC. The aim of the present study was to evaluate the 5-HT2AR protein expression and the Akt functional status in platelet homogenates of subjects diagnosed with schizophrenia (SZ), cannabis use disorder (CUD), or both conditions (DUAL), compared with age- and sex-matched control subjects.

Methods: Platelets of SZ, CUD and DUAL subjects and their sex and age-matched controls were purified by total blood centrifugation in a density barrier solution. Platelet-containing phase was recovered, washed and homogenized, and their containing proteins solubilized for western blot. Data were analyzed with GraphPad PrismTM version 9.0 (GraphPad Software, San Diego, CA, USA). Comparisons between groups were made using a two-way ANOVA followed by Tukey's post-hoc test.

Results: Results showed that CUD (+35 ± 12% vs. controls, *p<0.05) and SZ subjects (+59 ± 12% vs. controls, ****p<0.0001), but not DUAL subjects, displayed significantly higher 5-HT2AR protein immunoreactivity in platelets homogenates when compared with control subjects. Moreover, platelet homogenates from SZ subjects showed a decrease in total Akt immunoreactivity (-59±7% vs. controls, *p<0.05) compared with controls. An increase in phospho(Ser473)-Akt immunoreactivity was found in platelets of SZ (+45±21% vs. controls, *p<0.05), comparing with controls; while a significant decrease in DUAL (-70±8% vs. SZ, *p<0.05) compared to SZ subjects.

Conclusions: The present work demonstrates that schizophrenia subjects show different circulating markers pattern depending on the cannabis use disorder, and are in line with the hypothesis suggesting clinical differences among groups. These results may results useful for the development of new biomarkers in blood of subjects with schizophrenia that may help in the management of different clinical subgroups.

The authors declare they have no conflicts of interest. Funded by Spanish Ministry of Science and Innovation (PID2019-106404RB-I00), Spanish Ministry of Health (PNSD 2019I021) and Basque Government (2019111082).

ATTENTION PLEASE! CHRONIC AND ACUTE EFFECTS OF CANNABIS ON COGNITION IN PEOPLE WITH ADHD

Carrie Cuttler, Amanda Stueber, and Aria Petrucci

Department of Psychology, Washington State University, Pullman, WA, USA

Introduction: Individuals with attention deficit-hyperactivity disorder (ADHD) demonstrate difficulties with sustained attention and/or hyperactivity/impulsivity as well as an increased prevalence of cannabis use. Although impaired cognition is independently associated with ADHD, chronic cannabis use, and acute cannabis intoxication, the small existing literature suggests that people with ADHD use cannabis to self-medicate and may experience beneficial effects on cognition. The present study was conducted to examine the chronic and acute effects of cannabis on cognition in people with ADHD. We hypothesized that chronic cannabis use may be detrimental to cognition in individuals with and without ADHD, but that acute cannabis intoxication may enhance cognitive performance in those with ADHD relative to those without ADHD.

Method: Preliminary results are reported from an ongoing study in which non-users without ADHD (n=31), non-users with ADHD (n=14), cannabis users without ADHD (n=21) and cannabis users with ADHD (n=10) completed two separate 2-hour testing sessions over Zoom. Participants completed a baseline battery of cognitive tests while sober, including tests of memory (California Verbal Learning Test, Digit Span Forward [DSF] and Backwards [DSB]); attention (Test of Variables of Attention, Digit Symbol Substitution Test [DSST]); and inhibition/impulsivity (Stroop, Go/No-Go, Delay Discounting Test [DDT]). One-week later, cannabis users recompleted the tests after inhaling cannabis, while non-users completed the same tests again while sober.

Results: 2 x 2 ANOVAs conducted on the baseline (sober) session data revealed significant cannabis x ADHD interactions on performance on the DSB test (p = .04) and DSST (p = .04). Further probing revealed chronic cannabis-related impairments in working memory (DSB) test performance in individuals without ADHD but not in those with ADHD. In contrast, chronic cannabis-related errors on the DSST of attention were greater in the ADHD group than in the group without ADHD. Additional 2 x 2 ANOVAs conducted on session 2 change scores revealed significant interactions on DSF (p = .02), DSST (p = .02), and DDT (p = .03) performance. Probing of these interactions revealed beneficial effects of acute cannabis intoxication on working memory (DSF) in individuals without ADHD but not in those with ADHD. In contrast, cannabis users with ADHD showed a larger decrease in errors on the DSST under conditions of acute intoxication than those without ADHD (who demonstrate increased errors under conditions of intoxication). Finally, acute intoxication was associated with a greater propensity for smaller immediate rewards in cannabis users without ADHD, while intoxicated cannabis users with ADHD showed an increased propensity for larger delayed rewards relative to their sober counterparts.

Conclusions: Overall support for our hypotheses was mixed. Nevertheless, they suggest that chronic cannabis use may exacerbate problems with attention in individuals with ADHD but spare them from typical cannabis-related impairments in working memory, while acute cannabis intoxication may improve attention and reduce impulsivity in individuals with ADHD. These results are consistent with previous research demonstrating that some medical cannabis patients demonstrate beneficial, rather than detrimental, effects of cannabis on cognition.

REGRESSION MODELING TO IDENTIFY VARIABLES ASSOCIATED WITH CLINICAL IMPROVEMENT FOLLOWING 3 MONTHS OF TREATMENT WITH MEDICAL CANNABIS

M. Kathryn Dahlgren[1,2,3], Kelly A. Sagar[1,2,3], Ashley M. Lambros[1,2], Rosemary T. Smith[1,2], Celine El-Abboud[1,2], Deniz Kosereisoglu[1,2], Staci A. Gruber[1,2,3]

[1]Cognitive and Clinical Neuroimaging Core (CCNC), McLean Imaging Center, Belmont, MA
[2] Marijuana Investigations for Neuroscientific Discovery (MIND) Program, McLean Hospital Imaging Center, Belmont, MA
[3]Department of Psychiatry, Harvard Medical School, Boston, MA, USA

Introduction: Legalization of medical cannabis continues to expand across the US, and increasing numbers of individuals report using cannabis to treat a variety of medical symptoms. While studies have recently begun to examine the impact of medical cannabis (MC) treatment, a myriad of questions remain unanswered, including what variables are associated with clinical improvement.

Methods: As part of an ongoing, longitudinal study, 55 patients interested in using MC for specific symptoms (e.g., anxiety, mood, pain) were assessed prior to initiation of MC use and returned for follow-up visits after initiation of MC. The current analyses examined data from patients' first follow-up visit, which occurred after 3 months of regular MC treatment. Patients completed clinical scales to assess mood (Profile of Mood States [POMS]), depression (Beck Depression Inventory [BDI]), anxiety (Beck Anxiety Inventory [BAI]), and pain (Visual Analogue Scale [VAS]). Paired *t*-tests assessed change over the course of treatment. Multinomial regressions assessed variables associated with change over the course of treatment (i.e., difference scores: baseline minus 3 months) for each clinical scale via backwards stepwise models using the probability of the likelihood ratios (p(Out)=.05). After collinearity assessments, predictor variables included in the models were: age, gender, IQ, race (binary: White & non-White), past cannabis use (naïve, light, frequent), total number of indications for MC use, and MC use episodes/week.

Results: Clinical improvement was observed on all scales after 3 months of MC treatment relative to baseline. Specifically, assessments of mood (POMS; p=.037), depression (BDI; p<.001), and pain (VAS; p=.008) all demonstrated significant improvement, and there was a trend for improved anxiety (BAI; (p=.056). Regression analyses identified several variables associated with clinical improvement over the course of treatment. With regard to mood, younger age (p=.014), being female (p=.035), being non-White (p=.027), and fewer total indications for MC use (p=.015) were all associated with greater improvement on the POMS. For depressive symptoms, only younger age (p=.009) was associated with greater improvement on the BDI. Regarding anxiety, younger age (p=.002), being female (p=.011), and fewer total indications for MC use (p=.022) were all associated with greater improvement on the BAI. For pain, being non-white (p=.007), and reporting infrequent previous cannabis use (p=.012) were associated with greater improvement on the VAS.

Conclusions: Results suggest that MC treatment is an effective adjunctive treatment for mood, depression, anxiety, and pain in at least some patients. Study analyses identified characteristics of patients who are likely to experience the greatest clinical benefit from MC treatment. Overall, in the current study, being younger, non-White, female, and having fewer baseline indications for MC use were all associated with greater improvements in clinical state. Additionally, being non-White, and reporting only infrequent cannabis use were associated with greater improvement in pain ratings. Future research should continue to examine variables associated with clinical improvement during MC treatment.

Acknowledgements: This study was funded by private donations to the Marijuana Investigations for Neuroscientific Discovery (MIND) Program.

BETA-CARYOPHYLLENE AS A NOVEL ADJUNCT THERAPY FOR THE TREATMENT OF URINARY TRACT INFECTIONS

Kayle Dickson, Cassidy Scott, Hannah White and Christian Lehmann

Department of Microbiology and Immunology, Dalhousie University, NS, Canada

Introduction: Urinary tract infections (UTIs) represent one of the most common types of infections and present frequently in urgent care. Symptoms of bacterial cystitis include urinary frequency, urgency and painful urination. While these infections are typically manageable with antibiotic therapy, painful symptoms may persist. Beta-caryophyllene (beta-C) is a terpene found in a variety of plants, including cloves, black pepper and *Cannabis sativa*. It has well described local anesthetic and anti-inflammatory activity, which are mediated through interactions with cannabinoid receptor 2. Beta-C does not have psychoactive effects, making it of interest for the management of inflammatory conditions. In addition, a variety of anti-bacterial activities have recently been described for beta-C. The goal of this project is to investigate beta-C's potential as an adjunct therapy for the management of UTIs.

Methods: Bacterial cystitis was induced in a murine model of UTI via the transurethral inoculation of uropathogenic *Escherichia coli*. Animals received intraperitoneal treatment with beta-C (100mg/kg), low dose fosfomycin (10mg/kg), or combination therapy immediately after induction (six hour model) or after six hours (24 and 72 hour models). After six, 24 or 72 hours, mice were evaluated for changes in both bacterial burden and behavioral parameters. Bacterial burden in the urine, kidneys, spleen, and blood were evaluated using standard serial dilution techniques. Pain levels, compared to a baseline, were assessed using von Frey aesthesiometry (evoked pain) and a behavioral score (non-evoked pain). Plasma and bladder tissue samples were collected to evaluate the expression of inflammatory mediators via multi-plex cytokine assay. In addition, intravital microscopy was conducted at the 24 hour timepoint to assess changes in leukocyte adhesion and functional capillary density within the bladder microcirculation.

Results: A localized infection results from UTI induction, with significant increases in urinary bacterial burden after six and 24 hours. Inoculation resulted in limited ascension into the kidneys and did not result in systemic infection, as evidenced by the low bacterial burden in the blood and spleen and low levels of pro-inflammatory cytokines in both plasma and bladder tissue. The 24 hour timepoint was selected for further investigation based on preliminary findings. Treatment with beta-C reduced the bacterial burden within the urine and bladder tissues, and was not significantly different from treatment with fosfomycin alone. Beta-C also significantly improved non-evoked pain levels at this timepoint and in the preliminary six hour model, as assessed by behavioral scoring. At the 24 hour timepoint, beta-C also significantly reduced the response to evoked pain, performing better than treatment with fosfomycin alone. As assessed by intravital microscopy, UTI induction resulted in a significant increase in adherent leukocytes and impaired microcirculation within the bladder. Treatment with beta-C restored these parameters.

Conclusion: Beta-C may be able to act as an adjunct therapy for the management of UTIs, as it is able to modulate the bacterial burden, pain responses and inflammation levels of animals experiencing a UTI. Future work is needed to determine the mechanism of beta-C's antibacterial effects.

Acknowledgements: Funded by Panag Pharma Inc. and Mitacs.

BRIEF REPORT: PREVALENCE AND PATTERNS OF CANNABIS USE IN RURAL JAMAICA

Denise C. Vidot[1], Bria-Necole A. Diggs[1], Brandie Bentley[2], Johis Ortega[1], Juan Hernandez[1], Eulalia Kahwa[3], Jermaine Whyte[3] and Marvin Reid[3]

University of Miami[1], University of Illinois Urbana-Champaign[2], University of West Indies-Mona[3]

Introduction: The purpose of this study was to estimate the prevalence and patterns of cannabis use across four districts in rural Jamaica: Arcadia, St. Thomas, Cedar Valley, St. Thomas, Giblatore, St. Catherine, and Wakefield, St. Catherine.

Methods: Our study team administered surveys to a sub-population of 64 community members attending pop-up health centers within four rural counties of Jamaica. The study team collaborated with community health center staff to conduct community-based screenings, wellness checks, and administered demographic and self-reported substance use pattern surveys to a subsample of the health center participants. Univariate data analyses were employed.

Results: Of the participants surveyed at the health center (n=64), a majority were males (57.8%) with most participants falling within the 40-49 age range (28.1%). 81.4% of participants (n=35) reported using cannabis at least once in their lifetime. Three of the four rural districts had a prevalence of more than 80% of cannabis use within respondents' lifetime (Cedar valley, 100%; Giblatore, 82.8%; and Wakefield, 87.5%), while Arcadia reported 58.3%. Although analysis did not find any significant differences between districts, 66.7% of the overall population self-reported regular cannabis usage. When it came to route of administration ever used, joint+grabba ($p=0.042$) and edible ($p=0.020$) cannabis users varied by district. There were also significant ($p=0.001$) differences in the most common route of administration between joint/spliff, joint+grabba, pipe/bowl, and liquid modes among cannabis users in the four districts.

Conclusions:
These findings underscore the importance of understanding not only the prevalence, but also the patterns of cannabis use across rural geographic areas. The data collected for this study provides preliminary evidence to understand cannabis use patterns and trends, but also aids future studies and populations using cannabis to self-medicate or for recreational purposes.

Acknowledgements: Funding for this article was made possible (in part) by T37MD008647 from the National Institute on Minority Health and Health Disparities. 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.

PERCEIVED EFFECTIVENESS OF CANNABIS-BASED MEDICINE PRODUCTS: A QUESTIONNAIRE STUDY

Simon Erridge[1,2], Beata Ciesluk[3], Lucy J Troup[3], Mikael H Sodergren[1,2]

1. Medical Cannabis Research Group, Imperial College London, UK
2. Sapphire Medical Clinics, London, UK
3. Division of Psychology, School of Education and Social Sciences, University of the West of Scotland, UK

Introduction: Cannabis-based medicinal products (CBMPs) are prescribed with increasing frequency, however, there remains a paucity of high-quality randomised controlled trials. Clinical practice is therefore reliant upon the assimilation of real-world evidence to inform future clinical trials and drug development, in addition to current delivery of care whilst these are pending. This study aimed to assess the perceived effectiveness of CBMPs across a variety of chronic health conditions. Secondary analysis assessed the perceived effects of CBMPs in relation to past and current recreational and prescription drug use.

Methods: 2,319 patients actively treated with CBMPs were invited to take part in a cross-sectional questionnaire study. A multidisciplinary team of academic clinicians and psychologist developed the questionnaire to establish patient demographics and assess self-reported efficacy across 25 self-identified conditions. Patients could identify that CBMPs were a therapy for more than one condition. Efficacy was assessed across a 5-point Likert scale (1 - much better, 2 - somewhat better, 3 - stayed the same, 4 - somewhat worse, 5 - much worse). Patient and public engagement helped to assess content validity and study feasibility. The questionnaire was distributed electronically and hosted on Qualtrics (Seattle, Washington, United States). Descriptive analysis was performed on participant answers.

Results: 450 (19.4%) respondents completed the survey. 258 (57.3%) and 176 (39.1%) identified as male and female respectively. The mean age participants started consuming cannabis was 28.0 (±16.3) years old. The mean length of cannabis consumption to date was 12.9 (±11.8) years. The reasons why people sought treatment with CBMPs included family or friend recommendation (n=124; 27.6%), healthcare professional recommendation (n=111; 24.7%), other recommendation (n=58; 12.9%), personal research (n=344; 76.4%), and previous experience with cannabis (n=299; 66.4%). Patients were mostly administering CBMPs daily (n=415; 92.2%), whilst 6.9% of patients administered their treatment when symptomatic. The most common methods of administering CBMPs were via vapouriser (n=318; 70.7%) and oils/tinctures (n=288; 64.0%). 36.4% (n=164) of patients administered their prescription via a method (edibles, smoking, dab rig) which they are counselled against. The most common indication for which patients reported were insomnia (n=363; 80.7%), pain (n=357; 79.3%), anxiety (n=335; 74.4%), and depression (n=310; 68.9%). 92.0% (n=334), 95.8% (n=342), 93.7% (n=314), and 94.5% (n=293) of patients subjectively rated their condition much or somewhat better for insomnia, pain, anxiety, and depression respectively.

Conclusion: This study demonstrates an association with improvement in self-reported chronic health conditions following initiation of treatment with CBMPs on a non-validated 5-point Likert scale. The findings are significantly limited by the subjective nature of reporting and lack of clinical validation. In contrast, this provides insights into conditions which may help inform more bespoke analysis through validated questionnaire. This anonymous questionnaire also provides important insights into the different methods of administration patients may use once prescribed CBMPs, even if this is against clinical recommendation.

PATIENT PRIORITIES FOR RESEARCH: A FOCUS GROUP STUDY OF UK MEDICAL CANNABIS PATIENTS

Simon Erridge[1,2], Fabian Olsson[1] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group,
Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK

Introduction: The value of patient contributions to the design and conduct of research is well understood. Whilst research into medical cannabis and its constituent active pharmaceutical compounds has increased exponentially since the turn of the century, there has yet to be an in-depth evaluation of medical cannabis patient preferences with respect to future research. As such, prioritisation of research agendas has been largely driven by academia and industry. This primary aim of this study was to elicit priorities for research from medical cannabis patients in the United Kingdom and to explore the reasons underpinning those priorities.

Methods: Patients undergoing active treatment for health conditions with medical cannabis in the United Kingdom were invited to take part in focus groups from December 2021 to February 2022. Each session was transcribed verbatim. An inductive thematic analysis of responses was performed independently by two researchers (S.E. & F.O.). Participants also completed a ranking exercise whereby they assigned ten counters (each equivalent to £1 million GBP) to competing research priorities.

Results: 30 medical cannabis patients participated across 3 focus groups. The mean age of participants was 44.7 (±10.5). 18 (60.0%) and 12 (40.0%) of participants were male and female respectively. The following themes were identified as research priorities: condition-specific analysis, comparisons to other medications, comparisons between different medical cannabis products, randomised controlled trials, real-world evidence, plant biology and manufacturing, the endocannabinoid system, minor cannabinoids/terpenes/flavonoids, barriers to access, and effects on workplace function/driving. Participants assigned the highest proportion of research funding to the following questions: assessment of effect on specific symptoms (26 counters; 8.7%), understanding the effects on quality of life (20 counters, 6.7%), evaluating the knowledge of medical cannabis among healthcare professionals (20 counters, 6.7%), understanding the stigma of medical cannabis patients (20 counters, 6.7%), and assessment of the effects of medical cannabis on driving and/or job performance (20 counters, 6.7%).

Conclusions: This priority setting study highlighted specific themes for focusing future research on medical cannabis. Clinically, there was a directive towards ensuring that research is either condition- or symptom-specific. Participants also emphasised themes related to the social impact of medical cannabis, such as knowledge of medical cannabis among healthcare professionals, stigma, and effects on driving and in the workplace. These findings can help guide both research funders and researchers alike into effectively implementing research which fits within a more patient-centric model.

AN ANALYSIS OF CLINICAL OUTCOME MEASURES FOR SCOTTISH PATIENTS FROM THE UK MEDICAL CANNABIS REGISTRY

Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Sushil Beri[1,2], Jonathan Hoare[1,2], Shaheen Khan[2,4], Mark W Weatherall[2,5], Michael W Platt[1,2], James J Rucker[2,6,7] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Guy's & St. Thomas' NHS Foundation Trust, London, UK
5. Buckinghamshire Healthcare NHS Trust, Amersham, UK
6. Department of Psychological Medicine, Kings College London, London, UK
7. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Similar to other jurisdictions of the United Kingdom it became legal for unlicenced cannabis-based medical products (CBMPs) to be prescribed in Scotland for chronic health conditions in 2018. However, despite this, there still exists a paucity of high-quality randomised controlled trials and observational studies to inform clinical practice. The UK Medical Cannabis Registry was set-up in 2019 and is the largest real-world evidence platform collecting outcomes for patients prescribed CBMPs in the UK. This case series aims to analyse changes in patient reported outcome measures and the incidence of adverse events in patients prescribed CBMPs in the Scotland.

Methods: Patients enrolled on the UK Medical Cannabis Registry until February 2022 residing in Scotland were identified. Demographics, co-morbidities, and drug and alcohol data were extracted. Primary outcomes were changes in EQ-5D-5L Index Values, Generalised Anxiety Disorder-7 Questionnaire (GAD-7), and Single-Item Sleep Quality Scale (SQS). Adverse events were reported in accordance with CTCAE version 4.0. Statistical significance was defined as $p<0.050$.

Results: 722 patients from Scotland were identified for inclusion. The mean age was 43.3 (±13.8) years. Most patients were male (n=392; 54.3%). 328 (45.4%) were unemployed. The median Charlson Comorbidity score was 0.0 (IQR: 0.0-5.0). 525 (72.7%) participants were previous or current consumers of cannabis prior to baseline assessment. The median lifetime cannabis consumption for these patients was 6.0 (IQR: 2.0-20.0) gram years. The most common primary diagnoses were chronic non-cancer pain (n=259; 35.9%), fibromyalgia (n=78; 10.8%), anxiety (n=72; 10.0%), and neuropathic pain (n=53, 7.3%). Improvements were observed in EQ-5D-5L index values, GAD-7, and SQS at 1, 3, and 6 months after initiating treatment ($p<0.001$). The incidence of adverse events was 186.6% (n=1,347). The most frequent adverse events were fatigue (n=109; 15.1%), somnolence (n=88; 12.2%) and insomnia (n=81; 11.2%). The majority of adverse events were mild (n=624; 86.4%) or moderate (n=564; 78.1%) severity.

Conclusion: This case series of Scottish patients treated with CBMPs demonstrates an association with improved general health-related quality of life metrics at up to 6 months follow up. Most adverse events were mild or moderate in severity. This study provides valuable insights for future randomised controlled trials and current clinical practice.

AN ANALYSIS OF CLINICAL OUTCOME MEASURES FOR ENGLISH PATIENTS FROM THE UK MEDICAL CANNABIS REGISTRY

Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Sushil Beri[1,2], Jonathan Hoare[1,2], Shaheen Khan[2,4], Mark W Weatherall[2,5], Michael W Platt[1,2], James J Rucker[2,6,7] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Guy's & St. Thomas' NHS Foundation Trust, London, UK
5. Buckinghamshire Healthcare NHS Trust, Amersham, UK
6. Department of Psychological Medicine, Kings College London, London, UK
7. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: In England, the prescribing of cannabis-based medicinal products (CBMPs) is limited secondary to challenges in facilitating access. A large barrier to prescribing is a paucity of high-quality evidence on outcomes following treatment with CBMPs. Importantly, there is limited evidence assessing changes in health-related quality of life (HRQoL) of English patients to inform national guidance. This study aims to assess changes in HRQoL following treatment of chronic health conditions with CBMPs in patients from England enrolled on the UK Medical Cannabis Registry (UKMCR).

Methods: Patients in England treated with CBMPs up until February 2022 were identified from the UKMCR, the largest bespoke registry collecting outcomes on medical cannabis patients in the UK. Baseline demographics were assessed utilising descriptive statistics. Changes in HRQoL from baseline at 1, 3, 6, and 12 months were assessed utilising validated questionnaires [EQ-5D-5L, single-item sleep quality scale (SQS), General Anxiety Disorder-7 Scale (GAD-7)]. $p<0.050$ was defined as statistically significant.

Results: A total of 1,701 patients were included in the case series with a mean age of 42.6 (±14.8) years. 971 (57.1%) patients were male. 680 (40.0%) participants were unemployed. The mean body mass index was 27.2 (±6.8) kg/m^2. The most common primary diagnoses were chronic non-cancer pain (n=533; 31.3%), fibromyalgia (n=198; 11.6%), anxiety (n=181; 10.6%), and neuropathic pain (n=152, 8.9%). The median Charlson Comorbidity Index score was 0.0 (IQR: 0.0-5.0). 901 (53.0%) participants were prior consumers of cannabis at baseline assessment. The median lifetime cannabis exposure of previous and current consumers at baseline was 5.0 (IQR: 1.0-16.0) gram years. At 1, 3, 6, and 12 months there were observed improvements in EQ-5D-5L index values, GAD-7, and SQS ($p<0.001$). The incidence of adverse events was 193.8% (n=3,296). Fatigue (n=249, 14.6%), dry mouth (n=233, 13.7%), somnolence (n=201, 11.8%) and lethargy (n=198, 11.6%) were the most reported adverse events. Most adverse events were mild (n=1,354; 79.6%) or moderate (n=1,434; 84.3%).

Conclusion: This study is the first to report demographics and outcomes in English medical cannabis patients. It demonstrates a positive association between initiation of treatment for chronic illnesses and improvements in HRQoL. This builds upon existing studies which have demonstrated similar outcomes. High quality randomised controlled trials are necessary to further evaluate this association. This study also highlights the importance of robust pharmacovigilance in the prescribing of unlicenced medications, identifying fatigue, dry mouth, somnolence and lethargy as being the most frequently experienced adverse events. Most adverse events were mild or moderate.

CLINICAL OUTCOME ANALYSIS OF PATIENTS WITH AUTISM SPECTRUM DISORDER: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

Simon Erridge[1,2], Jess Kerr-Gaffney[3,4], Carl Holvey[2], Ross Coomber[2,5], Daniela Barros[2], Urmila Bhoskar[2], Gracia Mwimba[2], Kavita Praveen[2], Chris Symeon[2], Simmi Sachdeva-Mohan[2], Mikael H Sodergren[1,2] and James J Rucker[2,3,4]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. Department of Psychological Medicine, Kings College London, London, UK
4. South London & Maudsley NHS Foundation Trust, London, UK
5. St. George's Hospital NHS Trust, London, UK

Introduction: Autism spectrum disorder (ASD) is a common neurodevelopmental condition present in approximately 1.5% of the general population. Individuals with ASD are affected by a higher incidence of comorbid irritability, challenging behaviours, self-injury, and psychiatric conditions. However, there are limited therapeutics available to help reduce the severity or impact of these comorbidities. Pre-clinical models have suggested that the endocannabinoid system is implicated in difficulties in emotional regulation in ASD. Cannabis-based medicinal products (CBMPs) have therefore been identified as a promising novel therapeutic. However, there is a paucity of clinical evidence of their efficacy and safety. This case series aims to assess changes to health-related quality of life and the incidence of adverse events in patients treated with CBMPs for associated symptoms of ASD enrolled on the UK Medical Cannabis Registry (UKMCR).

Methods: Patients treated with CBMPs for ASD-related symptoms for a minimum of one month were identified from the UKMCR. Demographic details were extracted from the UKMCR. Primary outcomes were changes in validated patient reported outcome measures [Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), quality of life index (EQ-5D-5L)] at 1, 3, and 6 months compared to baseline. Adverse events were recorded and analysed using terminology from CTCAE v.4.0. Statistical significance was determined by $p<0.050$.

Results: 74 patients with ASD were included in the analysis. The mean age of participants was 32.7 (±11.6) years. 53 (71.6%) and 21 (28.4%) participants were male and female respectively. 46 (62.2%) were unemployed. 50 (67.6%) and 10 (13.5%) of participants were either current cannabis consumers or previous consumers. There were significant improvements in general health-related quality of life, anxiety, and sleep as assessed by the EQ-5D-5L, GAD-7, and SQS respectively at 1, 3, and 6 months ($p<0.050$). There were 180 (243.2%) adverse events reported by 14 (18.9%) participants. If present, adverse events were commonly mild (n=58; 78.4%) or moderate (n=81; 109.5%), rather than severe (n=38; 51.4%) or disabling (n=3; 4.1%).

Conclusions: This study demonstrated in patients with ASD an associated improvement in general health-related quality of life, and anxiety- and sleep-specific symptoms following initiation of treatment with CBMPs. The adverse event incidence was 243.2%, however these were reported by only 18.9% of participants, with the majority being mild or moderate. This suggests that CBMPs were well tolerated by most patients. These findings, whilst promising, require further evaluation within the setting of randomised controlled trials.

CLINICAL OUTCOME DATA OF CHILDREN TREATED WITH CANNABIS BASED MEDICINAL PRODUCTS FOR TREATMENT RESISTANT EPILEPSY – ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Jonathan Hoare[1,2], Shaheen Khan[2,4], Michael W Platt[1,2], James J Rucker[2,5,6], Mark W Weatherall[2,7], Sushil Beri[1,2,*] and Mikael H Sodergren[1,2,*]

[1]Medical Cannabis Research Group, Imperial College London, London, UK
[2]Sapphire Medical Clinics, London, UK
[3]St Georges NHS Healthcare Trust, London, UK
[4]Guy's & St Thomas' NHS Foundation Trust, London, UK
[5]Department of Psychological Medicine, Kings College London, London, UK
[6]South London & Maudsley NHS Foundation Trust, London, UK
[7]Buckinghamshire Healthcare NHS Trust, Amersham, UK
[*] considered joint senior authors

Background: Cannabis-based medicinal products (CBMPs) are increasingly used in clinical practice for children with treatment-resistant epilepsy (TRE). However, there is a paucity of high-quality research to support the use of cannabidiol (CBD) outside of Lennox-Gastaut and Dravet syndromes. Similarly, there is limited evidence of the benefits and risks of delta-9-tetrahydrocannabinol (Δ9-THC) therapy in children with TRE.

Methods: A case series of all children (<18 years old) with TRE from the UK Medical Cannabis Registry was analysed. Primary outcomes were ≥50% reduction in seizure burden, changes in the Impact of Paediatric Epilepsy Score (IPES) and incidence of adverse events. Statistical significance was determined at a p-value<0.050.

Results: Thirty-five patients were included in the analysis. Patients were treated with the following - CBD isolate oils (n=19), CBD broad-spectrum oils (n=17), and CBD/Δ9-THC combination therapy (n=17). Twenty-three (65.7%) patients achieved a ≥50% reduction in seizure burden, whilst 13 (37.1%) sustained a ≥90% reduction in seizure burden across all treatment cohorts. Four (11.4%) patients had complete remission. 94.1% (n=16) of patients treated with CBD and Δ9-THC observed a ≥50% reduction in seizure burden compared to 31.6% (n=19) and 17.6% (n=17) of patients treated with CBD isolates and broad-spectrum CBD products respectively (p<0.001). There was no significant difference in IPES score between baseline and any follow-up period (p>0.050). Twenty-six (74.3%) adverse events were reported by 16 patients (45.7%). The majority of these were mild (n=12; 34.2%) and moderate (n=10; 28.6%).

Conclusions: This represents the largest series of its kind in Europe. The results show a promising signal towards the effectiveness of CBMPs in children with TRE, particularly in the cohort of patients treated with Δ9-THC. However, these data should be contextualised within the limitations created by selection bias and the lack of an appropriate control arm for treatment. The results from this study could be utilised in the design of future phase II randomised controlled trials, particularly for dosing regimens. The short terms adverse effects appear well-tolerated, however long-term effects of CBMPs on neurodevelopment are still unknown. The UK Medical Cannabis Registry will form an important components of a pharmacovigilance strategy that will contribute to the long-term data in this patient population.

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

Sajed Mangoo[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Kavita Praveen[2], Chris Symeon[2], Urmila Bhoskar[2], Simmi Sachdeva-Mohan[2], James J Rucker[2,4,5] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Department of Psychological Medicine, Kings College London, London, UK
5. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Although second-generation antidepressants are effective in the management of depression, they have a relatively modest response rates and some patients are unable to tolerate associated side effects of treatment. Pre-clinical evidence suggests a role of cannabinoids in emotional regulation and reduction of depressive behaviours. However, there exists a paucity of high-quality clinical evidence of efficacy and safety of cannabis-based medicinal products (CBMPs) in the setting of depression. The primary aim of this study is to analyse health-related quality of life outcomes of patients prescribed CBMPs for the treatment of depression.

Methods: Patients treated with CBMPs for longer than one month with a primary diagnosis of depression were identified from the UK Medical Cannabis Registry. The primary outcome was changes from baseline in patient reported outcomes measures [Patient Health Questionnaire-9 (PHQ-9), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L] at 1, 3 and 6 months. Secondary outcomes included descriptive analysis of reported adverse events according to CTCAE v.4.0. $p<0.050$ was defined as statistically significant.

Results: 129 patients with depression were identified for inclusion. The mean age of participants was 35.6 (±11.1) years old. Most patients were male (n=95; 73.6%). 88 (68.2%) participants were consumers of illicit cannabis at the time of starting CBMPs, with a median lifetime cannabis exposure of 6.5 (IQR: 2.0-20.0) gram years. The median PHQ-9 score at baseline was 16.0 (IQR: 9.0-21.0). There were reductions in severity of depression symptoms according to PHQ-9 scores at 1-month (median: 8.0; IQR: 4.0-14.0; $p<0.001$), 3-months (median: 7.0; IQR: 2.3-12.8; $p<0.001$), and 6-months (median: 7.0; IQR: 2.0-9.5; $p<0.001$). Improvements were also seen in anxiety (GAD-7), sleep (SQS), and general health-related quality of life (EQ-5D-5L index value) measures at 1, 3, and 6 months ($p<0.050$). 153 (118.6%) adverse events were recorded by 18 (14.0%) participants. The majority of these were mild (n=76; 58.9%) or moderate (n=57; 44.2%).

Conclusion: A significant reduction in PHQ-9 scores was observed in patients treated with CBMPs for depression from moderately severe levels at baseline to mild at 1, 3, and 6 months. Moreover, there were noted improvements in general health related quality of life metrics. The incidence of adverse events was 118.6%, however the majority of these were mild or moderate, with no life-threatening or limiting adverse events recorded. This analysis provides insights for further study of CBMPs within the setting of randomised controlled trials.

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

Martha Nicholas[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Ezzat Awad[2], James J Rucker[2,4,5], Mark W Weatherall[2,6] and Mikael H Sodergren[1,2]

1. Medical Cannabis Research Group, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Department of Psychological Medicine, Kings College London, London, UK
5. South London & Maudsley NHS Foundation Trust, London, UK
6. Buckinghamshire Healthcare NHS Trust, Amersham, UK

Introduction: Headache disorders are a common cause of disability and reduced quality of life globally. Cannabis-based medicinal products (CBMPs) have an increasing evidence base, both pre-clinically and clinically, demonstrating a role in the treatment of chronic pain conditions. However, there is a paucity of research specifically focused on headache disorders. The primary aim of this study is to assess changes in validated patient reported outcome measures in patients with headache disorders. Secondary aims include assessment of clinical safety in this population.

Methods: A case series of patients with headache disorders was extracted from the UK Medical Cannabis Registry. Primary outcomes were changes in Headache Impact Test-6 (HIT-6), Migraine Disability Assessment (MIDAS), Generalised Anxiety Disorder-7 (GAD-7) questionnaire, Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L at 1, 3 and 6 months compared to baseline. Adverse events were analysed in accordance with Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. p-values<0.050 were determined as statistically significant.

Results: 97 patients were identified for inclusion in the case series with migraine (n=82; 84.5%), tension-type headache (n=6; 6.2%), and cluster headache (n=9; 9.3%). The mean age of the cohort was 37.9 (±11.1) years. 55 (56.7%) patients were male. Most patients (n=54; 55.7%) were consumers of cannabis prior to initiating treatment with CBMPs. Improvements were detected in HIT-6, MIDAS, SQS, and EQ-5D-5L index values at 1-, 3-, and 6-months following baseline (p<0.050). Reduction in GAD-7 scores were observed at 1-, and 3-months only (p<0.050). There were 111 (114.4%) adverse events recorded by 17 (17.5%) patients. The adverse events were commonly either mild (n=63; 64.9%) or moderate (n=39; 40.2%).

Conclusion: These results suggest an associated improvement in headache and migraine specific patient reported outcomes for patients with headache disorders following initiation of treatment with CBMPs. Furthermore, patients also demonstrated improvement in general health related quality of life metrics. Whilst an observational study design cannot ascertain causality, this study can support the development of randomised controlled trials and clinical practice for CBMPs in headache disorders.

AN UPDATED ANALYSIS OF CLINICAL OUTCOME MEASURES ACROSS PATIENT GROUPS IN THE UK MEDICAL CANNABIS REGISTRY

Fabian Olsson[1], Simon Erridge[1,2], James Tait[1], Carl Holvey[2], Ross Coomber[2,3], Sushil Beri[1,2], Jonathan Hoare[1,2], Shaheen Khan[2,4], Mark W Weatherall[2,5], Michael W Platt[1,2], James J Rucker[2,6,7] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Guy's & St. Thomas' NHS Foundation Trust, London, UK
5. Buckinghamshire Healthcare NHS Trust, Amersham, UK
6. Department of Psychological Medicine, Kings College London, London, UK
7. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Despite an increase in the prescribing of cannabis-based medicinal products (CBMPs) across multiple jurisdictions, there remains a paucity of high-quality longitudinal data of patient outcomes and safety following initiation of treatment. The UK Medical Cannabis Registry, established in 2019, is the largest CBMP-specific registry in the UK. The aim of this study is to assess efficacy and safety of treatment through bespoke analysis of patient reported outcome measures and recorded adverse events across a wide spectrum of conditions.

Methods: A case series of patients from the UK Medical Cannabis Registry was analysed. Patient demographic details, in addition to pre-existing co-morbidities, and drug and alcohol consumption was assessed. Primary outcomes were changes in the General Anxiety Disorder Scale (GAD-7), EQ-5D-5L, and Sleep Quality Scale (SQS) at 1, 3, 6, and 12 months compared to baseline. Secondary analysis included assessment of recorded adverse events (CTCAEv4 criteria). Statistical significance was defined as $p<0.050$.

Results: 2,833 patients were included in the analysis. The mean age of the cohort was 42.4 (±14.3) years old. 1,613 (56.9%) and 1,219 (43.0%) of the patients were male and female respectively. The most common primary diagnoses were chronic non-cancer pain (n=914; 32.3%), anxiety (n=318; 11.2%) fibromyalgia (n=306; 10.8%) and neuropathic pain (n=237, 8.4%). Prior to enrolment 1,584 (55.9%) patients consumed cannabis with median lifetime consumption of 5.0 (IQR: 1.1-18.0) gram years. Statistically significant improvements were observed in GAD-7, EQ-5D-5L index values, and SQS scores at 1, 3, 6, and 12 months ($p<0.050$). There were 5,172 (183.6%) reported adverse events, of which fatigue (n=409, 14.4%), dry mouth (n=347, 12.2%), somnolence (n=312, 11.0%) and lethargy (n=308, 10.9%) were the most reported. Most adverse events were mild (n=2,201; 42.6%) or moderate (n=2,191; 42.4%).

Conclusion: This study, the largest observational series of patients treated with CBMPs in the UK, demonstrates an association with improved general health-related quality of life up to 12 months. In addition, specific improvements in generalised anxiety and sleep quality were also observed. The majority of adverse events were mild and moderate. Whilst randomised controlled trials are essential to determine causality, this study helps inform current clinical practice and future trials, whilst also being a fundamental component of pharmacovigilance.

ASSESSMENT OF CLINICAL OUTCOMES IN PATIENTS WITH POST-TRAUMATIC STRESS DISORDER: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

Manaswini Pillai[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Kavita Praveen[2], Chris Symeon[2], Urmila Bhoskar[2], Simmi Sachdeva-Mohan[2], James J Rucker[2,4,5] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Department of Psychological Medicine, Kings College London, London, UK
5. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Post-traumatic stress disorder (PTSD) affects between 5-10% of adults during their lifetimes. Current data suggests that available medical therapies such as selective serotonin reuptake inhibitors are not appropriate first line agents if sustained and long-term symptom improvement is intended. Cannabis-based medicinal products (CBMPs) are a potential novel therapeutic for PTSD. However, there is limited high-quality clinical evidence of their utilisation in this setting. The primary aim of this study is to analyse data from the UK Medical Cannabis Registry, to assess changes in patient reported outcome measures (PROMs) in patients treated with CBMPs for PTSD. Secondary aims are to evaluate the incidence of adverse events.

Methods: Patients were identified from the UK Medical Cannabis Registry for inclusion and restricted to those who had started treatment at least 1 month prior to data extraction. Primary outcomes were changes in the following PROMS at 1, 3 and 6 months compared to baseline: Impact of Events Scale, Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L. Adverse events were recorded and analysed according to the Common Terminology Criteria for Adverse Events version 4.0. Statistical significance was defined as $p<0.050$.

Results: 162 patients with PTSD were included in the analysis. The mean age of the cohort was 37.6 (± 9.9) years. There were more male patients (n=97; 59.9%) than female patients (n=65; 40.1%). 81 (50%) patients were unemployed at point of enrolment. Most patients were cannabis consumers (n=122; 75.3%) at baseline. The median lifetime cannabis exposure was 10.0 (IQR: 2.4-25.0) gram years. Improvements were seen in the median Impact of Events Scale compared to baseline (59.0; IQR: 49.8-68.0) at 1 month (43.5; IQR: 30.0-57.0; $p<0.001$), 3 months (34.0; IQR: 19.5-50.0; $p<0.001$), and 6 months (32.0; IQR 20.0-50.0; $p<0.001$). Positive changes were also observed in each of Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L index values ($p<0.050$) at each recorded follow-up ($p<0.050$). 220 (135.8%) adverse events were reported by 33 (20.4%) patients, of which 30 (18.5%) were severe.

Conclusion: In patients treated with CBMPs for PTSD there was a statistically significant reduction in the Impact of Events Scale, a PTSD-specific PROM measuring of the effect of symptoms on quality of life. Moreover, there were improvements in general quality of life measures. Whilst randomised controlled trials with placebo comparators are still required to determine causality, these data may help inform future clinical studies and practice.

CLINICAL OUTCOME DATA OF ANXIETY PATIENTS TREATED WITH CANNABIS-BASED OILS AND DRIED FLOWER IN THE UNITED KINGDOM: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

Raphael Rifkin-Zybutz[1,2,3], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Daniela Barros[2], Urmila Bhoskar[2], Gracia Mwimba[2], Kavita Praveen[2], Chris Symeon[2], Simmi Sachdeva-Mohan[2], James J Rucker[2,4,5] and Mikael H Sodergren[1,2]

1. Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Department of Psychological Medicine, Kings College London, London, UK
5. South London & Maudsley NHS Foundation Trust, London, UK

Introduction: Generalized anxiety disorder (GAD) is a common mental health problem. Despite the advent of effective psychological and pharmacological treatments, a significant proportion of patients have symptoms refractory to best medical treatment. Cannabis-based medicinal products (CBMPs) have been identified as a novel therapeutic based on pre-clinical models of anxiety, however there is a paucity of high-quality evidence of its effectiveness and safety in patients. This study aims to evaluate clinical outcomes of patients with GAD enrolled in the UK Medical Cannabis Registry (UKMCR) treated exclusively with an inhaled dried flower (Adven EMT2, Curaleaf International, Guernsey,UK), a sublingual medium-chain triglyceride based oil (Adven 20 & Adven 50, Curaleaf International, Guernsey, UK), or combination of both CBMPs.

Methods: A prospective cohort study of patients from the UKMCR who were treated with dried flower, oils or both CBMPs for GAD was performed. Primary outcomes were changes in Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), quality of life index (EQ-5D-5L) at 1, 3, and 6 months compared to baseline. Change in scores were compared to baseline as assessed by paired t-tests according to data characteristics. The effect of use of different CBMPs on outcomes was assessed by linear regression adjusting for age, gender, previous cannabis consumption, and baseline scores. Adverse events were assessed in line with CTCAE version 4.0. Statistical significance was determined by $p<0.050$.

Results: 303 patients were included in the final analysis. 56 (18.5%), 110 (36.3%) and 137 (45.2%) patients were treated with a medium-chain triglyceride-based oil, dried flower, or a combination of CBMPs respectively. The mean age of participants was 37.0 (±11.5). 208 (68.6%) of participants were male. Patients receiving CBMPs had improvements in GAD-7, SQS, EQ-5D-5L at 1, 3, and 6 months compared to baseline ($p<0.001$). Those using dried flower only had lower anxiety scores at 1 month ($p<0.010$) compared to those using oils but differences across products for other time points were non-significant ($p>0.050$). There were no differences in outcomes according to other variables (age, gender, or previous cannabis consumption) at any time point ($p>0.050$). 571 (188%) adverse events were reported by 31 (10.2%) patients. There were no life-threatening adverse events, and the majority were mild (n=269; 88.8%) or moderate (n=242; 79.9%).

Conclusions: This study indicates an association between treatment with CBMPs and improvement in health-related quality of life, anxiety symptoms and insomnia in patients with GAD. Those prescribed dried flower only had a larger improvement in anxiety specific symptoms at one months, as measured by the GAD-7 scale compared to those prescribed oils or a combination of oils and dried flower. There was no detected effect of age, gender, or previous cannabis consumption, on response to CBMPs. Placebo-controlled trials are still necessary to distinguish the efficacy of CBMPs in the setting of GAD, in addition to identifying the optimum method of administration.

PERCEIVED STIGMA OF PATIENTS UNDERGOING TREATMENT WITH CANNABIS-BASED MEDICINAL PRODUCTS

Lucy J Troup[1], Simon Erridge[2,3], Beata Ciesluk[1], Mikael H Sodergren[2,3]

1. Division of Psychology, School of Education and Social Sciences, University of the West of Scotland, UK
2. Medical Cannabis Research Group, Imperial College London, UK
3. Sapphire Medical Clinics, London, UK

Introduction: Cannabis-based medicinal products (CBMPs) have been legal in the UK since November 2018 when they were rescheduled to allow for therapeutic use. However, despite this change there are still significant barriers to accessing CBMPs. These are thought to include cost, evidence, education, and stigma. Whilst there is a growing body of evidence of the therapeutic potential of CBMPs, little is understood about the effect of stigma on current and prospective UK patients. Evaluation in other countries has highlighted perceived stigma from healthcare professionals and society more broadly. This study therefore aimed to investigate the perceived stigma attached to patients prescribed CBMPs across a variety of domains and agencies, including healthcare settings, to establish its prevalence in a UK population.

Methods: 2,319 patients treated with CBMPs at Sapphire Medical Clinics were invited to take part in this cross-sectional questionnaire study. The questionnaire was developed utilising a multidisciplinary group of clinicians and psychologist to establish patient demographics, previous and current cannabis consumption, in addition to perceived stigma. Content validity and feasibility was confirmed through pilot testing by the Sapphire Medical Clinics Patient and Public Involvement Group (n=7). The survey was distributed via Qualtrics (Seattle, Washington, United States). Descriptive analysis was performed on responses.

Results: 450 (19.4%) participants completed the questionnaire study. 176 (39.1%) identified as female and 258 (57.3%) identified as male. The mean age they started consuming cannabis was 28.0 (±16.3). 84.4% (n=380) of participants believed that those who receive treatment with CBMPs are stigmatised. Overall, participants were comfortable speaking about their prescription with 81.3% (n=366) reporting feeling very comfortable or comfortable telling friends, 76.9% (n=346) telling family, and 61.3% (n=276) telling medical professionals. Participants largely thought that friends (n=372; 82.7%) and family (n=339; 75.3%) were very approving or somewhat approving of their CBMP prescription. However, participants thought that only 37.8% (n=170) of healthcare professionals and 32.9% (n=148) of society in general were very approving or somewhat approving of their CBMP prescription. 57.1% (n=257), 55.3% (n=249), and 40.2% (n=181) of participants were afraid of what the police or criminal justice system, other government agencies, and healthcare professionals respectively might think about them receiving treatment with CBMPs.

Conclusions: This study highlights that there is a high prevalence of perceived stigma towards patients treated with CBMPs from society, government officials, medical professionals, and the criminal justice system. Reduction of perceived stigma would likely increase appropriate access to CBMPs. In addition, this would reduce the anxiety of patients currently treated with CBMPs. Finally, it would improve the safety of treatment with patients being enabled to share their full medication history with healthcare professionals. Future work, such as education initiatives, should be undertaken to explore strategies to reduce stigma at an individual and community level to avoid discrimination of patients.

CLINICAL OUTCOME DATA OF PATIENTS WITH PRIMARY INSOMNIA DISORDER TREATED WITH CANNABIS-BASED MEDICINAL PRODUCTS: ANALYSIS FROM THE UK MEDICAL CANNABIS REGISTRY

Kavyesh Vivek[1], Zekiye Karagozolu[1], Simon Erridge[1,2], Carl Holvey[2], Ross Coomber[2,3], Ezzat Awad[2], James J Rucker[2,4,5], Mark Weatherall[2,6] and Mikael H Sodergren[1,2]

1. Medical Cannabis Research Group, Imperial College London, London, UK
2. Sapphire Medical Clinics, London, UK
3. St. George's Hospital NHS Trust, London, UK
4. Department of Psychological Medicine, Kings College London, London, UK
5. South London & Maudsley NHS Foundation Trust, London, UK
6. Buckinghamshire Healthcare NHS Trust, Amersham, UK

Introduction: Insomnia disorder, defined as the persistent dissatisfaction of sleep quality or quantity for greater than three months is thought to affect 10% of adults globally. The current pharmacological management of insomnia is limited by the risk of abuse associated with benzodiazepines and Z-drugs, the most frequently prescribed classes of medications for insomnia. Cannabis-based medicinal products (CBMPs) have been identified as potential therapeutic agents, however there is a paucity of high-quality longitudinal data to assess the outcomes in patients with insomnia disorder treated with CBMPs. The aim of this paper is to analyse change in sleep-specific and general quality of life metrics, in addition to adverse events in patients prescribed CBMPs for primary insomnia disorder.

Methods: This study describes a case series of patients with primary insomnia disorder enrolled in the UK Medical Cannabis Registry. Primary outcomes were changes from baseline in the Single-Item Sleep Quality Scale (SQS), Generalised Anxiety Disorder-7 (GAD-7), and EQ-5D-5L at 1, 3 and 6 months. Secondary outcomes included adverse event analysis according to CTCAE version 4.0 criteria. Statistical significance was identified as p-value<0.050.

Results: 61 patients were included in the final analysis. The mean age of the cohort was 41.3 (±13.0) years. The majority of patients were male (n=44; 72.1%). 31 (50.8%) patients were consuming cannabis at baseline, whilst 15 (24.6%) had previously consumed cannabis and 15 (24.6%) were cannabis-naïve. There was an improvement in self-assessed sleep quality according to the SQS from baseline (median: 2.5; IQR: 2.0-4.25) to 1 month (median: 6.0; IQR: 4.0-8.0; p<0.001), 3 months (median: 6.0; IQR: 5.0-8.0; p<0.001) and 6 months (median: 6.0; IQR: 5.0-8.0; p<0.001). There were also improvements in the EQ-5D-5L Index value and GAD-7 scores at 1, 3, and 6 months (p<0.001). There was a total of 30 (49.2%) adverse events recorded by 6 patients (9.8%), with most being mild (n=18; 29.5%) or moderate (n=10; 16.4%) in severity.

Conclusions: These results demonstrate a clinically significant improvement in self-assessed sleep quality up to 6 months following initiation of treatment with CBMPs. Moreover, patients experienced statistically significant benefits in general health-related quality of life and generalised anxiety symptoms. Adverse effects were experienced by fewer than 10% of participants, with most being mild or moderate in severity. This study complements recent randomised controlled trials on CBMPs for insomnia demonstrating that improvements are sustained at up to 6 months, however ongoing evaluation is necessary to identify the optimum cannabinoid composition of CBMPs for the treatment of insomnia, in addition to assessment of long-term efficacy and safety.

EVIDENCE-BASED CUSTOMER DISCOVERY CONFIRMS PERCEIVED CUSTOMER VALUE FOR A DECENTRALIZED RESEARCH PLATFORM FOR MEDICAL CANNABIS

Cheryl J. Fitzer-Attas[1], Shrey Joshi[2] and Connie Pascal[3]

[1]Platient Research Technologies LLC, South Brunswick, New Jersey, US,
[2]BioMedical Engineering, Rutgers University, New Jersey, US, and
[3]Department of Library and Information Science, Rutgers University, New Jersey, US

Introduction: The National Science Foundation's Innovation Corps program (I-Corps™) trains scientists & engineers in innovation skills and provides a framework for transforming their ideas into commercial successes. One of these skills, evidence-based customer discovery (EBCD) is a qualitative research methodology that focuses on understanding the problems potential customers face and what they value by interviews and insight gathering, all done before actually developing a product or service. Despite the global tidal wave of medical cannabis usage, its industry is highly-regulated and quasi-legal, leading to significant research hurdles and acute under-funding. Products often reach the market without the rigorous research that could support their broader acceptance by the medical community. The goal of this project was to use EBCD to assess potential customer segments, along with their obstacles and interests, when considering development of a decentralized on-demand research platform focused on clinical and plant medical cannabis data.

Methods: During participation in a Rutgers University regional I-Corps™ cohort, we defined potential customer segments and their corresponding value proposition hypotheses for our research platform. We then conducted semi-structured interviews with a diverse group of stakeholders (purposeful and snowball sampling) to test our hypotheses and generate insights. Interviews took place over the course of 15 weeks, were conducted via online video calls, recorded, and transcribed prior to tracking of insights using the software Innovation Within™. We had predetermined a requirement of >80% aligned insights for 'validation' of customer segments and value proposition hypotheses.

Results: We conducted 47 interviews with 15 researchers (academic/hospital settings), 14 employees of medical cannabis producers, 9 health-care providers (HCPs) who actively recommend or prescribe medical cannabis, 6 regulators/policymakers, and 2 healthcare consumers. Interviewees resided mostly in North America (72%), with lesser representation from the EU/UK (15%) and Israel (13%). Value propositions hypotheses were confirmed as 'valid' for researchers, producers, and HCP customer segments, with >80% alignment of insights. Important customer obstacles and potential platform solutions were extracted.

Conclusions: Based on lean startup methodologies, EBCD is a crucial first step in determining the potential success of a product or service before it is developed or reaches the market. We used direct dialog with key stakeholders to 'validate' and characterize specific customer segments and to 'validate' hypotheses for each of these customer groups based on the perceived value of a decentralized on-demand clinical research platform specific for medical cannabis. This process allowed us to gain qualitative evidence for product-market fit, and to learn about particular features of interest.

OREXIN-A, 2-AG AND 2-AG-DERIVED 2-AGP ARE INVOLVED IN OBESITY-ASSOCIATED ALTERATIONS OF HIPPOCAMPAL MEMORY IN MICE

Nicola Forte[1], Serena Boccella[2], Lea Tunisi[1], Alba Clara Fernández-Rilo[1], Maria De Risi[4,5], Monica Iannotta[2], Fabiana Piscitelli[1], Elvira De Leonibus [4,5], Sabatino Maione[2,8], Vincenzo Di Marzo[1,9,10,11] and Luigia Cristino[1].

1 Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche (CNR), Pozzuoli, NA, Italy. 2 Department of Experimental Medicine, Division of Pharmacology, University of Campania Luigi Vanvitelli, Napoli, Italy. 3 Department of Science and Technology, University of Sannio, Benevento, Italy. 4 Telethon Institute of Genetics and Medicine, Pozzuoli, Naples, Italy. 5 Institute of Biochemistry and Cell Biology, Consiglio Nazionale delle Ricerche (CNR), Monterotondo Scalo, Rome, Italy. 6 Department of Agricultural Sciences, University of Naples Federico II, Portici, NA, Italy. 7 Department of Veterinary Medicine and Animal Productions, University Federico II, Napoli, Italy. 8 I.R.C.S.S., Neuromed, 86077 Pozzilli, Italy. 9 Heart and Lung Research Institute of Université Laval, Québec City, QC, Canada. 10 Institute for Nutrition and Functional Foods, Centre NUTRISS, Université Laval, Québec City, QC, Canada. 11 Canada Excellence Research Chair on the Microbiome-Endocannabinoidome Axis in Metabolic Health, Université Laval, Québec City, QC 61V0AG, Canada.

Introduction: The decision "to eat or not" is derived from the integration in memory of different inputs (contextual, somatic, sensory, and socio-cultural). Every time, the outcome of this choice is stored in our memory and the same information will be used for future feeding-related decisions. Although the association between western-diet or high fat diet (HFD), obesity, and impairment of hippocampal-dependent learning and memory performance (HDLMP) has been reported in many works, the physiological mechanism underlying HDLMPP remains elusive. The hypothalamic peptide Orexin-A (OxA) and the endocannabinoid 2-arachidonoylglycerol (2-AG) have emerged as strong regulators of hippocampal functions. The biosynthesis of 2-AG, and subsequent CB1 activation are stimulated by OxA. OxA neurons of obese mice undergo a rewiring and CB1 receptor-mediated disinhibition, which, in turn, promotes an orexinergic drive to Lateral Hypothalamus output areas. We recently demonstrated how excessive OxA/eCB signaling alters adult neurogenesis, pattern separation, and episodic memory in obese mice. Moreover, 2-AG is partly converted into 2-arachidonoyl-sn-glycerol-3-phosphate (2-AGP), a lysophosphatidic acid (LPA) autocrine messenger in the hypothalamus of obese mice. 2-AGP induces LPA1 receptor (LPA1R) activation and pT231-Tau accumulation via the Pyk2-mediated pTyr216 GSK3β pathway, thus provoking a synaptic rewiring and reduction of the excitatory drive onto preopiomelanocortin neurons. We speculate that the role of Tau phosphorylation as a new form 2-AGP-mediated regulatory mechanism of synaptic plasticity could be extended to different brain areas and could contribute to the onset of nutritional-induced tauopathies.

Methods: Leptin knock-out (ob/ob), wt littermate, and C57Bl fed high-fat diet (HFD) mice were used in this study. We applied different biochemical, morphological, and e-physiological studies ranging from Western blot, immunohistochemistry, and electron microscopy, in vivo/in vitro electrophysiology coupled with pharmacology to study hippocampal memory-related plasticity in obesity. Furthermore, behavioral tests were performed to assess episode memory and memory capacity in obese mice: novel object recognition test, Morris Water Maze, and 6DOT test.

Results and Conclusions: We observed that the activation of either the OxA/2-AG/CB1 or the 2-AG/2-AGP/LPA1R pathway influence hippocampal plasticity and memory, in the former case by altering neurogenesis in the dentate gyrus and inducing disfunction in episodic memory, and, in the lattercase, by activating pT231-Tau accumulation in the CA3-CA1 region, thus negatively influencing memory capacity. Antagonism of LPA1R with AM095 restores LTP, short-term plasticity, and dorsal hippocampal memory-related functions in obese mice. Our data support the previously proposed link of neurodegeneration with obesity.

Acknowledgment: The financial support from EU, grant "PON IDF SHARID" n. ARS01_01270 – CUP B66G18000640005 and Joint International Research Unit (JIRU) for Chemical and Biomolecular Research on the Microbiome and its impact on Metabolic Health and Nutrition (MicroMeNu) is acknowledged. This study is also supported by the Sentinelle Nord Program of Université Laval (to N.F., L.C. and V.D.).

HEALTHCARE PROVIDERS' KNOWLEDGE AND ATTITUDES ON CANNABIS-BASED TREATMENTS

Albert Garcia-Romeu, Sandeep Nayak, David Mathai, Erin Wang and Natalie Gukasyan

Department of Psychiatry and Behavioral Sciences,
Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction: Despite limited clinical data, medical use of cannabis-based treatments for a variety of conditions has expanded rapidly. As a result, healthcare providers who may lack specialized training in therapeutic use and interactions of cannabis are increasingly serving patients who use cannabis-based treatments.

Methods: The current study was conducted via anonymous online survey circulated through Internet-based postings targeted broadly to healthcare providers in the U.S.A. (e.g., physicians, psychologists, counselors, social workers, therapists, nurses, pharmacists). Demographic data, and basic knowledge and attitudes surrounding cannabis-based treatments were queried.

Results: Presently, 175 people have completed the survey. Data collection is ongoing. The current sample has a mean (SD) age of 28 (11.5) years, is primarily female (67%), white (90%), and hold Master's (40%) or Doctoral (36%) degrees, with Physicians (21%), Registered Nurses (15%), and Therapists (13%) being most prevalent. Participants completed their professional training a mean (SD) of 12 (10.6) years ago. Most (84%) reported their patients use cannabis, and a majority (77%) stated they would like training in therapeutic use of cannabis, with 71% reporting they would be open to using cannabis-based treatments in their clinical practice. Most (87%) agreed cannabis shows promise as a medical treatment, and 95% felt that medical cannabis should be legally available, with 97% calling for more clinical research on cannabis. Only 29% of respondents reported formal clinical training in therapeutic use of cannabis, while larger proportions cited academic literature (67%), popular media (68%), and personal experience (77%) as their primary knowledge sources regarding cannabis. Most said they would trust information on therapeutic use of cannabis from professional organizations (80%), academic research centers (87%), and experienced clinicians (91%). A majority thought cannabis could be administered safely in outpatient hospital (74%), private practice (79%), and specialized clinic (88%) settings. The most commonly endorsed concerns regarding risks of cannabis use among patients were lack of training for healthcare professionals (63%), financial costs / insurance coverage (44%), psychosis (44%), and addiction or abuse potential (43%).

Conclusion: A majority of healthcare providers responding to the present survey reported cannabis use among their patients, and expressed openness to using cannabis-based treatments in clinical practice. However, most did not receive formal training in the therapeutic use of cannabis and cited the need for additional targeted education from trustworthy sources as well as further research. Though some concerns regarding risks were noted, many respondents agreed cannabis-based treatments could be safely administered in a variety of healthcare settings.

Acknowledgments: Support for this study was provided by the Johns Hopkins Center for Psychedelic and Consciousness Research with funding from Tim Ferriss, Matt Mullenweg, Craig Nerenberg, Blake Mycoskie, and the Steven and Alexandra Cohen Foundation.

PREVALENCE AND IMPACT OF CANNABINOID USE IN PATIENTS WITH SPINAL DISORDERS

Durga Monica Ghosh, Alex Upfill-Brown and Elizabeth Lord

David Geffen School of Medicine at the University of California, Los Angeles, CA, USA
Department of Orthopaedic Surgery, David Geffen School of Medicine at UCLA, CA, USA

Introduction: One in four Americans suffer from chronic back pain, with 40% reporting trauma or surgery as its origin. Cannabis is becoming widely decriminalized or legalized for medical and recreational use. While there is literature on medical cannabis for pain management, little is focused on pain following spinal surgeries. This observational study aimed to address whether cannabinoid use provides meaningful pain relief and a decrease in pain medication consumption post-operatively.

Methods: Patients 18 years or older with a history of spine fusion at a large academic spine center since 2013 were asked to complete a questionnaire and were incentivized through raffle entry and follow-up calls. The survey included questions regarding demographics, level of pain, types of pain medication used for management of spine pain, recreational, personal, and medical use of marijuana and cannabis products, and whether marijuana or cannabis use reduces pain. Patients were asked for quality-of-life scores via PROMIS Pain Behavior, Pain Interference, and Physical Function.

Results: A total of 7,371 patients were sent surveys, with 118 responding (1.6% response rate) from March to July 2021. Of the 118 patients surveyed, 61 patients identified themselves as having used cannabis and continue to use cannabis for pain. Of the respondents, 23.1% of patients reported using cannabis once or more daily, and 47% of patients reported using cannabis for pain more than once a month. Among spine patients, 55.5% reported a 50% or greater reduction in the use of prescription pain medications, specifically opioids, after using cannabis. PROMIS Scores for patients who underwent spine surgery indicate both cannabis users and nonusers experience more pain and more difficulties with physical function than the general population, as demonstrated by the T-Test Analysis of PROMIS Scores by Cannabis Users vs Nonusers. No statistical significance was found between users and nonusers.

Conclusion: Among spine patients, it was subjectively perceived that cannabis use had reduced their pain medication use. However, it was found that cannabis users did not have reduced pain levels based on PROMIS scores. There was no statistical difference in PROMIS Scores between users and nonusers, differing from the association with reported quantity of pain medication used. This continued research will be pioneering in giving providers and patients information regarding cannabinoid use in patients with spinal disorders and will determine if cannabis can decrease narcotic pain medication prescription.

CANNABINOID EXPOSURE AND SUBJECTIVE EFFECTS AFTER ACUTE *AD LIBITUM* ADMINISTRATION OF ORAL CANNABIS IN A NATURALISTIC SETTING

Laurel Gibson[1], Raeghan Mueller[2], Angela Bryan[1],
L. Cinnamon Bidwell[1] and Kent Hutchison[2]

[1]University of Colorado Boulder, Boulder, Colorado, USA
[2]University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA

Introduction: The legalization of cannabis has led to an expansive retail marketplace with increased production and sale of diverse cannabis products and administration methods, including edible cannabis products. Although edible cannabis products make up a large portion of legal market sales and are gaining in popularity, there is a paucity of research examining the acute effects of these products. The purpose of the present study was to examine the acute effects of three commercially available edible cannabis products with different levels THC and CBD.

Method: A federally compatible, naturalistic at-home administration procedure was used to investigate differences in plasma cannabinoid concentrations and subjective effects after *ad libitum* use of legal-market cannabis edibles. Participants were $N = 99$ regular cannabis users. Participants were randomized to self-administer one of three legal-market cannabis edible products: (1) a THC-only tablet, (2) a CBD-only tablet, or (3) a THC+CBD tablet. Participants were assessed before and 2-hours after *ad libitum* administration of their assigned product.

Results: 2h post-use plasma THC and CBD concentrations were strongly correlated with self-reported milligrams of THC ($p < .001$) and CBD ($p < .001$) consumed. Participants in the CBD condition reported feeling less 'high' relative to those in the THC ($p < .001$) and THC+CBD ($p < .001$) conditions. Participants in the THC+CBD condition reported similar levels of positive (high, elation) and negative (anxiety, paranoia) effects relative to participants in the THC condition ($ps > .27$). Notably, despite reporting similar levels of subjective effects after acute use, participants in the THC+CBD condition consumed less THC ($p = .003$) relative to participants in the THC condition.

Conclusions: This is the first study, to our knowledge, that has explored whether the acute effects of legal market cannabis edibles vary as a function of their cannabinoid content. Findings suggest that, among experienced users, edible cannabis products with both THC and CBD may be associated with less THC consumption, as users of THC+CBD products appear to experience positive drug effects at similar rates relative to users of products with higher levels of THC and no CBD. Given the potential harms associated with long-term THC exposure, THC+CBD products may pose less risks to the user relative to THC-only products, thus representing an important avenue for future harm reduction research.

Acknowledgements: Funded by the National Institute on Drug Abuse (Grant no. DA039707).

PRELIMINARY DATA ON THE IMPACT OF RECREATIONAL CANNABIS LEGALIZATION ON CANNABIS USE PATTERNS IN THE NY METROPOLITAN AREA

Rina Goldstein, Sean Madden and Nehal P. Vadhan

Center for Addiction Services and Personalized Interventions Research,
Feinstein Institutes for Medical Research, Glen Oaks, NY, USA

Introduction: In March of 2021, limited recreational cannabis use was legalized in NY State. The effects of legalization on local cannabis use behavior remains unclear. This study aims to assess reported changes in cannabis use patterns and motivation following legalization.

Methods: 290 cannabis users from the NY metropolitan area who had completed a baseline original online survey in 2018 on cannabis use patterns and related variables were targeted via email to complete a follow-up survey which included additional items related to legalization. 78 adults (age 23-57; M=49, F=28, T=1) have completed the survey in a 3-week recruitment period thus far.

Results: Respondents endorsed using cannabis 6.27 (SD = 1.5) days per week, on average. Regarding routes of administration, all participants reported smoking (100%), and about half reported vaping (53.8%) or ingesting (53.8%) their cannabis. Almost all respondents reported using cannabis for recreational purposes (97.4%) whereas a lesser majority reported using cannabis for medicinal purposes (80.8%). 64 (82.1%) respondents reported knowing about the legalization before it happened, and 14 (17.9%) only knew once it happened. Regarding changes in recreational cannabis use since the NYS legalization, 71 (91.0%) respondents reported that their use stayed the same, 4 (5.1%) reported that they increased their use, and 3 (3.8%) reported that their use had decreased. 5 (6.4%) respondents reported restarting their use since legalization. Among those who increased or restarted their use, 5 (6.4%) reported no more legal consequences, 3 (3.8%) reported less worry at work and more awareness of positive or medicinal effects, and 2 (2.6%) reported increased social acceptability. Among those that decreased their use, their reasons were unrelated to legalization (n=3).

Conclusions: These preliminary data suggested that cannabis use patterns in a sample of regular cannabis users were not significantly altered following legalization of recreational cannabis use in the New York metropolitan area. Most users were aware of the legalization prior to it happening, and of those who restarted their use, commonly cited reasons were related to fewer consequences since legalization. Final data collection will be used to confirm these results and explore further motivations for change in cannabis use.

MEDICAL CANNABIS LEGALIZATION IN THE UNITED STATES: EVIDENCE-BASED OR POLITICALLY DRIVEN?

G. Malik Burnett,[1] David A. Gorelick[2] and Kevin P. Hill[3]

[1]Center for Addiction Medicine, University of Maryland Medical Center, Baltimore, MD, USA; [2]Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA; [3]Department of Psychiatry, Harvard Medical School, Boston, MA, USA

Introduction: As of March 2022, 37 US states and 4 territories have legalized medical cannabis for various qualifying medical and psychiatric conditions. Legalization was done by political process (legislation, referendum), not the evidenced-based regulatory process used by the US Food and Drug Administration. We describe the medical cannabis landscape using descriptive statistics and compared the strength of evidence for the efficacy of medical cannabis for each qualifying condition and associations between strength of evidence and number of qualifying conditions per jurisdiction over time.

Methods: Current information about medical cannabis legal status and qualifying conditions was obtained by reviewing the text of each medical cannabis law (National Conference of State Legislatures web site https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx). Evidence of efficacy was obtained from 2 recent systematic reviews (US National Academy of Science [NAS], 2017; Montero-Oleas N, et al. BMC Complement Med Ther 2020;20:12). Strength of evidence was graded according to the system used by the US NAS. Formal statistical testing was not performed due to small sample sizes and skewed distribution of variables.

Results: The 41 US jurisdictions list 42 distinct qualifying conditions: 35 medical, 7 psychiatric. The median number of conditions per jurisdiction is 13, range 0-29. Six conditions are listed by ≥ 80% of jurisdiction; 9 conditions by < 20% of jurisdictions. Almost two—thirds (65%) of qualifying conditions have insufficient or limited evidence of efficacy; 9% have limited evidence of harm. Only one-quarter (25.5%) of conditions have at least moderate evidence of efficacy. The mean (SD) proportion of conditions per jurisdiction with at least moderate evidence for efficacy is 39.1% (10.2%), range 22%-67%. The pattern of number of conditions and level of evidence of efficacy per jurisdiction have not changed meaningfully over the past 2.5 decades. The 6 commonest (≥80% of jurisdictions) conditions have more favorable strength of evidence of benefit (half with substantial evidence) than do the 10 rarest (<20% of jurisdictions) conditions (20% with even moderate evidence). The 5 qualifying conditions with substantial evidence of efficacy are more commonly listed (all >50% of jurisdiction) than are the 3 qualifying conditions with limited evidence of harm (67% in <10% of jurisdictions).

Conclusions: The majority of qualifying conditions for medical cannabis have little or no scientific evidence of efficacy; 9% have limited evidence for harm. While conditions with substantial evidence of efficacy are more commonly listed than those associated with harm, the overall pattern suggests that legal decisions about qualifying conditions for medical cannabis are often driven more by politics than by science.

Acknowledgements: No external funding was received for this study.

A MAJOR QUESTION: ACCURACY OF MINOR CANNABINOID CONTENT ON CANNABIS-CONTAINING PRODUCT LABELS

Deniz Kosereisoglu [1,2], Rosemary T. Smith[1,2], Kelly A. Sagar[1,2,3], Ashley M. Lambros[1,2], M. Kathryn Dahlgren[1,2,3], Celine El-Abboud [1,2] and Staci A. Gruber [1,2,3]

[1]Cognitive and Clinical Neuroimaging Core (CCNC), McLean Hospital, Belmont, MA, USA; [2]Marijuana Investigations for Neuroscientific Discovery (MIND) Program, McLean Hospital, Belmont, MA, USA; [3]Department of Psychiatry, Harvard Medical School, Boston, MA, USA

Introduction: Although legalization of medical cannabis (MC) and cannabinoid-containing products continues to expand across the globe, a lack of standardization for product labels currently exists, raising concerns regarding inaccurate labeling of these products. Previous studies have examined label accuracy for Δ-9-tetrahydrocannabinol (Δ-9-THC) and cannabidiol (CBD), yet no studies thus far have examined the label accuracy of minor cannabinoids, which are also hypothesized to have significant biobehavioral effects and are increasingly sought by patients. Accordingly, we analyzed the accuracy of cannabinoid content claims on product labels, including Δ-9-THC, CBD, and minor cannabinoids (cannabidiolic acid [CBDa], cannabidivarin [CBDV], tetrahydrocannabinolic acid [THCa], tetrahydrocannabivarin [THCV)] cannabinol [CBN], cannabichromene [CBC], cannabigerol [CBG] and cannabigerolic acid [CBGa]).

Methods: Samples of 111 MC products (56 tinctures, 8 capsules/tablets, 14 vape cartridges, 9 patches/topicals, 17 edibles, and 7 flower/kief products) were submitted to an independent laboratory (ProVerde Laboratories, Inc.) for quantification of cannabinoid constituents via ultra-performance convergence chromatography. Label accuracy was assessed by comparing cannabinoid content on product labels to the quantification of individual cannabinoids, and labels were determined to be accurate (cannabinoids accurately labeled or omitted correctly) or inaccurate (cannabinoids omitted incorrectly, over-labeled by >10%, or under-labeled by >10%).

Results: Only 40.54% of products were labeled correctly (accurately labeled or omitted correctly) for Δ-9-THC content, 33.33% for CBD, and 65.09% on average for minor cannabinoids. Interestingly, for minor cannabinoids, this finding was driven by "omitted correctly" labelling (56.76%); when present, minor cannabinoids were accurately labeled only 8.33% of the time. In terms of inaccurate labels, concerningly, products were frequently under-labeled or omitted cannabinoids that were actually present ("omitted incorrectly"): Δ-9-THC (45.94%), CBD (39.64%), and minor cannabinoids (30.29%). Over-labeling inaccuracies were less common: Δ-9-THC (13.51%), CBD (27.03%), and minor cannabinoids (4.62%).

Conclusions: As MC patients often base their product choice and dosage for anticipated symptom relief on product labels, inaccurate cannabinoid labeling raises significant concerns regarding proper and consistent dosing, including increased risk of unwanted side effects (e.g., intoxication) or unexpected drug-drug interactions. The widespread inaccuracies of MC product labels in this sample highlight the need for labeling regulation and standardization across products.

Acknowledgments: This project was funded by private donations to the MIND Program. We thank Christopher Hudalla, PhD, ProVerde Laboratories, for laboratory services and consultation.

SEX-DEPENDENT DIFFERENCES IN ACUTE OBJECTIVE AND SUBJECTIVE INTOXICATION AMONG HIGH POTENCY CANNABIS FLOWER USERS

Charleen J. Gust, Laurel P. Gibson, Jarrod M. Ellingson, Sophie L. YorkWilliams, Cristina Sempio, Jost Klawitter, Angela D. Bryan, L. Cinnamon Bidwell and Kent E. Hutchison

University of Colorado Boulder, Colorado, USA

Introduction: Public acceptance and legalization of cannabis use, both for medical and recreational purposes, has changed dramatically over the past two decades. In the United States, thirty-seven states and the District of Colombia have legalized medical cannabis, and the commercial production and distribution of cannabis for recreational purposes is legal in 18 of these states. This rapid liberalization of U.S. cannabis policy has been met with a substantial increase in THC potency. However, given that most published studies have evaluated the effects of cannabis using products that are not representative of those available on the legal market, the risks associated with high THC potency cannabis have received little scientific attention. Additionally, research on how the risks associated with high potency products differ between men and women is quite scarce. To address this gap, the present analysis uses data from a novel, naturalistic research methodology to: (1) determine acute objective and subjective intoxication as well as cognitive effects following high THC potency cannabis flower use; and (2) examine the sex-dependent nature of these effects.

Methods: N=56 participants (33 male, 23 female) were included in analyses. Following their baseline appointment, all participants were asked to obtain one gram of cannabis flower (either 16% or 24% THC) from a local study-partnered dispensary. At the experimental appointment, which took place in a mobile pharmacology lab, participants completed a blood draw to assess plasma cannabinoid levels and measures of subjective effects (vigor, tension, feel high, physically stoned, and mentally stoned) and cognitive performance (delayed verbal recall, working memory, and inhibitory control) at three time points: pre-use, acute post-use, and one-hour post-use.

Results: In both men and women, plasma THC levels exhibited a quadratic effect of time (b = 268.84, $p < .001$). THC levels peaked at the acute post-use assessment and dropped an hour after use. Controlling for potency and THC levels at baseline, there was a marginal gender by quadratic change interaction on THC levels (b = -61.75, p = .105), with men displaying a stronger quadratic effect for THC than women. The association between peak THC and the five subjective intoxication measures did not vary as a function of sex (all ps > .39). Finally, the association between peak THC and the three cognitive performance measures also did not vary as a function of sex (all ps > .54).

Conclusions: Findings indicate that though men displayed higher plasma THC levels at the acute post-use assessment, there were no sex differences in cognitive performance or subjective drug effects following *ad libitum* cannabis use. These results have both clinical and public health implications and represent a contribution to the sparse literature on high THC potency forms of cannabis, as well as the potential for sex-dependent differences in response to cannabinoids.

CHRONIC CANNABIDIOL TREATMENT SEX-DEPENDENTLY ALTERS HPA AXIS RESPONSIVITY WITHOUT MODULATING FEAR MEMORY OR ANXIETY-LIKE BEHAVIOUR

Bryan W. Jenkins[1], Hayley Wilson[2], Kate Nicholson[1], Amy E. M. Newman[2] and Jibran Y. Khokhar[1]

[1]Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada
[2]Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

Background: Cannabidiol (CBD) may be a promising therapeutic for post-traumatic stress disorder and other anxiety-related disorders, however the preclinical evidence is mixed and underlying mechanisms are unknown. Considering evidence that CBD treatment regulates hypothalamus-pituitary-adrenal (HPA) axis gene expression in mice, we hypothesize that chronic CBD treatment in mice will sex-dependently alter HPA axis responsivity and shift baseline corticosterone (CORT) levels over time, reducing measures of fear conditioning (FC) and anxiety-like behaviour.

Methods: Female and male mice underwent daily intraperitoneal (i.p.) injections of vehicle or CBD (30mg/kg) for 25 days. At acute and chronic timepoints, blood samples were collected for analysis of CORT levels, while HPA axis responsivity was assessed using a dexamethasone (DEX, 0.1mg/kg, i.p.)/adrenocorticotropin (ACTH, 0.5mg/kg, i.p.) challenge. After chronic treatments, mice underwent elevated plus maze (EPM) and FC testing.

Results: As expected, female mice had higher baseline corticosterone levels and greater HPA axis responsivity than male mice. CORT levels were higher for all mice at the chronic timepoint. Female mice also spent more time than males in the open arms of the EPM. Chronic CBD treatment increased HPA axis responsivity selectively for male mice compared to vehicle-treated controls, while not affecting acute or chronic baseline CORT levels. Chronic CBD treatment did not alter behaviour in the EPM or FC freezing behaviour in female or male mice at the dose tested.

Conclusion: With higher CORT levels at the chronic timepoint for all mice and chronic CBD treatment selectively enhancing male HPA axis responsivity, chronic CBD treatment in female mice may have blunted female HPA axis responsivity to increased CORT levels. The lack of behavioural effect of chronic CBD treatment may highlight differential mechanisms of CBD on stress-related behaviours.

QUANTITATION OF 17 PHYTOCANNABINOIDS IN HEMP-DERIVED OILS: LC-MS/MS METHOD VALIDATION AND PRODUCT DATA

Erin Johnson[¶], Michael Kilgore[¶] and Shanna Babalonis[§]

Departments of Pharmacology and Nutritional Science[¶] and Behavioral Science[§], University of Kentucky College of Medicine, Lexington, KY, USA

Introduction: Cannabidiol (CBD) products are widely available and are used for several therapeutic indications. However, quantitation of the minor cannabinoid content in commercially available products has not been previously reported. To fill this gap: 1) a liquid-chromatography tandem mass spectrometry (LC-MS/MS) was developed for the quantification of 17 phytocannabinoids, 2) this method was validated for the analysis of hemp-derived oil products, 3) a total of 80 commercially available products were tested and Epidiolex was included as an FDA-approved standard.

Methods: A liquid extraction method was developed to isolate phytocannabinoids from an oil matrix. The extracts were analyzed by LC-MS/MS using a targeted method on a Thermo TSQ Vantage system with ionization in both positive and negative modes. Separation was carried out using a Phenomenex Kinetex® C8 column and 12-min gradient program. The method was validated for the quantification of 17 phytocannabinoids by internal standard method with a selection of stable-labeled phytocannabinoid analogs. The method validation included experiments of selectivity, recovery, matrix effects, accuracy, and precision. All products were analyzed for concentrations of cannabidivarinic acid (CBDVA), cannabidivarin (CBDV), Δ^9-tetrahydrocannabivarinic acid (THCVA), Δ^9-tetrahydrocannabivarin (THCV), cannabidiolic acid (CBDA), CBD, cannabigerolic acid (CBGA), cannabigerol (CBG), cannabinolic acid (CBNA), cannabinol (CBN), Δ^9-tetrahydrocannabinolic acid-A (THCA-A), Δ^9-THC, Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabicyclolic acid (CBLA), cannabicyclol (CBL), cannabichromenic acid (CBCA), and cannabichromene (CBC).

Results: The recovery of the 17 phytocannabinoids targeted in the method ranged from 87% to 100% with matrix effects ranging from -1% to +3%. Linearity was demonstrated by coefficient of determination (R^2) of at least 0.98 across all batches and all analytes with linear or quadratic regression and 1/x or $1/x^2$ weighting as appropriate.

Epidiolex® contained 96.1 mg/mL of CBD, 0.022 mg/mL of Δ^9-THC, 0.354 mg/mL of CBDV, and 0.007 mg/mL of CBG. Across the unregulated products, the most frequently detected cannabinoids (aside from CBD and Δ9-THC) were CBDV (100% of samples tested), CBG (77%), CBC (72%), CBN (67%) and CBL (67%). Concentrations of these minor cannabinoids varied widely, from trace concentrations to several mgs/mL (e.g., CBC: 0.006 – 3.330 mg/mL).

Conclusions: The developed method is suitable for the analysis of 17 targeted phytocannabinoids in hemp-derived oil products. The concentrations of minor cannabinoids varied greatly across products, with some containing quite high concentrations, while others containing little to no minor cannabinoids. Although the therapeutic effects of commercially available CBD products are typically attributed to the CBD content, minor cannabinoids may be contributing to the purported therapeutic efficacy of CBD formulations.

Acknowledgements: Dr. Shanna Babalonis was supported by National Institute on Drug Abuse grants R21 DA045101 and R01 DA045700.

Δ^9-TETRAHYDROCANNABINOL, CANNABIDIOL, AND THEIR COMBINATION RESULT IN SEXUALLY DIMORPHIC MODULATIONS OF PLASMA LIPIDS

Clare T. Johnson, Hannah R. Bentz, Gabriel H.D. Abreu, Ken Mackie,
Hui-Chen Lu and Heather B. Bradshaw

Introduction: Male and female mice have differing behavioral responses to cannabinoids (CBs) and differing endogenous cannabinoid (eCB) signaling systems. Our lab has previously shown that Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and the combination of THC+CBD modulate lipid signaling molecules in neural cell lines. Here, we hypothesized that CBD, THC, and their metabolites would accumulate differently in plasma when each CB was administered alone vs. in combination, and that they would be metabolized at different rates in male and female mice. Further, we hypothesized that plasma levels of endocannabinoids (eCBs), free fatty acids, and related lipid signaling molecules would be altered in a sex- and drug-dependent manner.

Methods: Male and female CD1 mice were administered CBD (10mg/kg), THC (10mg/kg), or THC+CBD (10mg/kg) via i.p. injection. 2hr post-administration, animals were sacrificed, and their blood collected in heparin-containing tubes that were then centrifuged for 5 min. The upper fraction containing plasma was collected and stored at -80˚C until further processing. To extract lipids, 75µL of plasma + 1,925µL of methanol + 5µL of 1µM internal standard (d8-AEA) were combined and incubated in the dark on ice for 15 min before being centrifuged at 19,000G, 20˚ C for 20 min. The resulting lipid-containing supernatants were decanted into 6 mL of water to make a ~75% organic solution. Lipids were partially purified from this mixture using C-18 solid phase extraction columns, eluting with 65%, 75%, and 100% methanol (1.5 mL). Lipids were quantified using HPLC-MS/MS. Data were analyzed by drug treatment relative to vehicle using one-way ANOVAs with Fishers LSD post-hoc tests.

Results: CBD plasma concentrations were significantly higher in the THC+CBD treatment group in both males and female compared to CBD alone. CBD metabolites 7-OH-CBD and 7-COOH-CBD were also significantly higher in the female THC+CBD group than female CBD. THC concentrations were significantly higher in male plasma than female plasma in the THC alone groups, but levels did not differ between the THC+CBD groups. Additionally, 11-OH-THC concentrations were higher in females in the THC+CBD group compared to females in THC alone. CBD had no effect on levels of NAEs in either females or males; however, THC+CBD significantly increased levels of PEA, SEA, OEA, and LEA in females with no effect in males. THC alone had a particularly dramatic effect on 2-acyl-sn-glycerol species in male plasma, with significant or trending decreases in all 4 species measured (2-AG, 2-LG, 2-OG, and 2-PG), whereas only levels of 2-LG was significantly decreased after THC treatment in females.

Conclusion: Taken together, these data illustrate that 10mg/kg of CB treatment has significant effects on endogenous lipids in plasma 2 hours after injection and that these changes are sex dependent. Likewise, concentrations of CBs are differentially regulated by CB species and treatment modality and that CB metabolites are the most variable by sex.

Acknowledgements: This project was funded through a T32 (T32DA024628) training grant

NABILONE IMPAIRS SPATIAL AND VERBAL WORKING MEMORY IN HEALTHY SUBJECTS

Faiz M Kassim[1], Sophie Tod[1], Matthew A Albrecht[2], Sean Hood[1], Joseph W.Y. Lee[1], Jennifer Rodger[1] and Mathew T Martin-Iverson[1]

[1]University of Western Australia, Perth, Australia
[2]Curtin University, Perth, Australia

Introduction: The present study aimed to investigate the effect of acute cannabis intoxication on verbal (VWM) and spatial (SWM) working memory by administering oral doses of synthetic cannabinoid agonist, nabilone (1-2 mg, PO). The study also aimed to investigate the effect of nabilone on schizotypy (psychotic-proneness) scores and resultant effects on WM, VWM and SWM.

Methods: Healthy participants completed spatial span and digit span across different delay conditions for WM (n = 28) and psychological testings for schizotypy measures (n = 25) in each of two days after receiving nabilone (1-2 mg, PO) or placebo in a randomized, double-blind, counterbalanced, crossover manner.

Results: Nabilone impaired VWM ($p = 0.03$), and SWM ($p = 0.00016$). Nabilone did not significantly change schizotypy scores. Schizotypy scores were negatively correlated with WM ($\rho = -0.43$, $p = 0.028$) and VWM ($\rho = -0.35$, $p = 0.07$) but not with SWM ($\rho = -0.24$, $p = 0.23$) across averaged delay condition.

Conclusion: low doses of synthetic cannabinoid (nabilone) impaired SWM and VWM indicating that exogenous activation of the cannabinoid system deteriorates cognitive performance. Furthermore, the relationship between WM impairment and schizotypy in healthy individuals suggests that WM deficit may be a core cognitive dysfunction in people with schizophrenia spectrum disorder.

Acknowledgements: Funded by University of Australia (RTP scholarship for the first author)

DO CANNABIS "BREATHALYSERS" WORK? RELATIONSHIP BETWEEN BREATH CANNABINOIDS, PLASMA CANNABINOIDS, AND DRIVING PERFORMANCE

Richard C. Kevin[1], Rebecca Gordon[1], Thomas R. Arkell[1,2], Frederick Vinckenbosch[2], Eef L. Theunissen[2], Johannes G. Ramaekers[2] and Iain S. McGregor[1]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, NSW, Australia
[2]Faculty of Psychology and Neuroscience, Maastricht University, The Netherlands

Introduction: The effects of cannabis on driving performance are increasingly pertinent as cannabis is legalised or decriminalised for medicinal and recreational use. Breath sampling devices can trap small amounts of cannabinoids in exhaled breath, and it has been suggested that such devices could be used alongside ethanol "breathalysers" and roadside drug testing. However, it is unclear if breath cannabinoid concentrations correlate with blood cannabinoid concentrations or if they can predict driving impairment.

Methods: As part of a double-blind, within-participants, randomized clinical trial, participants vaporised THC-dominant, CBD-dominant, THC/CBD-equivalent, or placebo cannabis, before undertaking two on-road driving tests (40 min and 4 h post-vaping). Breath samples were collected from 14 participants using SensaBues breath collection devices at the end of each driving test. Cannabinoids were extracted from these devices and analysed via LC-MS/MS for THC, CBD, and THC/CBD phase I metabolites. Blood samples were also taken throughout the study, and plasma was quantitatively analysed for cannabinoids by LC-MS/MS.

Results: CBD and THC were readily detectible in breath, 7-COOH-CBD, 11-OH-THC, and 11-COOH-THC were occasionally detected, and 6- and 7-OH-CBD were not detected (limits of detection ranging between approximately 1-20 pg/collection pad depending on analyte). Bivariate correlations between breath concentrations and plasma concentrations failed to reach statistical significance for all analytes. Interestingly, breath 11-OH-THC was positively correlated with standard deviation of lateral position (SDLP, "lane weaving"), a measure of driving impairment, for the first driving test only ($r = .74$, $p < .001$). However, breath data was highly variable for all detected analytes, and 11-OH-THC concentrations fell below limits of detection and quantification for most samples.

Conclusions: In this study, there was no clear relationship between cannabinoid concentrations in breath and blood. Yet there did appear to be a relationship between breath 11-OH-THC and driving impairment as measured by SDLP, although we note that this only occurred during the first driving test, and that this relationship was underpinned by a small number of datapoints. Considering the overall variability of the breath data, and the lack of predictive utility for blood concentrations (compared to the close relationship between breath and blood alcohol concentration, for example), it may be difficult or impossible to implement roadside breath testing for cannabis-induced driving impairment.

CANNabinoid Drug Interaction Review (CANN-DIR™) Web App

Paul T. Kocis and Kent E. Vrana

Penn State College of Medicine, Department of Pharmacology, Hershey, PA, USA

Introduction: Recognizing the opportunity to increase patient safety by reducing unintended drug-drug interactions (DDI), we have developed the CANNabinoid Drug Interaction Review (CANN-DIR™) Web App to identify potential DDI. CANN-DIR™ is based on an extension of the authors' prior work entitled '*Delta-9-Tetrahydrocannabinol and Cannabidiol Drug-Drug Interactions'. Med Cannabis Cannabinoids 2020;3:61-73. doi: 10.1159/000507998*
CANN-DIR™ was developed to screen for potential drug-drug interactions from the perspective of how a cannabinoid (THC, CBD, *x:y* THC:CBD) may affect the metabolism of another concomitantly prescribed medication. CANN-DIR™ is freely accessible from both mobile and desktop devices by the following URL: https://CANN-DIR.psu.edu

Methods: CANN-DIR™ was based on FDA-approved prescribing information for prescription cannabinoids (dronabinol, nabilone, & prescription CBD). The Summary of Product Characteristics (SmPC) was the source of drug-drug interaction information for the combined Δ^9-THC & CBD product nabiximols. We also included the FDA 'Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers'.

Results: The drug interaction RESULTS screen lists the cannabinoid (precipitant) medication that either INHIBITS, INDUCES, or competes as a SUBSTRATE for a specific enzyme/receptor for the medication (object) of interest. The use of directional arrows and capitalization provide visual cues to help identify the predicted increase (↑) or decrease (↓) effect on the metabolism of the medication of interest.

Conclusions: As Δ9-THC and CBD over-the-counter (OTC) products and prescription medications are becoming increasingly available from a pharmacy, dispensary, Internet, local retail store, or by illicit means, there is an increased likelihood of an unintended drug-drug interaction when coadministered with another herbal, OTC, or prescription medication. This online tool provides a free publicly accessible mechanism by which a healthcare provider, caregiver, or patient can determine if there is a potential drug-drug interaction between their prescription medications and a cannabinoid product.

CANN-DIR™ is the only known drug interaction web app specific to the cannabinoid class of medications and is meant to provide a readily available resource when evaluating other medications. Individual patient characteristics also need to be included in this medical decision making. If a potential drug-drug interaction is identified, it does not necessarily translate into a contraindicated combination nor a clinically significant interaction, but an opportunity to evaluate the dose of either the cannabinoid or concomitantly prescribed medication.

Acknowledgements: K.E.V. and with the Penn State College of Medicine are the recipients of research support from PA Options for Wellness (a state-approved medical marijuana clinical registrant). We acknowledge Penn State College of Medicine's Center for Medical Innovation.

SERUM MARKERS OF BONE TURNOVER FOLLOWING ADMINISTRATION OF ORAL MEDICAL CANNABIS PRODUCTS IN HEALTHY ADULTS

Justyna Kulpa, Graham Eglit, Laura MacNair, Helena Yardley, Mark Ware, Marcel O. Bonn-Miller and Erica N. Peters

Human and Animal Research Program, Canopy Growth Corporation, Smiths Falls, Canada

Introduction: Bone tissue quantity and structural integrity are maintained via a continual cycle of osteoclastic bone destruction and osteoblastic bone formation. While cannabidiol (CBD) has been shown to maintain bone integrity in preclinical models, little is known about the effects of delta-9-tetrahydrocannabinol (THC) on bone. Here we explored the effects of two commercially available cannabis-based products on normal bone homeostasis through evaluation of markers of bone resorption (carboxyl-terminal collagen crosslinks; CTx) and bone formation (procollagen type 1 N-terminal propeptide; P1NP) in healthy men and women.

Methods: The present study is a secondary analysis from two Phase 1 double-blind, placebo-controlled, multiple-dose trials of two oral medical cannabis products: Spectrum Yellow oil (0.9 mg THC, 20 mg CBD per 1 mL of oil), and Spectrum Red softgels (2.5 mg THC, 0.3 mg CBD per softgel). Participants (n=38 males, 43 females; age=18 to 53 years; BMI=18.3 to 29.6 kg/m^2) were randomized to receive 5, 10, 15, or 20 mg total THC (corresponding CBD levels varied as a function of relative CBD dose within each administered product), or placebo daily (dosed BID) for 7 days. Serum bone markers (CTx, P1NP) were assessed prior to the first dose (baseline), and 32-hours (day 8) and 5 days (day 13) following discontinuation.

Results: Collapsed across group and timepoint, significant sex differences in CTx (F=21.02, p<.001) and P1NP (F=6.33 p=.01) were observed (both higher in males); therefore, sex was included as a covariate in analyses. For CTX, there was a significant day x group interaction (F=4.12 p=.003), wherein levels of CTX were lower in Spectrum Red (t=2.59, p=.029) and Yellow (t=2.90, p=.012) groups than placebo on day 8, but there was no difference among any group at baseline or on day 13, suggesting a dampening of CTX levels in the presence of Spectrum Red and Yellow. For P1NP, there was no treatment x day interaction (p=.122) and no difference between treatments when marginalized over levels of day (all ps>.43). Bone marker values outside the normal reference range (RR) were observed at all timepoints, including at baseline; CTx >RR (n=71) was predominantly (85.9%) observed in male participants, while P1NP >RR (n=100) was more evenly distributed between sexes (53.0% in males). None of the changes in CTx or P1NP was considered clinically significant.

Conclusions: To our knowledge, these are the first interventional human data on the effect of cannabinoids on serum biomarkers of bone turnover. Short-term treatment with CBD- or THC-dominant medical cannabis products did not result in clinically significant changes in bone turnover markers, but did attenuate increase of a bone resorption marker, possibly suggesting improved bone metabolism. CTx changes were disproportionately observed across sexes and may have been confounded by higher baseline levels in males or diurnal variation. Further research over longer dosing durations in individuals exhibiting bone-specific conditions (e.g., osteoporosis) is needed.

CANNABIDIOL ENHANCEMENT OF EXPOSURE THERAPY IN TREATMENT REFRACTORY PATIENTS WITH ANXIETY DISORDERS: A RANDOMISED CONTROLLED TRIAL

Caroline M.B. Kwee[1,2], Johanna M.P. Baas[1], Febe E. van der Flier[2], Lucianne Groenink[3], Puck Duits[2], Merijn Eikelenboom[4], Date C. van der Veen[5], Mirjam Moerbeek[6], Neeltje M. Batelaan[4], Anton J.L.M. van Balkom[4] and Danielle C. Cath[5,7]

[1] Department of Experimental Psychology and Helmholtz Institute, Faculty of Social and Behavioral Sciences, Utrecht University, Utrecht, The Netherlands;
[2] Altrecht Academic Anxiety Center, Utrecht, The Netherlands;
[3] Department of Pharmaceutical Sciences, Division of Pharmacology, UIPS, Utrecht University, Utrecht, The Netherlands;
[4] Department of Psychiatry, Amsterdam Public Health Research Institute, VU University Medical Center and GGZ inGeest, Amsterdam, The Netherlands;
[5] University of Groningen, University Medical Center Groningen, Groningen, The Netherlands;
[6] Department of Methodology and Statistics, Faculty of Social and Behavioral Sciences, Utrecht University, Utrecht, The Netherlands;
[7] GGZ Drenthe, department of specialist trainings, Assen, The Netherlands.

Introduction: Preclinical research suggests that enhancing CB1 receptor agonism may improve fear extinction. In order to translate this knowledge into a clinical application we examined whether cannabidiol (CBD), a hydrolysis inhibitor of the endogenous CB1 receptor agonist anandamide (AEA), would enhance the effects of exposure therapy in treatment refractory patients with anxiety disorders.

Methods: Patients with panic disorder with agoraphobia or social anxiety disorder were recruited for a double-blind parallel randomized controlled trial at three mental health care centers in the Netherlands. Eight therapist-assisted exposure in vivo sessions (weekly, outpatient) were augmented with 300 mg oral CBD (n = 39) or placebo (n = 41). The Fear Questionnaire (FQ) was assessed at baseline, mid- and post-treatment, and at 3 and 6 months follow-up. Primary analyses were on an intent-to-treat basis.

Results: No differences were found in treatment outcome over time between CBD and placebo on FQ scores, neither across (β = 0.32, 95% CI [-0.60; 1.25]) nor within diagnosis groups (β = -0.11, 95% CI [-1.62; 1.40]). In contrast to our hypotheses, CBD augmentation did not enhance early treatment response, within-session fear extinction or extinction learning consolidation. CBD plasma measurements showed that overall, trial participants adhered to the assigned drug treatments. Incidence of adverse effects was equal in the CBD (n = 4, 10.3%) and placebo condition (n = 6, 15.4%).

Conclusions: In this first clinical trial examining CBD as an adjunctive therapy in anxiety disorders, CBD did not improve treatment outcome. Given the lack of clarity regarding the therapeutic window of oral CBD, the possibility of suboptimal dosing in the current study cannot be excluded. More studies with integrated pharmacokinetic and pharmacodynamic outcomes are needed for persuasive dose recommendations.

Acknowledgements: Supported by ZonMW and the Dutch Brain Foundation, Programme Translational Research, project number 40–41200–98-9269.

CANNABIDIOL IN CLINICAL AND PRECLINICAL ANXIETY RESEARCH. A SYSTEMATIC REVIEW INTO CONCENTRATION-EFFECT RELATIONS USING THE IB-DE-RISK TOOL

Caroline M.B. Kwee[1,2], Joop MA van Gerven[3], Fleur L.P. Bongaerts[1], Danielle C. Cath[4,5], Gabriël Jacobs[3], Johanna M.P. Baas[1] and Lucianne Groenink[6]

[1] Department of Experimental Psychology and Helmholtz Institute, Faculty of Social and Behavioral Sciences, Utrecht University, Utrecht, The Netherlands;
[2] Altrecht Academic Anxiety Center, Utrecht, The Netherlands;
[3] Centre for Human Drug Research, Leiden, The Netherlands;
[4] University of Groningen, University Medical Center Groningen, Groningen, The Netherlands;
[5] GGZ Drenthe, department of specialist trainings, Assen, The Netherlands;
[6] Department of Pharmaceutical Sciences, Division of Pharmacology, UIPS, Utrecht University, Utrecht, The Netherlands.

Introduction: Preclinical research suggests that cannabidiol (CBD) may have therapeutic potential in pathological anxiety. Dosing guidelines to inform future human studies are however lacking. We aimed to predict the therapeutic window for anxiolytic effects of CBD in humans based on animal models.

Methods: We conducted two systematic reviews in Pubmed and Embase up to August 2021, into pharmacokinetic (PK) and pharmacodynamic (PD) data of systemic CBD exposure in both humans and animals, which includes anxiolytic and potential side effects. Risk of bias was assessed for effects on anxiety outcomes (SYRCLE's RoB tool and Cochrane RoB 2.0), PK outcomes, and harm-related outcomes. A control group was an inclusion criterion in outcome studies across species. In human outcome studies, randomisation was required. We excluded studies that co-administered other substances. We used the IB-de-risk tool for a translational integration of outcomes.

Results: We synthesized data from 87 articles. Most studies (70.3%) reported null effects of CBD on anxiety outcomes. There was no identifiable relation between anxiety outcomes and drug levels across species. In all species (humans, mice, rats), anxiolytic effects of CBD seemed to be clustered in certain differential concentration ranges, which differed between species.

Conclusions: A straightforward recommendation for optimal dosing was not possible, because there was no consistent linear effect of CBD on anxiety reduction, and concentration-effect relations were variable across species. Currently, these results raise questions about the broad therapeutic use as an anxiolytic. Meta-analytic studies are needed to quantitatively investigate drug efficacy. Acute and (sub)chronic dosing studies with integrated PK and PD outcomes are required for substantiated dose recommendations.

Acknowledgements: Supported by the research budget of Utrecht University and by a research grant of Espria/ MHC Drenthe (GR 18-130a; GR 18-130b).

CANNABIDIOL (CBD)-ASSOCIATED LIVER ENZYME ELEVATION: A SYSTEMATIC REVIEW OF CLINICAL TRIALS

Lindsay A. Lo, April Christiansen, Lauren Eadie, David Kim,
Alasdair M. Barr and Caroline A. MacCallum

Department of Medicine, University of British Columbia, ON, CA

Introduction: Preliminary studies on cannabidiol (CBD) in children with seizures, and more recently in several adult samples, have noted incidental findings of elevated liver enzymes (LE), predominantly in the transaminases alanine transaminase and aspartate aminotransferase. While LE elevation is a common phenomenon with the administration of many drugs and is often acute and reversible, a significant elevation in liver enzymes with the administration of a new drug can be indicative of a drug-induced liver injury (DILI). This has brought forward questions about the liver-safety of CBD, especially with higher-doses (1500 mg/day CBD) and concomitant medication use (e.g., anti-epileptics). Given the escalating use of CBD preparations and several concerning reports of abnormal liver function test results, we conducted an in-depth systematic review of the literature on CBD and liver safety.

Methods: Studies assessing CBD-associated changes in LE were identified through database searches of CENTRAL, CINAHL, EMBASE, Medline, MedRXIV, Web of Science, and Clinicaltrials.gov. Human clinical trials or drug safety and monitoring studies that initiated daily CBD therapy for a maximum of 6 months with serial LE measurements were included. The study selection, data extraction, and quality assessment were conducted by two independent reviewers following PRISMA guidelines. The primary outcomes of interest were the proportion of DILI and LE elevations >3x upper limits of normal (ULN) . DILI was defined as 3x ULN AST or ALT and 2x ULN bilirubin based on the American Association for the Study of Liver Diseases guidelines.

Results: A total of 2194 studies were screened, 27 studies met inclusion criteria. Out of 1402 participants on daily CBD therapy, there were 0 cases of confirmed DILI, a total of 14 cases (1%) of suspected DILI from 7 studies, and a total of 156 cases (11.1%) of LE elevations over 3x ULN from 18 studies. LE elevations were most detected within the first 6 weeks of initiation and resolved spontaneously while continuing CBD, or with reduction of either concomitant anti-epileptic medication or CBD dose. Studies reporting higher CBD doses, such as 1500 mg/day or 20 mg/kg/day, more commonly reported LE elevations. Anti-epileptics, particularly valproate, appear to be a risk factor to LE elevations. In the 156 cases of LE elevations, 122 (78%) of participants were on valproate.

Conclusion: While LE elevations over 3x ULN may occur, they are usually transient in nature occurring during the initiation and titration phase of CBD. Those at risk were typically on valproic acid or using doses of CBD >1000mg per day. More importantly, the progression to DILI appears to be rare. LEs should be monitored in these patients at risk.

CANNABIDIOL TRIGGERS MITOCHONDRIAL DYSFUNCTION AND CELL DEATH IN HORMONE-REFRACTORY PROSTATE CANCER BY TARGETING VOLTAGE-DEPENDENT ANION-SELECTIVE CHANNEL (VDAC1) AND HEXOKINASE II (HK-II)

Ali Mokhtar Mahmoud[1], Magdalena Kostrzewa[1], Marianna Cerasuolo[2], Roberto Ronca[3] and Alessia Ligresti[1]

[1]Institute of Biomolecular Chemistry, National Research Council of Italy, Italy
[2]University of Portsmouth, School of Mathematics and Physics, Hampshire, UK
[3]University of Brescia, Department of Molecular and Translational Medicine, Italy

Introduction: Hormone refractory prostate cancer (HRPC) is a major hurdle in prostate cancer (PCa). Metabolic reprogramming plays a key role in PCa oncogenesis and resistance, however, the interaction between metabolism and oncogenic signalling is not fully understood. Cannabidiol (CBD) and cannabigerol (CBG) were reported to inhibit tumor growth in human prostate cancer cells and xenograft models[1,2] although by different, not completely elucidated, mechanisms. CBD gained substantial interest in clinical use for efficacy and tolerability in neurological diseases. Currently, a phase I/Ib study is evaluating the safety and effectiveness of Epidiolex (CBD) in biochemically recurrent PCa patients (Clinical Trials Identifier: NCT04428203). Meanwhile, our group is investigating whether CBD-mediated cell death via mitochondria-targeting can interfere with metabolic reprogramming in HRPC cells.

Methods and Results: Previously at *ICRS 2021*[3], we showed that combined treatment with purified botanically derived CBD and CBG (GW Research Ltd, Cambridge, UK; ≥ 98%, 1:1, 37.5+37.5 mg/kg, i.p. twice per week for 6 weeks) reduced tumor relapse ($p=0.0052$) in TRAMP animals with hormone refractory disease. Similar results were observed *in vitro* where treatments (CBD and CBG, 10-30 μM) induced a greater pro-apoptotic effect ($p<0.01$) in HRPC compared to parental non-HRPC cells. We reported that in HRPC cells, CBD (3-6 μM) increased glycolytic rate ($p<0.001$) and inhibited oxidative phosphorylation ($p<0.001$), whereas CBG did not. In addition, CBD in HRPC cells decreased mitochondrial ATP production ($p<0.01$), membrane potential ($p<0.05$), and mitochondrial mass ($p<0.01$). Finally, we showed that CBD (6 μM) in HRPC cells modulated oncogenic related signalling pathways; increased mRNA expression of HIF-1a, PTEN and AMPK, increased protein levels of PTEN and decreased protein levels of activated Akt.

Herein, we demonstrate that CBD-mediated cell death in HRPC cells originates from the early targeting of mitochondria, as demonstrated by CBD (6 μM) accumulation after 3-hour in mitochondrial fraction via LC-MS quantification. We show that VDAC1-mediated signalling is crucial for CBD mitochondrial-induced pro-apoptotic action as demonstrated by the significant ($p<0.01$) reduction of cell death and caspase 3/7 activation mediated by CBD treatment if pre-incubated with VDAC1 inhibitor DIDS*. Preliminary data indicate also that CBD (6 μM) up-regulates HK-II expression and influences its mitochondrial recruitment in HRPC cells; we are now assessing if this effect is VDAC1-mediated. Finally, although we found that CBG effects in HRPC cells are in general less pronounced than CBD, *in silico* studies indicate that, if optimally combined, these two phytocannabinoids exert therapeutic potential and reduce PCa cell growth during hormone refractory phase of disease.

Conclusion: These findings support the existence of metabolic and oncogenic vulnerabilities in HRPC that can potentially be therapeutically targeted by phytocannabinoid-based treatments.

References: 1- De Petrocellis et al., Br. J. Pharmacol. (2013). 2- Sharma et al., Pharmacol & Pharm. (2014). 3- Mahamoud AM et al, ICRS Proceeding (2021).

Acknowledgments: The authors thank GW Research Ltd. for provision of materials (CBD and CBG). AL is the recipient of a research grant from GW Research Ltd. * TRAMP= transgenic adenocarcinoma of the mouse prostate model. DIDIS = 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid disodium salt hydrate (Sigma).

COMPARISON OF CANNABIS AND PSILOCYBIN ADVERSE EVENTS IN A NATIONAL REPORTING SYSTEM

Jahan Marcu[1], Teresa Simon[2,3], Andres Gomez[2,3], Matt Wabwire[2] and John Simon[4]

[1]Marcu Enterprises USA, Marcu and Arora, LLC USA,
[2]Physicians Research Center, LLC USA,
[3]Adjunct Fellow Univ of PA, Center for Public Health Initiatives, USA,
[4]Drexel University MPH Candidate, USA

Background: In the United States, jurisdiction that have legalized adult use and medical cannabis are now also considering legislative changes to allow access to psychedelics, such as psilocybin containing mushrooms and products thereof. In the clinic setting, reported side effects of cannabis-derived products and psilocybin appear similar but these may be different under real world circumstances. Given the increasing interest in these products, and real-world evidence suggesting concomitant use, we explored the prevalence of cannabis and psilocybin -related adverse events in a National Reporting Database.

Traditionally, a product is studied in Phase 1-3 studies, where the benefit risk profile is established prior to national approval and monitored subsequently after approval. One of the mechanisms for monitoring safety is the Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS). FAERS is a United States national database originally designed to support the FDA's post marketing safety surveillance program

Methods: We utilized FAERS to see what data is reported regarding psilocybin and cannabis. FAERS database searches was conducted with product names (aka search terms): psilocybin; for cannabis terms included: cannabis sativa and 11-Nor-9-Carboxy-delta9-tetrahydrocannabinol.

Results: Our preliminary FAERS data contains, for example, psilocybin as the "suspect product active ingredient" with 34 AEs and 0 deaths being reported. Most frequent AEs for psilocybin were reported as substance abuse, drug dependance and euphoric mood. Where THC is a suspect drug, there were 52 cases, associated with 35 AEs and 24 deaths. For cannabis there are over 5,000 cases we are presently analyzing. Most frequent AEs for cannabis were reported as toxicity to various agents, drug abuse, substance abuse.

Conclusion: As of the writing of this abstract, the data from FAERS may not adequately represent the true safety nor risk profile of cannabis and psilocybin. Based on the limitations of FAERs, we have designed a data collection tool for collecting more granular information on cannabis and psilocybin product safety. Available pilot data utilizing this data collection tool may be shared during this presentation. The purpose of this presentation is to raise awareness of the public FAERS database as well as to introduce a potential safety monitoring tool. We hope that a comparison of AEs between psilocybin and cannabis would provide insight into public health efforts.

GET YOUR THINKING CAP ON: IMPLEMENTATION OF THE CANNABIS AWARENESS PROGRAM (CAP) FOR HIGH SCHOOL STUDENTS

Jahan Marcu[1], Teresa Simon[2], Mathew Wabwire[2], Sara Jane Ward[3], and Tim Smale[4]

[1]Marcu Enterprises USA, Marcu and Arora, LLC USA,
[2]Physicians Research Center, LLC USA,
[3]Department of Neural Sciences, Lewis Katz School of Medicine,
Temple University. Philadelphia PA,
[4]President of Freestyle Consulting Inc

Introduction: The cannabis awareness program (CAP) is an educational series focused on making participants aware of the effects of cannabis on the body and the brain, especially the developing brain. Participants in this education series also learn about historical uses and issues regarding medical cannabis. Modern scientific evidence on health is discussed in the context of current events surrounding cannabis policy and global trends.

Methods and Results: Our group, led by Tim Smale, presented a series of six cannabis awareness educational sessions between November 2021 and January 2022 in a pilot program with his former high school, St. Francis de Sales School in Toledo, Ohio, a Catholic college preparatory high school in Toledo, Ohio. The participants (adults and children) were surveyed, and overall thought the program was presented well and thoroughly covered the material. Attendance included Fr. Ronald Olszewski, Foundation Chairman, Caleb Fortner, Dean of Men, Antonio Caputo, Assistant Dean of Men, and St. Francis School seniors. Many reported that they felt they were left with a good understanding of the primary issues surrounding cannabis use amongst adolescents. The program was well received and may be integrated into high school health class curriculum. More pilot education sessions are currently being planned in other states/school districts for this year.

Conclusion: The purpose of this presentation will be to share the curriculum of the cannabis education, pilot data, and survey questions to be used to collect knowledge, attitudes, and outcomes. Additionally, the future directions and ongoing development of this program will be discussed. It is our hope that attendees of the ICRS meeting can help to provide insight and guidance for our project's success and impact for the benefit of public health.

CANNABIS USE PATTERNS, PERCEPTIONS, AND RELATED HEALTH OUTCOMES AMONG SPANISH SPEAKERS IN THE UNITED STATES AND INTERNATIONALLY

Renée Martin-Willett, Elizabeth Zambrano Garza, L. Cinnamon Bidwell

University of Colorado Boulder, Boulder CO, U.S.A.

Introduction: Cannabis and health research continues to be largely conducted with young, white male research participants, largely ignoring people of color or communities for whom English is not a primary or native language. Little is known of the usage patterns, perceptions, and medically-related use in primarily Spanish-speaking communities, nor of whether location of residence may be associated with these topics. This study describes the development of an online Spanish-language cannabis and health survey with particular attention to medically motivated and recreational use, mode of administration, and potency of products. This study also describes the unique challenges of conducting research on cannabis and health in Spanish speaking communities and the community engaged research strategies being used.

Methods: The survey was developed in English and professionally translated for linguistic equivalency, followed by revision by a diverse Spanish speaking focus group to improve cultural equivalency (n=7), and finally back translated by bilingual research staff. Participants were recruited through community engaged research partnerships, and then transitioned to survey platforms such as mTurk and Prolific due to COVID-19 restrictions.

Results: 535 individuals completed the survey, including 238 residing in the United States (U.S.) (M_{age}= 31.2, SD=9.88; 51.3% female and non-binary), 222 residing outside of the U.S. (M_{age}=28.3, SD=9.24; 51.4% female and non-binary) and 76 who did not report country of residence (M_{age}= 26.6, SD=7.38; 25% female and non-binary). U.S. respondents were significantly older than the other respondents (2.87, p<0.01). Overall use was mostly recreational, while the U.S. group was significantly more motivated by medical or combined medical and recreational reasons to use cannabis than the other two groups (p=0.02), and smoked or vaporized significantly more often (though not as much) than the other two groups (p<0.01). There were no significant differences between use patterns of other types of cannabis such as edibles, topicals, or concentrates. While all the groups mostly received information about cannabis from the media, the groups differed on other sources of information (e.g., medical professionals, family or friends) (p<0.01). The most common reason for medical use was anxiety or depression (14% of sample), and there were no significant differences between groups for reasons driving medically-motivated use.

Conclusions: The current study takes a community engaged, equity-focused approach to provide critical, early data from an understudied group. It reveals that Spanish speaking communities in the U.S. and internationally have both shared and divergent cannabis use patterns both medically and recreationally, particularly for smoking or vaporizing and where groups sought information about cannabis use. Challenges to collecting these data include lack of availability of alternate forms of cannabis in different countries, cultural stigma surrounding cannabis, or worries around study participation related to immigration. Future work should continue to leverage engaged partnerships to foster trust in the community. These data will help to improve representation in the field of cannabis and health research, and empirically inform future research with this diverse and growing international community.

Acknowledgements: This study was funded by the Colorado Department of Public Health and Environment, State of Colorado (CDPHE2902; PI: Bidwell)

HOW LONG DOES A SINGLE ORAL DOSE OF CANNABIDIOL PERSIST IN PLASMA? FINDINGS FROM THREE RECENT CLINICAL TRIALS

Danielle McCartney, Richard C. Kevin, Anastasia S. Suraev, Ayshe Sahinovic, Peter T. Doohan and Iain S. McGregor

Lambert Initiative for Cannabinoid Therapeutics, University of Sydney, Sydney, Australia

Introduction: The lipophilic phytocannabinoid, Δ^9-tetrahydrocannabinol (THC), has a long 'window of detection' in plasma (e.g., >5-days in daily cannabis users) owing to the fact that it is readily stored in, and only slowly liberated from, adipose tissue. Cannabidiol (CBD) is also lipophilic – but typically consumed at much higher doses than THC. It could therefore have a similarly long (if not longer) window of detection. This would have important implications for the design of clinical trials involving CBD; specifically, the length of the washout period used in crossover studies. A washout period is appropriate if it allows one treatment to clear before the next is administered. Here, we measured plasma cannabinoid concentrations ≥7-days after a single, oral dose of CBD and estimated the window of detection.

Methods: Three randomised, double-blind, placebo-controlled, crossover clinical trials (CTs) were previously conducted. The participants in these CTs had not used cannabinoids in the last 3-months and completed either two (CTs 1 & 2) or four (CT 3) treatment sessions where they were administered a single, oral dose of placebo or CBD. CBD doses were – CT 1: 300 mg; CT 2: 200 mg; and CT 3: 15, 300 and 1500 mg. Sessions were separated by ≥7 days. Blood was drawn to determine plasma cannabinoid concentrations. Participants were included in this secondary analysis if their plasma cannabinoid concentrations were measured ≥7 days after CBD use (i.e., they received CBD before placebo). Measures obtained after more than one dose of CBD (i.e., in CT 3) were only included if the previous dose(s) had washed out.

Results: Thirty-two participants (n=18 male; age: 37±11 y; body mass index [BMI]: 24±4 $kg \cdot m^{-2}$) were included in this analysis; 14 of whom provided repeated measures. Overall, 10, 10, 18 and nine received 15, 200, 300 and 1500 mg CBD, respectively. Six of the latter nine also provided a second blood sample ≥14 days after CBD use (Time-2). In total, 0% (15 mg), 60% (200 mg), 28% (300 mg), 100% (1500 mg; Time-1) and 57% (1500 mg; Time-2) of participants had detectable levels of CBD in plasma (i.e., >0.25 $ng \cdot mL^{-1}$) ≥7 days post-treatment. One had 1.9 $ng \cdot mL^{-1}$ in plasma 56-days after the 200 mg dose. A mixed-effects regression analysis containing six fixed effects: dose ($mg \cdot kg^{-1}$) (p<0.001), washout (days) (p=0.003), washout ($days^2$) (p=0.026), BMI (p=0.105), sex (p=0.363) and age (p=0.057), and random effects to account for dependency, (R^2_c=0.57) suggests a 12-day washout period would be sufficient to reduce plasma CBD concentrations to zero if a 30-year-old male (70 kg; BMI <25 $kg \cdot m^{-2}$) used a single, oral dose of 300 mg.

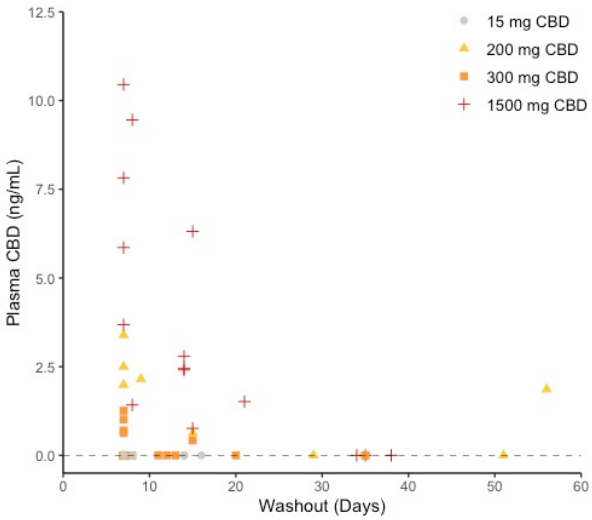


Fig 1. Plasma CBD concentrations (all shown)

Conclusions: CBD has a long window of detection in plasma. Single-dose crossover studies should therefore be conducted with caution – and chronic-dosing crossover studies, avoided.

PHARMACOKINETICS AND TOXICITY EVALUATION AFTER INHALATION OF DELTA-9-TETRAHYDROCANNABINOL AEROSOLS GENERATED FROM A PRESSURIZED METERED DOSE INHALER (PMDI)

Jake McDonald

Lovelace Biomedical
Albuquerque, New Mexico. USA

Introduction: The objective of this research was to evaluate the maximum tolerated dose of delta-9-tetrahydrocannabinol (THC) aerosols generated using a pressurized metered dose inhaler (pMDI) exposure system in male and female Sprague Dawley rats following once daily nose-only inhalation exposure at up to 30 mg/kg/day for one day and then on study days 3-7.

Methods: Animals were dosed with a custom-made nose-only inhalation system and rodents were exposed to 5, 15 and 30 mg/kg/day. The TK1 samples were collected on day 1 and the TK2 samples were collected on day 7. THC analysis was performed by LCMS. The data were analyzed both as group averages and by group averages separated by animal sex. Pharmacokinetics were analyzed by win/nonlin. On day 7 clinical pathology samples were collected and analyzed as well as organ weights and histopathology.

Results: Noncompartmental results were determined to include Cmax, AUC, Tmax and half-life for three different doses and two different timepoints. Overall, the data suggest that THC undergoes apparent first order elimination and is rapidly absorbed over the dose range evaluated. Specifically, the doses for the TK1 and TK2 sampling were significantly different based on the real time findings within the study. Of note is the high dose group TK1 sampling. There is an apparent increase in concentration as a function of time. These data were reviewed for the biosample analysis and confirmed to be accurate. This apparent increase in systemic concentration may have factored into the observed clinical observations in this dose group. Clinical pathology results showed a slight increase in red blood cell counts at high doses. There were decreased organ weights in kidney, liver and spleen. Histologically, scattered changes of inflatrates, degeneration, and hyperplasia of the epithelia were observed in nasal turbinates and lungs.

Conclusion: In the analysis of impact of dose on the NCA it is interesting that the dose normalized C_{max} is similar for TK1 and similar within TK2. However, the values are different between TK1 and TK2 with TK2 having an increase dose normalized C_{max}. Similarly, for the dose normalized AUC the values across all doses and both TK1 and TK2 are similar. No significant differences were observed on a per sex basis. High doses of inhaled THC caused respiratory and systemic responses that decreased with lower doses.

'NEXT DAY' EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON COGNITIVE FUNCTION AND SAFETY-SENSITIVE TASKS: A SYSTEMATIC REVIEW

Danielle McCartney, Anastasia S. Suraev and Iain S. McGregor

Lambert Initiative for Cannabinoid Therapeutics, University of Sydney, Sydney, Australia

Objective: Many individuals perform cognitively-demanding and/or safety-sensitive tasks after recent cannabis use. While it is known that cannabis and its main intoxicating component, Δ^9-tetrahydrocannabinol (THC), can *acutely* impair cognitive and psychomotor performance, possible 'next day' (or residual) effects are heavily debated. We conducted a systematic review of studies investigating the 'next day' effects of cannabis/ THC (hereafter termed THC) use on cognitive function and safety-sensitive tasks.

Methods: Studies that measured performance on 'safety-sensitive' tasks (e.g., simulated or on-road driving, simulated flying/piloting) and/or discrete neuropsychological tests >8-hours post-(last) THC administration using an interventional design were identified by searching the online databases Scopus and Web of Science from inception until October 25th, 2021. All participant populations and comparator conditions (e.g., placebo, baseline) were accepted. However, studies were excluded if THC was co-administered with another active treatment. *Risk of bias in included studies* was evaluated using the Revised Cochrane Risk of Bias tool (RoB 2.0) and the RoB 2.0 for crossover trials, as appropriate. The results of the included studies were synthesised qualitatively; that is, described in terms of whether THC was found to have a significant effect (i.e., $p<0.05$) on performance relative to the primary comparator, taken as placebo in placebo-controlled trials and baseline (i.e., pre-treatment) in non-placebo-controlled trials. Meta-analysis was not performed as studies often failed to report the information required to calculate an effect estimate (e.g., mean±SD performance scores).

Results: Twenty studies (n=458 participants) were reviewed. Most used randomised (N=11) or non-randomised (N=5) double-blind (DB), placebo-controlled (PC) designs. THC was administered via either inhalation (N=13) or oral ingestion (N=7). The median [IQR] THC dose was 16 [11–26] mg, when reported (N=15). The total number of performance tests administered between >8–12, >12–24 and >24–48-hours post-treatment was 98, 158 and 89, respectively. No studies had an overall 'low risk' of bias although two received 'low risk' ratings on most domains assessed. Nine had 'some concerns', and 11, a 'high risk' of bias. Only five studies detected negative 'next day' effects of THC. Two of these had a 'high risk' of bias and none used a randomised DB, PC design (N=2 non-randomised DB; N=2 non-randomised single-blind; N=1 'pre-/post-treatment'). Negative effects were observed at THC doses ranging between 5–20 mg on a total of 12 tests: 10 neuropsychological tests and two simulated flying tasks. Ten of these 12 tests were conducted between >8–12 hours post-treatment with the remainder conducted between >12–24. The two 'higher quality' studies observed no 'next day' effects of THC – but neither did they detect acute effects.

Conclusion: A very small number of low-quality studies have detected significant 'next day' effects of THC on safety-relevant tasks. However, most studies, including those of higher quality, show no effect. Overall, THC appears unlikely to elicit clinically-significant 'next day' effects as confirmed in a recent trial by our own group (Suraev, *et al* this meeting).

Acknowledgements: Supported by the Lambert Initiative for Cannabinoid Therapeutics, a philanthropically funded center at the University of Sydney, Australia.

DOUBLE YOUR FUN WITH EDIBLES? THE RELATIVE POTENCY OF 11-OH-THC COMPARED TO THC – A META-ANALYSIS

John M. McPartland

GW Pharmaceuticals, Histon, Cambridge, United Kingdom
College of Medicine, University of Vermont, Burlington, VT, USA

Introduction: After consuming an edible, THC is hepatically metabolized to 11-hydroxy-THC, to a much greater extent than when THC is inhaled. The metabolite is cannabimimetic. Pharmacokinetic studies of THC → 11-OH-THC are numerous, but pharmacodynamic (PD) studies of 11-OH-THC are relatively few—a lacuna in the literature, considering the popularity of edibles. Some PD studies have made direct comparisons between 11-OH-THC and THC. These studies were collated and analyzed using Relative Potency (RP)—a metric that measures the ability of an unknown sample to produce a response compared to a reference sample, when tested under the same conditions. RP is not an absolute value, it is a ratio between an unknown and a reference.

Methods: PubMed and Google Scholar served as search engines. Studies meeting inclusion criteria made direct comparisons between 11-OH-THC (the unknown) and THC (the reference). PD studies included *in vitro* ligand binding at CB_1 (binding affinity or K_i), *in vitro* ligand efficacy at CB_1 receptors (FsAC or [^{35}S]GTPγS assays), and *in vivo* studies (rodent tetrad and other behavioral assays). For each retrieved study, we calculated RP (11-OH-THC response divided by THC response). The RP ratio was expressed as a quotient (*i.e.,* 2-to-1 ratio expressed as 2), and quantitatively synthesized across studies.

Results: Forty-eight studies met inclusion criteria, from which RPs were calculated:
In 13 *in vitro* affinity studies, mean RP = 1.82 ± 0.215 SE. In 9 *in vitro* efficacy studies, mean RP = 2.24 ± 0.609. In 26 *in vivo* animal studies, mean RP = 5.94 ± 1.20. High variance in the *in vivo* studies was dissected by subgroup analysis; heterogeneity was partially due to species differences (RP mice > rats) and sex differences (RP females > males). Three studies were conducted with human participants; descriptive rather than quantitative, RP could not be calculated.

Discussion: A synthesis across studies suggests that 11-OH-THC produces 2- to 5-fold greater cannabimimetic effects than THC at CB_1. This may be one reason why overdoses happen more frequently with oral versus inhaled consumption. Epidemiology studies show that edible overdoses happen more frequently in women, and rodent studies suggest that females are more sensitive to 11-OH-THC than males. More work is needed! No *in vitro* affinity or efficacy studies have been done with 11-OH-THC at human CB_1 or CB_2. *In vivo* human studies have made *indirect* comparisons, by administering cannabis, measuring changing blood levels of THC and 11-OH-THC over time, and correlating these changing levels with cannabimimetic effects—not the same as direct comparisons.

ASSOCIATIONS BETWEEN CHRONIC CANNABIS USE AND RESTING EEG

Conor H. Murray[1] and Ziva D. Cooper[1,2]

[1]UCLA Cannabis Research Initiative, Jane and Terry Semel Institute,
Department of Psychiatry and Biobehavioral Sciences, UCLA, USA
[2]Department of Anesthesiology and Perioperative Medicine, UCLA, USA

Introduction: Adolescence is a critical period of neurodevelopment that includes marked changes in the expression and function of the endocannabinoid system. Behaviorally, our work has shown that adolescents (ages 18 to 20), relative to adults (ages 30 to 40), are more sensitive to the effects of delta-9-tetrahydrocannabinol (THC). Unexpectedly, beyond THC effects, we found also that the adolescents differed from adults in resting-state neurophysiology, with age-dependent reductions in broadband power (reduced amplitudes from delta to gamma), a finding that may be attributed to cortical maturation. It has been suggested that chronic cannabis use during adolescence interferes with neural development. However, whether chronic cannabis use during adolescence interferes with the maturation of neural oscillations has yet to be explored.

Methods: We analyzed data from N = 82 participants between the ages of 21 and 55 from the Los Angeles area. We examined age of onset of cannabis use, cannabis use disorder (Cannabis Use Disorder Identification Test, CUDIT-R), and total number of lifetime cannabis use. In all participants, we recorded 5 minutes of resting-state electroencephalography (EEG; Muse S, Interaxon Inc.). The magnitude, or power, of the neural oscillations across the delta, theta, alpha, beta, and gamma frequency bands for each participant will be included in a generalized linear model with an interaction term for age and cannabis use (average use per year). To detect modification of age effects by cannabis use, the model will be compared to a reduced model (age and cannabis use as covariates) using a likelihood ratio test, with a significant result indicating a moderation effect on resting-state neurophysiology by cannabis use.

Results: The 82 participants (48 males, 34 females) ranged in age from 21 to 53 years (mean = 29 years) with an average onset of cannabis use at age 16. Participants averaged >12 on the CUDIT-R, indicating possible cannabis use disorder, and averaged over 2,000 reported lifetime uses of cannabis. Our prior work, which uncovered age-dependent reductions in broadband power, examined participants that reported less than 20 total lifetime use of cannabis. In line with this previous report, we anticipate age-dependent reductions in EEG power across delta, theta, alpha, beta and gamma frequency bands, with a moderating effect by cannabis use.

Conclusions: Earlier studies found no effect of acute THC exposure on resting-state EEG in people with minimal cannabis use experience. This work will define if frequent cannabis use during early adulthood has similar or divergent effects on EEG power, a marker of neural activity. Findings will inform the impact of adolescent cannabis use on neurodevelopment.

Acknowledgements: This work has been funded by the National Institutes of Health (Grant no. R01 DA047296; R01 DA047296) and the Semel Charitable Foundation.

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

[1]J. Patrick Neary, [1]Jyotpal Singh, [2]Jane Alcorn, [2]Stephanie Vuong[3] and Lanishen Bhagaloo

[1]Faculty of Kinesiology & Health Studies, University of Regina, Regina, SK, Canada
[2]College of Pharmacy & Nutrition, University of Saskatchewan, Saskatoon, SK, Canada
[3]Gateway Alliance Medical Clinic, Regina, SK, Canada

Introduction: Cannabidiol (CBD) can improve physiological parameters and symptoms in humans with post-concussion syndrome (PCS). The literature implies that cerebrovascular activity is modulated by phytocannabinoids. PCS can result in altered blood pressure (BP) and cerebral haemodynamic responses. Understanding the effects of phytocannabinoids on these physiological variables in PCS is required. We used a repeated squat-stand maneuver to exaggerate cerebral autoregulation and applied wavelet transformation to analyse the wavelet amplitude in cerebral oxygenation and beat-to-beat BP to assess cerebrovascular and cardiovascular effects of CBD.

Methods: Cerebral oxygenation (HbO_2; oxyhemoglobin) was monitored in the right prefrontal cortex using near-infrared spectroscopy and beat-to-beat BP was collected using finger photoplethysmography. Three participants performed an isometric squat, holding for 10 seconds and standing for 10 seconds, repeated 15 times at 20 second Intervals (0.05 Hz) for a total of 5 minutes. Wavelet transformations (median ± SD) were applied to separate the physiological activity into Intervals I (0.6–2 Hz; cardiac activity), II (0.145–0.6 Hz; respiratory function), and III (0.052–0.145 Hz; smooth muscle cell activity). CBD was self-administered by the participants daily for 2 months with dosage ranging from 25mg to 400mg per day, with one participant taking 20mg CBD:1mg tetrahydrocannabinol (THC) per day up to 40mg CBD:2mg THC per day. Venous blood samples were collected to analyse plasma CBD (peak) concentrations. Participants were assessed every 17 ± 6 days for up to 70 days.

Results: At a concentration of 3.27ng/mL, Participant 1 (20mg CBD:1mg THC-40mg CBD:2mg THC) showed a 26% increase at Interval III for BP and a 53% decrease in HbO_2 amplitude at Interval I. At a concentration of 5.7ng/mL, Participant 2 (100mg-200mg CBD) showed a 21% increase at Interval I for BP, and a 39% decrease in HbO_2 amplitude at Interval III. At a concentration of 12.2ng/mL Participant 3 (25mg-50mg CBD) showed a 24% increase at Interval I for BP, and a 17% decrease in HbO_2 amplitude at Interval III.

Conclusion: Phytocannabinoids influenced the wavelet amplitudes of the BP and HbO_2 on a case-by-case basis in females with PCS. These results suggest that participants taking a combination of CBD/THC showed changes at different Intervals for BP and HbO_2 as compared to the participants taking full spectrum CBD. Full spectrum CBD induced the greatest change in the BP cardiac Interval and HbO_2 smooth muscle cell Interval. CBD/THC influenced the HbO_2 cardiac activity and BP smooth muscle cell activity. More research is needed to understand the pathophysiological response in accordance with the plasma concentrations of the phytocannabinoids in a larger study.

Acknowledgments: Thanks to the volunteer participants.

MEDICINAL CANNABIS USE IN NEW ZEALAND FOLLOWING THE INTRODUCTION OF THE MEDICINAL CANNABIS SCHEME

Karen Oldfield, Harry Delany, Allie Eathorne, Giles Newton-Howes and Irene Braithwaite

Medical Research Institute of New Zealand, Wellington, New Zealand

Introduction: The medicinal cannabis scheme (MCS) was introduced in New Zealand (NZ) in April 2020. This follows a global trend of the acceptance of the use of cannabis as a medicine and aimed to ensure the quality of the medicinal cannabis products accessed by New Zealanders. In other jurisdictions, understanding cannabis use has powerfully improved changes in approach to both perceptions and regulations. As such, we proposed a longitudinal cohort study of medicinal cannabis users in NZ, characterising the cannabis products they are using, how this affects their health and their quality of life outcomes. The current cross-sectional study examined the feasibility of such a longitudinal cohort study in NZ.

Methods: Online cross-sectional survey, comprising of feasibility questions and conditions and current use questions (adapted CAMS 2018 survey) distributed through social media advertising, targeting medicinal cannabis users in NZ, undertaken from 24th August 2021 to 3rd October 2021.

Results: Three hundred and fifteen surveys were started. Two hundred and forty-seven started the feasibility section (208 (66%) completing) and 182 started the conditions and current use (116 (36%) completing the full survey). The mean age of respondents was 41 years, range 18-77, with 66% female. Twenty-five percent identified as Māori. Of those who completed the feasibility questions, 86% indicated willingness to participate in a longitudinal study, preferring to undertake this remotely for up to 24 months. The mean age of first cannabis use for any reason was 19 years with first use for medicinal reasons 34 years, with 17% reporting that they had no prior recreational cannabis use. The most common conditions indicated for medicinal use were pain (83%), sleep (76%) and mental health (61%); primarily anxiety, depression and post-traumatic stress disorder (PTSD). Access for medicinal use was from friends and family (58%), growing their own (33%) or from a recreational dealer (37%). Only 20% reported accessing medicinal cannabis from a prescriber that was dispensed from a pharmacy. The primary reason for not accessing prescribed medicinal cannabis was cost. Respondents indicated they had decreased use of other medications since starting medicinal cannabis. Reported side effects were mild and tolerable, with dry mouth, drowsiness and increased appetite most common. Four in five respondents thought that cannabis should be legal for all purposes in NZ. Preference was for oral and vaporised products, with almost half indicting they would like to grow their own legal cannabis.

Conclusion: Access to prescribed products in NZ appears to be low, with medicinal cannabis users reporting preferences to access cannabis illicitly due to cost. Users of medicinal cannabis express willingness to participate in longer term research exploring their health and social outcomes following the use of cannabis as medicine, identifying pertinent areas where future research is needed, in both health and regulations.

Acknowledgements: The Medical Research Institute of New Zealand (MRINZ) receives funding from a Health Research Council of New Zealand Independent Research Organisation Grant (Grant no: 18/002).

MEDICAL CANNABIS AUTHORIZATION PATTERNS, SAFETY, AND ASSOCIATED EFFECTS IN OLDER ADULTS

Laura MacNair[1], Maja Kalaba[1], Erica N. Peters[1], Matthew T. Feldner[1], Graham M.L. Eglit[1], Lucile Rapin[2], Erin Prosk[2] and Mark A. Ware[1]

[1]Canopy Growth Corporation, Ontario, Canada
[2]Santé Cannabis, Quebec, Canada

Introduction: Use of medical cannabis is increasing in the older adult population; however, few investigations have examined cannabis use patterns, safety, and associated effects in this demographic.

Methods: This is a sub-analysis of 201 older adults (aged ≥ 65 years) from a retrospective observational study of 639 patients who were legally authorized a medical cannabis product and then followed by a physician over 3 months. Cannabis authorization patterns, adverse events (AEs), Edmonton Symptom Assessment Scale-revised (ESAS-r), and Brief Pain Inventory Short Form (BPI-SF) data were collected. Mixed effects models with random intercepts for participant and a predictor for time were fit to evaluate baseline and three-month change in Δ-9-tetrahydrocannabinol (THC) dose, cannabidiol (CBD) dose, ESAS-r items, and BPI-SF items.

Results: The most common symptom for which medical cannabis was authorized was pain (170, 84.6%). Sixty-seven of the 201 patients at baseline completed the 3-month follow-up. At baseline and at the 3-month follow-up, CBD-dominant products were authorized most frequently (106, 57%) followed by 1:1 THC to CBD products (76, 41%), and then THC-dominant products (3, 2%). Patients were authorized an average daily dose of 2.44 mg THC (95% confidence interval [CI], 1.98-2.99) and 8.45 mg CBD (95% CI, 8.36-10.22) at baseline, which more than doubled at three months for both THC (6.27 mg, 95% CI, 4.76-8.17) and CBD (20.63 mg, 95% CI, 17.34-26.47). The most frequent AEs were dizziness (18.2%), nausea (9.1%), dry mouth (9.1%), and tinnitus (9.1%). Significant reductions in ESAS-r scores were observed over time in the domains of pain (p = .019) and tiredness (p = .001), but not for drowsiness (p = .225) or well-being (p = .079). Significant reductions in BPI-SF scores over time were observed for worst pain (p = .007), average pain (p = .001), pain right now (p = .001), and pain severity (p = .001).

Conclusions: Overall, medical cannabis was safe, well-tolerated, and associated with clinically meaningful reductions in pain in this sample of older adults. Future controlled research could examine reasons for attrition and explore strategies to promote retention of older adults in medical cannabis treatment.

Acknowledgements: This research was funded by Canopy Growth Corporation. The authors would like to acknowledge all patients and health care providers for their data collection efforts. They also thank Laura Ruiz and the Santé Cannabis team for their data extraction efforts.

AUTOBIOGRAPHICAL MEMORY FOR EMOTIONAL AND NEUTRAL EVENTS IN CHRONIC CANNABIS USERS

Aria S. Petrucci[1], Daniela J. Palombo[2] and Carrie Cuttler[1]

[1]Department of Psychology, Washington State University, Pullman, WA, USA
[2]Department of Psychology, University of British Columbia, Vancouver, BC, CAN

Introduction: A small literature demonstrates that cannabis users recall autobiographical (AB) memories (i.e., memories for personal life events) with less episodic detail and memory specificity than non-users, indicating a pattern of over-general memory. However, over-general AB memory can stem from impairments in several, specific phenomenological aspects of memory (e.g., vividness, sensory detail), and it is not clear which aspects may contribute to over-general memory in cannabis users. Moreover, a robust literature in the general population has shown that emotional events are recalled with a greater sense of vividness and sensory detail than neutral events due to neurobiological changes that occur during acute stress. Because chronic cannabis users display blunted neurobiological responses to acute stress, they may not show hallmark patterns of memory enhancement for emotional events. The present study is the first to examine the effects of chronic cannabis use and emotional valence on AB memory vividness and sensory detail.

Methods: In an online platform, a preliminary sample of chronic cannabis users (>4 uses per week for >1 years) and non-users (no past-year use and ≤10 lifetime uses) recalled two positive, two negative, and two neutral events that they experienced during the past year. For each remembered event, participants completed a self-report measure of AB memory phenomenology (Memory Experiences Questionnaire – Short Form) which probes vividness and sensory detail. For each memory, vividness and sensory detail sum scores were calculated and then averaged by valence to produce a mean vividness and sensory detail score for memories of each valence. Data were analyzed with 2 x 3 mixed factorial ANOVAs with cannabis use status (non-user, chronic user) as the between-subjects factor, event valence (positive, negative, neutral) as the within-subjects factor, and vividness and sensory detail as the dependent variables. Planned one-way repeated measures ANOVAs and pairwise *t*-tests were used to further examine main effects of valence on vividness and sensory detail separately in chronic cannabis users and non-users.

Results: Results revealed a significant main effect of valence on both vividness ($p = .01$) and sensory detail ($p = .008$). Neither the main effect of cannabis use status nor the interaction between cannabis use status and valence were significant when examining vividness or sensory detail. Follow up tests revealed a significant effect of valence on vividness ($p = .005$) and sensory detail ($p = .001$) in non-users but not chronic users ($p = .32$, $p = .34$, respectively). Specifically, non-users rated negative and positive events with greater vividness and sensory detail than neutral events (all $p < .01$). Non-users' ratings of vividness and sensory detail for positive events were not significantly different than their respective ratings for negative events.

Conclusion: Results indicate that non-users recall emotional (positive and negative) events with greater reported vividness and sensory detail than neutral events, but this pattern of emotional memory enhancement is not observed in chronic cannabis users. This may be due to blunted stress reactivity for emotional events that is associated with chronic cannabis use.

CANNABIS USE AND THE INCIDENCE OF MENTAL HEALTH DIAGNOSES: A 12-YEAR STUDY OF PEOPLE WHO USE UNREGULATED DRUGS

Hudson Reddon[1,2], Zach Walsh[3], Stephanie Lake[1,4,5], Cameron Grant[1], Maria Eugenia Socias[1,2], Dan Werb[6,7], Kanna Hayashi[1,8] and M-J Milloy[1,2]

1. British Columbia Centre on Substance Use, 1045 Howe Street, Vancouver, BC V6Z 2A9, Canada
2. Department of Medicine, University of British Columbia, Vancouver, BC V6Z 1Y6, Canada
3. Department of Psychology, University of British Columbia, Kelowna, BC, V6T 1Z4, Canada
4. UCLA Cannabis Research Initiative, Jane and Terry Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, Los Angeles, CA, 90095, USA
5. Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, 90095, USA
6. Centre on Drug Policy Evaluation, Unity Health Toronto, Toronto, ON, M5B 1T8, Canada
7. Division of Infectious Diseases and Global Public Health, UC San Diego, La Jolla, CA, 92093, USA
8. Faculty of Health Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada

Introduction: We undertook the present study to estimate the association between cannabis use and the incidence of mental health diagnoses among marginalized people who use unregulated drugs (PWUD).

Methods: The data for this investigation were collected from two prospective cohorts of PWUD in Vancouver, Canada between December 2005 and November 2018. Study participants completed follow-up visits biannually after baseline. Among participants without a history of mental health disorders, we used extended Cox regression models to estimate the association between high-frequency cannabis use and incident mental health disorder diagnoses.

Results: We enrolled 2184 participants of whom 803 (36.8%) were diagnosed with a mental health disorder over follow-up. The incidence density for any mental health disorder diagnosis over follow-up was 11.1 (95% confidence interval [CI]: 10.6-11.7) per 100 person years, and depression was the most commonly diagnosed mental health disorder. In the multivariable analysis, cannabis use was not significantly associated with incident mental health disorder diagnosis during follow-up (hazard ratio=0.43, 95% confidence interval: 0.12-1.48, P=0.180).

Conclusions: Cannabis was not significantly associated with incident mental health disorder diagnosis in the present study. These findings contribute to a large but discordant body of evidence evaluating the chronic mental health impacts of cannabis use. Further analysis of cannabis use for the purposes of harm reduction and self-medication would be useful to characterize this relationship with more certainty among marginalized groups such as PWUD.

PHARMACOKINETICS AND ORAL BIOAVAILABILITY OF CANNABIDIOL IN HORSES AFTER INTRAVENOUS AND ORAL ADMINISTRATION WITH OIL AND MICELLAR FORMULATIONS

Sánchez de Medina, Antonia[1,2]; Serrano-Rodríguez, Juan Manuel[3]; Díez de Castro, Elisa[1,2], García-Valverde, María Teresa[4]; Saitua, Aritz[1]; Becero, Mireia[1]; Muñoz, Ana[2,5]; Ferreiro-Vera, Carlos[4] and Sánchez de Medina, Verónica[4]

[1] Clinical Veterinary Hospital. University of Cordoba. Córdoba, Spain.
[2] Department of Animal Medicine and Surgery, Veterinary Faculty. University of Cordoba. Córdoba, Spain.
[3] Department of Nursing, Pharmacology and Physiotherapy. Pharmacology Area, Veterinary Faculty. University of Córdoba. Córdoba, Spain.
[4] Phytoplant Research S.L.U., Córdoba, Spain.
[5] Equine Sport Medicine Center CEMEDE, Department of Animal Medicine and Surgery, Veterinary Faculty. University of Córdoba. Córdoba, Spain.

Introduction: Intravenous (IV) pharmacokinetics (PK) and oral (PO) bioavailability of cannabidiol (CBD) have not been investigated in horses and may represent a starting point for clinical studies, especially in this species due to the potential use of oral route for veterinarians and owners. This issue is of great importance since there is a lack of knowledge of the PK properties of CBD in veterinary pharmacology. As a consequence, the current research aims: a) to characterize PK after IV and PO administration of CBD in healthy horses; b) to determine PO bioavailability; c) to evaluate the absorption with different CBD formulations and d) to simulate different treatments and plasma levels as points of reference for further clinical assays.

Methods: Single IV experiment and two-way randomized PO assays, Latin-square design were used. Eight healthy horses received IV CBD at 1 mg/Kg dose, in addition to PO CBD in sesame and micellar formulations, both at 10 mg/Kg. CBD concentrations were measured using LC-MS/MS and subsequently were analyzed by non-linear mixed effect pharmacokinetic models. Parameters obtained were used to simulate a 14-day treatment and calculate the maximum and minimum concentrations at steady state.

Results: CBD kinetics was described by three compartment models resulting a volume of distribution of 48 L/Kg, a plasma clearance of 1.2 L/h/Kg with a long half-life of 22 h. However, PO bioavailability was low, ranging from 10 to 13% for micellar and oil formulations, respectively. Moreover, micellar formulation presented higher absorption rate constants and maximum plasma concentration (C_{max}) with shorter times to reach that value, what was confirmed with the simulations where higher concentration ranges at steady state for micellar formulation were predicted.

Conclusions: It was concluded that PO bioavailability of CBD in horses is low but falls within the range described for other species as humans and dogs. Micellar formulation showed faster absorption than sesame oil formulation, suggesting that higher concentrations could be achieved in multiple dose treatments. These finding indicated that CBD could be of interest in equine clinical pharmacology, although further studies are necessary in this context to evaluate its use in horses.

PHARMACOKINETICS OF CANNABIDIOL IN SPRAGUE-DAWLEY RATS AFTER PULMONARY AND ORAL ADMINISTRATION

Daniela Schwotzer[1], Wendy Dye[1], Jacob Jantzi[1], Hammad Irshad[1], Kristen R. Trexler[2], Timothy W. Lefever[2], Marcel O. Bonn-Miller[2], Mark Ware[2] and Jacob McDonald[1]

[1]Lovelace Biomedical, Albuquerque, New Mexico, USA

[2]Canopy Growth Corporation, Smiths Falls, Ontario, Canada

Introduction: Cannabidiol (CBD) is of increasing interest for its beneficial health effects, including but not limited to; anti-depressant, anxiolytic, anti-inflammatory. CBD containing products are available in different formulations, i.e. dermal, oral, inhalation. There is limited research on the differences of CBD absorption, as well as distribution and excretion of CBD and its major metabolites 6-Hydroxy-cannabidiol (6-OH-CBD), 7-Hydroxy-cannabidiol (7-OH-CBD), and 7-carboxy-cannabidiol (7-COOH-CBD) across different routes of administration. The presented research therefore focused on the pharmacokinetics of CBD and metabolites in plasma, brain tissue and urine after inhalation and oral administration.

Methods: For the inhalation study 65 rats were exposed to filtered air or CBD formulated in Propylene Glycol (PG) at a target concentration of 1.0/1.3 mg/L CBD/PG via nose-only inhalation for 14 consecutive days. Exposure duration was 180 minutes per day for filtered air and 12, 23, 45, 90, and 180 minutes per day for CBD in PG. For the oral study 24 animals received a single oral gavage of 10 mg/kg CBD in Medium Chain Triglyceride (MCT) oil. Blood was collected at multiple time points up to 960 minutes after single and 14-day inhalation exposure and after single dose oral gavage. Urine and brain tissue were collected the day after final inhalation and after oral administration. Pharmacokinetics were determined from blood, brain tissue and urine samples.

Results: Inhalation exposure resulted in average daily presented/deposited CBD doses ranging from 8.9/0.9 mg/kg to 138.5/13.9 mg/kg. CBD peak plasma concentrations (up to 3300 ng/mL) were reached at 5 min post exposure and consistently decreased until 16 hours. Metabolite concentrations were 10-100-fold lower than CBD. There was no difference in CBD concentrations between sexes or accumulation from Day 1 to Day 14. After oral administration peak plasma concentrations were lower compared to inhalation (96.5 ng/mL) and were reached at 2 hours post dosing. CBD was detected at low levels in brain after 14-day inhalation (>3.5 mg/kg deposited dose) and after single oral administration of 10 mg/kg. CBD metabolites were identified in brain tissue at the highest inhalation dose level only, and at much lower concentrations than parent CBD. CBD was detectable in urine for all dose groups after inhalation. No metabolites were found. No CBD or metabolites were detected in urine on the day after oral dosing.

Conclusions: The present research indicates presence of CBD in blood and brain after inhalation and oral administration with CBD plasma levels and bioavailability being higher and more rapid after inhalation than after oral administration.

AN INVESTIGATION OF COVID-19'S IMPACT ON DEPRESSIVE SYMPTOMS AMONG CANNABIS USERS USING MACHINE LEARNING

Kassidy Seol and Xueyu Zhu

Department of Mathematics, The University of Iowa, IA, USA

Introduction: Psychological depressive symptoms (PDS), such as those of major depressive episodes (MDE), have been one of the most common mental health concerns, affecting more than a quarter of adults in the United States. The therapeutic benefit and/or risk of PDS with cannabis use remains unclear. With 46 states either legalizing or decriminalizing the consumption of cannabis for medical or recreational use, it is imperative for the public health to understand the relationship between PDS and cannabis usage and whether this may have evolved after COVID-19. During the pandemic, marijuana sales showed a steady growth despite the recession, and the rate of PDS among adults escalated from around 19% to around 28%. Using popular machine learning algorithms, this study investigated COVID-19's impact on depression by conducting a comparative analysis on key sociodemographic risk factors of PDS among the cannabis users between 2019 and 2020.

Methods: The National Survey on Drug Use and Health is one of the largest annual surveys sponsored by the Substance Abuse and Mental Health Services Administration. We first trained and compared three different supervised machine learning algorithms: logistic regression (LR), k-nearest neighbors (KNN), and random forest (RF) and identified the optimal classifier based on accuracy, sensitivity, specificity, precision, F-score, receiver operating characteristics (ROC), and the area under the ROC curve (AUC). The chosen classifier was then used as a common analysis framework to compare the key sociodemographic risk factors of PDS before and after COVID-19 first became a global pandemic.

Results: The RF classifier was identified as the most optimal machine learning algorithm compared to the other two classifiers, with all performance metrics close to 0.99. LR demonstrated accuracy, sensitivity, specificity, precision, and F-score of 0.87, 0.90, 0.77, 0.92, and 0.91, respectively. KNN showed the worst performance, with all the statistical metrics mostly being between 0.71 to 0.86. Through RF, we have discovered two sets of 11 key predictors out of the 2,741 and 2,890 survey questions for 2019 and 2020, respectively, that are closely related to the development of PDS among those who depend on or abuse cannabis. One of the most important predictors for both 2019 and 2020 was IMPGOUT, which pertained to difficulty going out of the house. Additionally, there was a distinct shift from work negligence to difficulty finding employment from 2019 to 2020, with the variable for the number of days missed from work (WRKSKIPMO) being identified as a key risk factor for 2019 and the variable for wanting specific employers (WRKOKPREH) being identified for 2020. Another difference between the features is the presence of two "social" variables: difficulty participating in a social activity and the act of giving away/sharing marijuana. Both were identified in 2019, but no such predictors were identified in 2020.

Conclusion: Through machine learning algorithms, we have shown that community socialization facilitated by marijuana use was an important predictor of depressive symptoms but became less significant after COVID-19. We have also shown that other intrinsic risk factors for PDS, like IMPGOUT, persist despite social upheavals, but further study is still needed to better understand how social changes could impact sociodemographic risk factors in vulnerable subgroups. Such understanding may help in identifying factors early and developing individualized treatment plans.

AN UPDATE TO SAFETY DATA ON CANNABIS-LIKE PRODUCTS IN THE FDA FAERS DATABASE

Teresa A. Simon[1,2], Andres Gomez[1,2], John Simon [1,3] and Jahan Marcu[1,4]

[1]Physicians Research Center, LLC USA,
[2]Adjunct Fellow Univ of PA, Center for Public Health Initiatives, USA,
[3] Drexel University MPH Candidate, USA
[4]Marcu Enterprises USA, Marcu and Arora, LLC USA

Introduction: Health Alerts on the dangers of the consumption of delta-8 Tetrahydrocannabinol (THC) were issued from both the United States Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) on September 14, 2021;15 states have banned delta-8 THC; prior work using the FDA Adverse Event (AE) Reporting System and the lack of a national cannabis database, the authors continue to monitor cannabis-derived cases reported in FAERS. While delta-8 THC is an isomer of delta-9 THC, some believe it is legal to synthesize delta-8 THC under the 2018 Hemp Farm bill. The FDA and Drug Enforcement Agency (DEA) websites list delta-8 THC as schedule 1 drug. Despite this conundrum and a lack of safety data, delta-8 THC and other derived products (e.g., HHC isomers, D10 THC, etc.) have exploded into the market.

Methods: An updated FAERS database query was performed on January 22, 2022, and included all cases reported up to September 30, 2021. Product names (aka search terms): Delta-8 tetrahydrocannabinol (THC); Delta- 9 THC, Marinol and cannabidiol (CBD) were applied. We used proportional reporting ratio (PRR) to compare the proportion of spontaneous reports for a Delta-8, Delta-9 THC and Marinol linked to a specific adverse outcome, compared to the same values for the same event pairs for cannabidiol (CBD). For a marketed product, a PRR of 2 for a drug event combination indicates the proportion of reports for the drug-event combinations is twice the proportions of the event to the comparator and is considered a potential safety signal. For this analysis we report PRRs greater than 10.

Results: FAERS Data as of September 30,2021 showed cases by "suspect product active ingredient". Dyspnea was the most common (18%) AE in Delta-8 THC cases; seizure (30%) for CBD, nausea (12%) and vomiting (11%) for Marinol and toxicity to various agents (42%) for Delta-9 THC. When Delta-8 THC is compared to CBD, the highest PRR occurs in the respiratory system (14.49); CBD compared to Marinol the greatest PRR was 10.4 in vascular system. The types of AE reported within the vascular system were diverse. The most common event was hypertension (N=3). CBD compared to Delta-9 most PRR ranged from 0.0 to 3.5;

Conclusion: Delta -8 THC continues to have a high number of respiratory adverse events (N=89) reported in the only national safety database (FAERS). A PRR of 14 indicates respiratory events are 14 times more common in those where Delta-8 THC was a suspect product. It is unclear if Delta-8 THC or another factor, such as an adulterant, is the cause of these events. These events could have more serious downstream effects such as cardiac events as a result of hypoxia reported in susceptible users. Based on the limitations of FAERs and our experience analyzing the AE data, we have designed a data collection tool for collecting more granular information on cannabis-derived products pointing to safer use for all. Available pilot data utilizing this newly developed collection tool will be shared during this presentation.

"*FREE WEED THAT GETS YOU HIGH*": DID THE FDA AND CDC HEALTH ALERTS ON DELTA-8 THC REACH MAINSTREAM SOCIAL MEDIA?'

Teresa A. Simon[1,2], Anthony Silverstone[1,3] Yomei Shaw[1,4] and Jahan Marcu[1,5]

[1]Physicians Research Center, LLC USA,
[2]Adjunct Fellow Univ of PA, Center for Public Health Initiatives, USA,
[3] Fairfield University MPH Candidate, USA,
[4]FORWARD, The National Databank for Rheumatic Diseases, USA
[5]Marcu Enterprises USA, Marcu and Arora, LLC USA

Introduction: Social media enables public sharing of information, including experiences regarding substance use. Twitter is currently among the most popular social media platforms worldwide, and researchers have published numerous papers exploring methods for investigating health outcomes and attitudes (i.e., sentiment) on twitter. We tracked health-related information and communication regarding delta-8 THC and other cannabis products. On September 14, 2021, both the FDA and CDC issued health advisories related to artificially derived delta 8-THC products, after poison control centers across the nation reported over 600 cases related to exposure to these products. The health alert seemed to receive minimal, if any, national news coverage. We sought to determine the impact of the CDC and FDA health advisories, as well as the sentiment and attitudes towards delta-8 THC, as mentioned on Twitter.

Methods: Five hundred tweets before and after September 14th, 2021 (date of CDC and FDA health advisory) were extracted from Twitter using web scraping. Posts were filtered using key search terms such as: "Delta 8", "D8", Delta 8 THC", and "D8THC". A Likert scale of 1-5 was used to rate the tweets (the value of 1 being the most positive result, and 5 being the least positive result). Each tweet was scored by 3 independent researchers. Knowledge and attitude change before and after the national health advisory on September 14, 2021, were analyzed quantitatively and qualitatively.

Results: There were several cautionary comments prior to September 14th with an increase in warnings and precautions in the post-health advisory timeframe. During the 30-day post-advisory period some tweets specifically named the FDA/CDC health alerts while others were general warnings. Preliminary data shows the most common reasons for using delta-8 THC included: getting high, inducing sleep, and coping with pain, anxiety, depression, and PTSD. During the pre- and post-period tweets, the comments from positive reviews on its effects as a recreational drug to warnings of its potency and side effects. The adverse effects mentioned in tweets include: induced anxiety, lung pain, harshness, and dehydration. Interestingly, both pre and post health advisory tweets mention the lack of regulations, non-existent standards, and to "*stick with regular weed*."

Conclusion: To our knowledge, FDA and CDC delta-8 THC health alerts were not covered by the national news associations. Twitter, a social networking service with over 330 million active users, is a platform that showed an increase in delta-8 THC warnings post FDA/CDC Health Alert. Based on the types of tweets regarding the use of delta-8 THC, Twitter could become an avenue to quickly disseminate information on the risks and potential benefits of delta-8 THC.

HAIR REGROWTH WITH NOVEL HEMP EXTRACT - A CASE SERIES

Gregory L. Smith

Callitas Therapeutics

Introduction: Cannabidiol (CBD), tetrahydrocannabivarin (THCV) and cannabidivarin (CBDV) are all phytocannabinoids with novel therapeutic effects on hair regrowth through the endocannabinoid system. Topical application of these cannabinoids easily reaches hair follicles where they act as partial or full CB1 antagonist and agonist of vanilloid receptor-1 (TRPV1) and vanilloid receptor-4 (TRPV4). All these ECS receptors relate to hair follicle function. Blocking the CB1 receptor on the hair follicle has been shown to result in hair shaft elongation; in addition, the hair follicle cycle (anagen, catagen, and telogen phases) is controlled by TRPV1. This study was conducted on subjects with androgenetic alopecia (AGA), as a follow-up to a prior study using high-CBD hemp extract without CBDV or THCV. That study showed an average 93.5% increase in hair numbers after six months of use. This subsequent study is being done to determine if daily topical application of a hemp-oil high in CBD, THCV and CBDV concentrations would result in improved hair regrowth in the area of the scalp most affected by AGA.

Methods: A case series study was done of 31 subjects with AGA. They used a once-daily topical hemp extract formulation, averaging about 33 mg per day for 6 months. A hair count of the greatest area of alopecia was carried out before treatment was started and again after 6 months of treatment. To facilitate consistent hair count analysis, a permanent tattoo was placed at the point for maximum hair loss on the scalp. The subjects were also asked to qualitatively rate their psychosocial perception of 'scalp coverage' improvement after the study was completed. The qualitative scale included – "very unhappy" "unhappy" "neutral" "happy" and "very happy." The subjects were photographed in a standard manner before and after the study. The photographs were compared for improvements in 'scalp coverage' by an independent physician. The qualitative scale included "none", "mild", "moderate", "extensive" improvement of scalp coverage.

Results: The results revealed that all subjects had some regrowth. This ranged from 31.25% (from 16 to 21 hairs) to 2000% (from 1 to 21 hairs). The average increase was statistically significant 246% (15.07 hairs /cm2 increase) in men and 127% (16.06 hairs /cm2) in women. There were no reported adverse effects. All subjects rated their psychosocial perception of the effects of the hair loss, as 'happy', or 'very happy.' Independent review of the photographs revealed evidence of 'mild' to 'extensive' scalp coverage improvements for all of the subjects.

Conclusions: Though the exact mechanism of therapeutic effects is not known, THCV and CBDV are most likely functioning as full CB1 receptor neutral antagonists, and CBD is most likely functioning as a partial CB1 receptor antagonist and potentially via Wnt messaging. All three cannabinoids were functioning as TRPV1 agonists. This topical hemp formulation was superior to oral finasteride, 5% minoxidil once daily foam and CBD topical extract alone. Since this hemp extract works through novel mechanisms entirely different from both finasteride and minoxidil, it can be used in conjunction with these current drugs and would be expected to have synergistic effects. However, safety and efficacy of this combination would be to be evaluated.

PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS OF HEMP-DERIVED CANNABIDIOL (CBD) TOPICAL PRODUCTS

Tory Spindle[1], Hayleigh Tilton[1], Spencer Lin[1], Ed Cone[1], Ruth Winecker[2], Eric Welsh[3], Lynn Wagner[3], Ron Flegel[4] and Ryan Vandrey[1]

[1]Behavioral Pharmacology Research Unit, Johns Hopkins University School of Medicine;
[2]RTI International;
[3]Department of Defense (DoD);
[4]Substance Abuse and Mental Health Services Administration (SAMHSA)

Introduction: Cannabidiol (CBD) products intended for topical application (e.g., creams, lotions, patches, etc.) have proliferated in the U.S. since hemp (cannabis containing <0.3% THC) was legalized in 2018 via the "Farm Bill." However, despite their popularity, controlled clinical studies on this diverse product category are lacking. This study characterized the pharmacokinetic (PK) and pharmacodynamic (PD) effects of various commercially-available CBD-dominant topical hemp products with low concentrations (<0.3%) of THC.

Methods: Thirteen infrequent cannabis/CBD users were randomized to use either a CBD-dominant cream (N=8; CBD dose = 11 mg per application; THC dose = 0.7 mg per application), a placebo cream (N=2), or a CBD-dominant lotion (N=3; CBD dose = 95 mg per application; THC dose = 4.2 mg per application). Participants completed an 8-hr drug application session in the laboratory, followed by a 10-day outpatient drug application period (b.i.d. dosing); participants returned to the lab on Days 2, 3, 7, 10, and 17 (1-week washout). During each application, participants applied ¼ tsp of their assigned product on each of their upper arms, an area known to promote transdermal drug absorption (the surface area of the application site was kept consistent at 5 sq. in for each participant). Blood, urine, and oral fluid (OF) were collected and PD effects were assessed during the laboratory session and the outpatient visits.

Results: Subjective/cognitive effects and vitals were similar at baseline and all post-dosing timepoints. Trace concentrations of THC were detected in OF for all CBD-dominant cream participants but THC concentrations never exceeded 4 ng/mL (threshold for a "positive" cannabis test). Two of the three participants who used the CBD-dominant lotion had several OF samples with THC concentrations >4 ng/mL. All urine specimens for 5 CBD-dominant cream participants were negative (screening cutoffs: 20, 50, 100 ng/mL); remaining urine and blood samples are pending analysis. Overall, among active product participants, OF CBD concentrations increased steadily throughout the study, peaked during the outpatient phase, and decreased markedly at the washout visit.

Conclusions: The topical CBD products examined in this study did not produce significant PD effects. The CBD-dominant cream did not produce positive results on urine or OF drug tests for cannabis/THC, but positive OF results were observed in two of the three CBD-dominant lotion participants. The observed increases in OF cannabinoid concentrations (particularly CBD) is suggestive of transdermal absorption. More research is needed to explore how factors such as formulation and dose impact PK and PD effects of topical cannabinoid products.

Acknowledgements: Funded by the Department of Defense (DoD) and the Substance Abuse and Mental Health Services Administration (SAMHSA)

FATTY AMIDE HYDROLASE REGULATES TEARING IN A SEX-DEPENDENT MANNER IN MICE

[1,2]Jenna Billingsley, [1,2]Kelsey Andreis, [1,2]Amanda Thayer,
[1,2]Jim Wager-Miller, [1,2,3]Heather Bradshaw and [1,2,3]Alex Straiker

[1]The Gill Center for Biomolecular Science,
[2]Department of Psychological and Brain Sciences,
[3]Program in Neuroscience, Indiana University, Bloomington, IN 47405.

Cannabis users frequently report dry mouth and dry eye. We recently showed that cannabinoid CB1 receptors regulate tearing in mice. Interestingly this regulation occurs in a sex-dependent manner, with CB1 receptor activation reducing tearing in males, but increasing tearing in females. One unresolved question is what endogenous cannabinoid mediates the reported effects. Two endocannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG) are each implicated as endogenous cannabinoid messengers, or endocannabinoids. Each is regulated by distinct synthetic and metabolic machinery. Anandamide is synthesized by NAPE-PLD and metabolized by fatty acid amidohydrolase (FAAH). 2-AG is synthesized by diacylglycerol lipases a and b, and metabolized by any of several serine hydrolases, particularly monoacylglycerol lipase (MAGL). To determine which endocannabinoid mediates CB1 receptor regulation of lacrimal gland function we tested tearing in wild type and transgenic mice. We examined expression of FAAH in lacrimal gland using immunohistochemistry and quantitative PCR. We also examined the levels of cannabinoid-related lipids using lipidomics.

Our chief findings are that FAAH blocker URB597 and FAAH knockout mice each increase tearing in females, but not males. This suggests that anandamide mediates the CB1-dependent increase in tearing. FAAH protein expression is restricted to the endoplasmic reticulum of epithelial cells, but we do not detect a difference in mRNA expression for FAAH or NAPE-PLD by sex. Our lipidomic experiments comparing baseline levels of cannabinoid-related lipids in male vs. female mice show relatively higher levels of several acylethanolamines, the lipid family that includes anandamide, in females, but not of anandamide itself. Levels of several acylglycerols, including the endocannabinoid 2-AG, were higher in males.

Our results indicate that the CB1 receptor-mediated increases in tearing in female mice likely involve anandamide and FAAH. However, anandamide does not appear to mediate the CB1 inhibition of tearing in male mice, suggesting that another endocannabinoid mediates this effect.

ECOLOGICAL MOMENTARY ASSESSMENT OF ANXIETY AND DEPRESSION IN NEWLY INITIATED MEDICINAL CANNABIS PATIENTS: MOMENTARY AND LONG-TERM CLINICAL EFFECTS

Justin C. Strickland, Rhiannon E. Mayhugh, Renuka Surujnarain and Ryan Vandrey

Department of Psychiatry & Behavioral Sciences,
Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction: Access to medicinal cannabis is expanding rapidly; yet little controlled data exist on therapeutic efficacy and how variations in cannabinoid composition alter the balance between medicinal benefit and performance impairment. Participants in this study (N=31) prospectively completed assessments of mood, sleep, cannabis use, and subjective feelings of impairment using a phone application immediately before and for 8 weeks after newly initiating medicinal cannabis.

Methods: All participants met criteria for clinically relevant anxiety and/or depression and were recently registered with the Maryland medicinal cannabis program. Mood and cannabis-related impairment measures were assessed at wake up, bedtime, and two random midday timepoints each day. Subjective measures were also collected immediately before and at the expected time of peak post-dose effects based on route of administration for each episode of cannabis use. In-depth clinical assessments of mood and health were completed at baseline prior to cannabis use initiation and 1, 3, and 6 months after initiation. Generalized linear mixed effect models were used to evaluate momentary relationships between acute cannabis exposure and clinical effects, longitudinal changes in health, and prediction of clinical changes by momentary effects.

Results: Reductions in anxiety and depressive symptoms were observed post-administration (632 independent use events) that were similar across routes of administration, $p < .05$. Impairment (e.g., subjective high, perceived driving impairment) was detected and differed by route of administration and gender, with less impairment reported with oral administration and among women, $p < .05$. Evaluation of dose effects showed greater decreases in perceived driving ability and increases in subjective high when higher THC doses were administered, but no differences by CBD dose, $p < .05$. Clinical assessments of anxiety and depression indicated reductions at each follow-up, but the magnitude of reduction was larger at the 1 and 3 month follow-ups (d_z=0.78-1.25) than 6 month follow-up (d_z=0.48-0.62). Models predicting clinical efficacy based on mean levels and variability of momentary effects did not indicate direct correspondence.

Conclusions: Positive clinical effects of medicinal cannabis products were detected. Possible separation of acute versus chronic effects within-person highlight the importance of distinguishing levels of analyses when determining medicinal cannabis clinical outcomes. These data also replicate canonical cannabinoid effects, thus demonstrating the feasibility of using remote data collection methods to evaluate drug effects over extended periods to balance the rigor of real-time pharmacodynamic measurement with the generalizability of real-world environments.

Acknowledgements: Funded by Thomas Jefferson University Lambert Center.

CHRONIC AND ACUTE EFFECTS OF CANNABIS ON SYMPTOMS OF ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

Amanda Stueber, Aria Petrucci, and Carrie Cuttler

Department of Psychology, Washington State University, Pullman, WA, USA

Introduction: Attention-deficit/hyperactivity disorder (ADHD) is characterized by symptoms of inattention, and/or hyperactivity and impulsivity. Past research indicates that people with ADHD use cannabis more frequently, and are at increased risk of developing cannabis use disorder. Moreover, there is evidence that people with ADHD may be using cannabis to self-medicate. For instance, our recent retrospective self-report study revealed that people with ADHD report that acute cannabis use decreases their symptoms of hyperactivity and impulsivity, but not inattention. In contrast, they reported that chronic cannabis use has few effects on their ADHD symptoms. The goal of this study was to examine chronic and acute effects of cannabis on people with ADHD. Specifically, we sought to 1) compare symptom ratings of chronic cannabis users and non-users with ADHD under sober conditions, and 2) prospectively examine changes in self-reported ADHD symptoms after acute cannabis intoxication in cannabis users relative to sober non-users.

Method: People with an ADHD diagnosis who use cannabis and who don't use cannabis completed two separate testing sessions over Zoom. During the first session, participants rated their symptoms of inattention, hyperactivity, impulsivity and irritability at three timepoints when all participants were sober. During the second session, participants were asked to provide baseline ratings of the severity of their symptoms of inattention, hyperactivity, impulsivity, and irritability. The group of cannabis users was then observed inhaling a cannabis product over Zoom while the non-users remained sober. All participants re-rated each of these symptoms immediately after, 20 minutes after, 40 minutes after, and 60 minutes after the cannabis use sessions.

Results: Comparisons of symptom ratings in the first session (when all participants were sober) revealed no significant differences in symptom ratings of chronic cannabis users with ADHD and non-users with ADHD. In contrast, comparisons of symptom rating change scores in the second session revealed acute beneficial effects of cannabis intoxication on symptoms of impulsivity, hyperactivity, and irritability. Specifically, relative to sober non-users with ADHD, people with ADHD who inhaled cannabis showed significant reductions in impulsivity ratings 40 minutes after use ($p = .04$), significant reductions in hyperactivity 40 minutes after use ($p = .005$) and 60 minutes after use ($p = .04$), as well as significant reductions in irritability immediately after cannabis use ($p = .004$), 20 minutes after use ($p = .02$), 40 minutes after use ($p = .04$), and 60 minutes after use ($p = .02$). In contrast, acute cannabis intoxication did not significantly affect ratings of inattention.

Conclusions: Results are consistent with a band-aid model of cannabis use for mental health. Specifically, acute cannabis intoxication appears to temporarily reduce symptoms of impulsivity, hyperactivity, and irritability in people with ADHD. However, chronic use of cannabis does not have any apparent longer-term beneficial effects on these symptoms. These findings may also help to account for the increased risk of cannabis use disorder in people with ADHD, since they would need to regularly use cannabis to experience its beneficial acute effects.

Funding: Washington State University's Alcohol and Drug Abuse Research Program

UK MEDICAL CANNABIS REGISTRY: A PATIENT EVALUATION

James Tait[1], Simon Erridge[1,2]and Mikael H Sodergren[1,2]

[1] Imperial College Medical Cannabis Research Group, Department of Surgery and Cancer, Imperial College London, London, UK
[2] Sapphire Medical Clinics, London, UK

Introduction: The UK Medical Cannabis Registry is the largest real world data platform for medical cannabis outcomes in the UK. It uses an online platform to collect patient reported outcome measures, adverse events and changes to prescription medications from patients prescribed medical cannabis for a range of conditions. This study aims to assess the effectiveness, efficiency and future research priorities for registry participants.

Methods: A cross-sectional survey was designed to assess the functionality and accessibility of the data collection platform as well as patient priorities for future research. Surveys were electronically distributed to 1627 patients who had been enrolled for three months or greater. Responses to closed questions were analysed utilising descriptive statistics. For open-ended questions an inductive thematic analysis was performed.

Results: 600 responses were recorded, of which 554 (92.3%) had used the online data entry platform. 272 (90.4%) and 256 (85%) patients believed it was easy to input medication names and dosages respectfully. 52 (8.67%) patients recorded an adverse event, of which 38 found it easy to do so (73.1%). 535 (96.6%) patients had completed health questionnaires with 490 (91.6%) patients finding this easy to do so. 553 (92.2%) patients strongly agreed or agreed that contributing to the UK Medical Cannabis Registry would impact the medical care of future patients. 'Assessing the impact of medical cannabis on quality of life generally' was identified as the top research priority for 357 (59.3%) patients.

Conclusions: This study demonstrates that the majority of patients enrolled in the UK Medical Cannabis Registry found the online data collection platform easy to access and use when completing patient reported outcome measures, adverse events and changes in prescribed medications. Patients believe that through contributing to the UK Medical Cannabis Registry they are having a positive impact on other individuals. Finally, priorities for future research for patients included assessment of quality of life and condition specific outcomes.

EVIDENCE FOR SUBSTANTIAL NASAL ABSORPTION OF Δ^9-TETRAHYDROCANNABINOL AND ITS ACTIVE METABOLITE FOLLOWING AEROSOL ADMINISTRATION

Alexa Torrens[1], Christina Ruiz[2], Faizy Ahmed[1], Stephen V. Mahler[2] and Daniele Piomelli[1]

[1]Department of Anatomy and Neurobiology and [2]Department of Neurobiology and Behavior, University of California, Irvine, CA, USA

Introduction: Smoking and vaping are the most common routes of cannabis self-administration amongst teenage users (Knapp et al., J Adolesc Health. 64 (2019) 487-493). Animal studies have shown that the pharmacokinetic profile of passive aerosol exposure to Δ^9-THC is comparable to clinical data in regards to the time it takes to reach maximum concentration and the half-life time of elimination in plasma after consuming a cannabis cigarette (Baglot et al., Sci Rep. 11 (2021) 23990; Ruiz et al., Psychopharmacology. 238 (2021) 3595-3605). Despite their value, these experiments have generally discounted species-specific differences in breathing mechanics and more specifically the fact that rodents, unlike humans, are obligate nose breathers. For this reason, we investigated whether Δ^9-THC absorption after passive aerosol administration might also occur through the nasal cavity.

Methods: Δ^9-THC was administered (100 mg/mL in 30 min) to adolescent (30-day-old) male and female Sprague-Dawley rats. Δ^9-THC and its first-pass metabolites – 11-hydroxy-Δ^9-THC (11-OH-THC) and 11-nor-9-carboxy-Δ^9-THC (11-COOH-THC) – were quantified in plasma, lung, cerebellum, olfactory bulb and nasal epithelium by liquid chromatography/tandem mass spectrometry. Data were analyzed by two-way analysis of variance with Bonferroni or Tukey post-hoc tests where appropriate. $P < 0.05$ was considered statistically significant.

Results: Δ^9-THC accumulation was substantially greater in nasal mucosa than in any other tissue at each time point following administration. For example, 5 min after the aerosol session the concentration of Δ^9-THC in nasal mucosa of female mice was 19,175 ± 293 pmol/g compared to 349 ± 38 pmol/mL in plasma ($P < 0.001$), 4,168 ± 502 pmol/g in lungs ($P < 0.01$) and 698 ± 129 pmol/g in cerebellum ($P < 0.001$). The results were similar in male mice and for both sexes at 15 min. Concentrations of the active metabolite, 11-OH-THC, were also significantly higher in nasal mucosa of both sexes compared to the other tissues at each time point ($P < 0.01$). Furthermore, 11-OH-THC concentrations decreased more than 40% in the plasma and lung, slightly decreased by 10 to 15% in the brain, and either stayed the same or slightly increased in the nasal epithelium after 15 min.

Conclusions: Δ^9-THC and its active metabolite, 11-OH-THC, accumulate in nasal mucosa of male and female adolescent mice after passive aerosol administration, reaching substantially higher concentration than those measured in plasma and lungs. The data also suggest (1) that Δ^9-THC may be metabolized to 11-OH-THC in nasal mucosa; and (2) Δ^9-THC might directly enter the brain via the cribriform plate. The human relevance of these findings remains to be established, but they should be taken into account when evaluating the ecological relevance of passive aerosol administration in animals.

Acknowledgements: Funded by the National Institute on Drug Abuse (Grant DA044118) and National Institutes of Health (R01 GM11558884).

THE CHARACTERIZATION OF CANNABIS USERS AND PRODUCTS AND THE EXPERIENCE OF NEGATIVE MENTAL EMOTIONS AFTER CANNABIS USE

Nir Treves, Noa Yakirevich-Amir, Wiessam Abu Ahmad, Omer Bonne, Elyad Davidson, Keenan Keeling, Branden Hall, Tyler Dautrich and Ilan Matok

Division of Clinical Pharmacy, School of Pharmacy, The Hebrew University
Department of Psychiatry, Hadassah Hebrew University Medical Center
Department of Anesthesia, Hadassah Hebrew University Medical Center
MoreBetter LTD

Introduction: Although cannabis products are marketed for mental-related conditions, one of the main concerns of cannabis use is its adverse mental effects. Studies suggest an association between cannabis use and the risk for mental diseases or exacerbation of existing mental conditions. This database study characterizes users who experienced negative mental emotions after cannabis exposure.

Methods: We used a self-report database of the ReleafApp by MoreBetter LTD, which enables users to track real-time cannabis user experience. From a list of 42 possible side effects on which the user may report, we focused on a group of negative mental emotions as an outcome that may indicate mental deterioration. Multivariable logistic regression models with mixed effects analyses were performed to study the association between user's traits, cannabis products, and reporting negative mental emotions.

Results: 144,954 sessions of cannabis consumption were reported by 6,191 users. Females and non-binary gender were associated with an increased risk for negative mental emotions than males (OR=1.16, 95%CI: 1.05-1.28, OR=1.48, 95%CI: 1.26-1.34). Older age was associated with less negative mental emotions than young age (OR=0.88, 95%CI: 0.78-0.99, OR=0.71, 95%CI: 0.62-0.81, in 30-40 and 40-50, respectively compared to the age group of 18-30 years). New users were associated with an increased risk for negative mental emotions compared to experts (OR=1.35, 95%CI: [1.12-1.64]). Oral cannabis products were associated with negative mental emotions compared to flowers consumption. Nevertheless, the experience of negative mental emotions was not associated with a change in the response to the symptoms for which the cannabis was used. In a cluster analysis, negative mental emotions were more correlated with one other than with other emotions.

Conclusions: Cannabis use in females and non-binary users was associated with negative mental emotions. Oral cannabis products are also associated with negative mental emotions, compared to flowers products. Older age and previous experience with cannabis were associated with fewer negative mental emotions. Further studies should examine the abovementioned traits in the context of cannabis use and mental illness.

EVALUATION OF SEX DIFFERENCES IN THE POTENTIAL OF Δ^9-TETRAHYDROCANNABINOL, CANNABIDIOL AND CANNABIDIOLIC ACID TO REDUCE NAUSEA-INDUCED CONDITIONED GAPING REACTIONS IN SPRAGUE-DAWLEY RATS

Erin M. Rock, Cheryl L. Limebeer and Linda A. Parker

Department of Psychology and Collaborative Neuroscience Program,
University of Guelph, Guelph, ON, Canada

Introduction: Cancer patients report nausea as a side-effect of their chemotherapy treatment. Using the pre-clinical rodent model of acute nausea—lithium chloride (LiCl)-induced conditioned gaping—our group has demonstrated that Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabidiolic acid (CBDA) may have anti-nausea potential. However, we have not evaluated the potential role of sex differences in mediating the effects of cannabinoids.

Methods and Results: We first compared the conditioned gaping reactions produced by varying doses of LiCl in male and female rats using the taste reactivity test (Experiment 1). LiCl produced dose-dependent conditioned gaping that did not differ in strength among male and female rats. The highest dose (127.2 mg/kg) produced the most robust conditioned gaping and was used in subsequent experiments with cannabinoid pretreatments. Next, we examined the potential of THC (Experiment 2; 0.0, 0.1, 0.5 or 1 mg/kg), CBD (Experiment 3; 0.0, 1, 5, 20 mg/kg), and CBDA (Experiment 4; 0.0, 0.01, 0.1, 1 μg/kg) to reduce conditioned gaping in both male and female rats. THC, CBD and CBDA dose-dependently reduced conditioned gaping in both male and female rats in a similar manner. In both male and female rats, pretreatment with THC (0.1, 0.5, 1 mg/kg) produced hyperlocomotion during conditioning, with no other pretreatments impacting activity. Although there were no sex differences in the effect of the cannabinoids on the LiCl-induced nausea reaction of conditioned gaping, during both conditioning and test, female rats consistently displayed more rearing behavior than males.

Conclusions: These preclinical results suggest that cannabinoids may be equally effective in treating nausea in both males and females.

Acknowledgements: Funded by research grants from the Natural Sciences and Engineering Research Council (03629) and Canadian Institutes of Health Research (388239) to LAP.

TOPICAL CHRONIC EXPOSURE TO BETA-CARYOPHYLLENE, BUT NOT TO CANNABIDIOL, INDUCES DERMATITIS IN MICE

Sara Jane Ward[1], Saadet Inan[1] and Ajay P. Nayak[2]

1. Center for Substance Abuse Research, Department of Neural Sciences, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA
2. Center for Translational Medicine, Department of Medicine, Division of Pulmonary, Allergy & Critical Care Medicine, Jefferson University, Philadelphia PA, USA

Introduction: Due to the established anti-inflammatory properties of cannabinoids, increasing interest has developed in identifying the potential for cannabinoids in the treatment of inflammatory skin disorders. However, exposure to cannabis components can also result in allergy-like reactivity including urticarial rash, contact dermatitis, angioedema, rhinitis, exacerbation of asthma and rarely anaphylaxis. With increasing access of cannabis for medicinal and recreational use, more individuals are likely to demonstrate allergic reactions. While high molecular weight protein allergens of cannabis have been reported, they do not explain the entire spectrum of symptomatic exposures to cannabis. The present study was conducted to identify the low molecular weight major allergens of cannabis and examine their mechanisms of action. We hypothesized that chronic administration of the terpene and CB2 receptor agonist beta-caryophyllene (BCP), but not cannabidiol (CBD), would produce allergic dermatitis in a murine itch model.

Methods: To test our hypothesis, we applied vehicle, CBD (0.1-10 mg/ml), or BCP (0.1-10 mg/ml) to the neck of mice to induce allergic dermatitis as in dinitrofluorobenzene-induced allergic dermatitis model. Two days before any topical application, the rostral area of the neck and the abdomen of mice were shaved. Initial sensitization with vehicle, CBD, or BCP (100 µl) was conducted on abdominal skin. One week later, neck applications have been started. Vehicle, CBD (0.1-10 mg/ml), or BCP (0.1-10 mg/ml) (50µl) was topically applied to the neck of mice 2 times a week for 5 weeks) Applications occurred on Tuesdays and Thursdays. Every Friday, mice were placed into observation boxes and the number of scratching bouts directed to the neck was counted for 60 min. Skin was also evaluated and dermatitis was scored. At the end of observations on week 5, the mice were euthanized using CO_2 followed by cervical dislocation. Neck skin tissue and plasma were collected for histological and molecular analyses. Additional studies were also conducted in CB2 receptor knockout mice and wild-type C57Bl/6 control, to examine the requirement of CB2 for observed outcomes.

Results: Chronic topical application of vehicle or CBD (0.1 – 10 mg/mL) did not produce dermatitis-like skin changes in the neck of the mice. In contrast, chronic administration of BCP at 10 mg/mL induced dermatitis beginning of week 3 and worsened by week 5. Dermatitis score and the number of scratching bouts were significantly higher in mice treated with BCP (10 mg/ml) compared to other concentrations of BCP, vehicle, and CBD at week 3 through week 5. Microscopic examination of the tissue sections stained with H&E revealed accumulation of inflammatory infiltrates in the epidermal compartments of murine skin following epicutaneous administration of BCP in a dose dependent manner. Further, we observed significant increase in diffused epidermal hyperplasia and elongation of rete ridges in the skin tissues from mice dosed with BCP relative to mice dosed with same concentration of CBD. Further, treatment with BCP and not CBD resulted in significant epithelial barrier disruption as noted by diminished staining of filaggrin in epidermal compartments. Finally, we observed significant recruitment of myeloid cells expressing CD11b in skin tissue of mice treated with BCP and interestingly, albeit to a lesser extent for CBD. On examination of the requirement of CB2 for these outcomes, when BCP 10 mg/ml solution was applied chronically to C57BLJ CB2 KO and their WT littermates, dermatitis and significant scratching behavior were observed in WT mice from beginning of the week 4 compared to CB2 KO mice. Although significantly less dermatitis and scratching behavior was noted for CB2 KO mice, (relative to WT mice), a delayed and diminished response was observed in CB2 KO mice treated with BCP.

Conclusions: Our results suggest that chronic topical exposure to BCP, and not CBD, produces an allergic dermatitis-like response in male Swiss Webster mice. Data from CB2 receptor knockout mice suggest that this effect may be partially mediated by chronic activation of the CB2 receptor.

ADVERSE EVENTS OF CANNABIS SINCE LEGALIZATION: A THREE-YEAR REVIEW

Maja Kalaba, Adela Leiva Centeno, Jessica Hart and Mark A. Ware

Canopy Growth Corporation, Smiths Falls, Ontario, CANADA

Introduction: Since cannabis legalization in Canada in 2018 there has been an increase in availability and consumption of regulated cannabis products. Product safety is a concern of patients, consumers, health professionals and regulators. Here we describe the adverse events (AEs) collected by a Canadian licensed producer (Canopy Growth Corporation (CGC)) over a three-year period in Canada.

Methods: From October 17, 2018 – December 31, 2021, AEs were collected from solicited and unsolicited sources from the Canadian medical and recreational markets and entered in the CGC global pharmacovigilance database. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), and seriousness criteria were defined by the International Conference of Harmonization (ICH). Causality assessments for serious AEs are based on the WHO-UMC system for standardized case causality assessment, and all AEs included in the analysis were verified by healthcare professionals. Where an individual was consuming multiple products, the AE reported was counted for each suspected product. For this analysis, AEs due to lack of efficacy, product-related quality issues (with no health impact) and unverified cases were excluded.

Results: A total of 5109 individual case safety reports comprising 12,590 AEs were received during the reporting period. Over this time period 146,866 patients had a medical account and purchased CGC medical cannabis; additionally, in 2020 alone, CGC sold over 11 million units to the recreational market. Among all reported AEs, 11,914 (95%) were assessed as non-serious. Of the 676 (5%) serious AEs, 223 (33%) were assessed as unlikely/not related and only 2 (0.3%) were assessed as related/certainly related to the cannabis product(s) consumed.

Year	**2018**	**2019**	**2020**	**2021**	**Total**
Total AEs	608	3887	4567	3528	12 590
Non-serious AEs	586	3722	4359	3247	11 914
Serious AEs	22	165	208	281	676
	Causality Assessment of Serious AEs				
Related/Certain	0	0	0	2	2
Probable/Almost Certain	13	94	17	26	150
Possible	7	36	104	154	301
Unlikely	0	27	73	52	152
Not related	2	8	14	47	71

Conclusions: Consistent with prior work, the vast majority of AEs reported with CGC products were non-serious. Risk factors such as age, gender and product profile type (classified by content of tetrahydrocannabinol and cannabidiol) as well as the nature (MedDRA coding) of the AEs are being explored. These longitudinal data demonstrate the importance of pharmacovigilance programs for licensed cannabis producers. The emerging data should be considered in the development of responsible use and safety guidelines for cannabis products.

Please read and follow our ICRS Code of Conduct:

https://icrs.co/ICRS.BoD/ICRS Code of Conduct.pdf

All Delegates participating in the ICRS Symposium – either virtually or in-person – give tacit approval to follow our rules.

TWEET US! Use: **#ICRS2022GALWAY**

How to Install NUI Galway's Eduroam WiFi:

1. Go to the Eduroam website and download the installer for your device type:

https://cat.eduroam.org

Mobile users can download the "geteduroam" app from the Apple App Store or Google Play Store.

2. Search for Galway, select National University of Ireland, Galway and enter the following:

Domain = Eduroam // Username = 9876001t / Password = nfqtj7387

3. To connect, select Eduroam from your device's list of available WiFi networks.

4. Alternatively, 5G/NUIG WiFi is available via:

Domain = NUIGConference1
or
NUIGConference2

Password = conference

INSTRUCTIONS FOR JOINING THE VIRTUAL PLATFORM:

The system is mobile-compatible, but to enjoy a comfortable and flawless viewing experience, we recommend using a computer or a tablet running the Chrome browser. In case you participate through a public internet connection, it is recommended that you ensure that the internet usage settings allow video conferences.

On the ICRS2022 Symposium date, clicking on this link will lead to the login page, on which you will be required to input your e-mail address and the password: **icrs2022**. You will then enter the virtual lobby.

Welcome to the ICRS2022 Lobby. The Lobby's toolbar will lead you to the various locations.

Clicking on the Sponsors button will bring you into the exhibition area.

You may visit the various Sponsors in the exhibition and enter each of them by clicking on the booth itself.

Main Hall - Clicking on the Main Hall button will open the main stage as per the day of the event. In the hall you may watch presentations and send questions by chat in each session.

Clicking on Program will open the full agenda, where you may find the full timetable of presentations and speakers, divided by session and dates.

In the ePoster library, you may view the posters and accompanying videos of posters by clicking on the poster you are interested in, and then the Videos tab on the left of the screen.

You can locate the desired poster by its general subject or by its authors' names, using the search assistant.

The top right icon will take you to your profile. Your name will be pulled from the registration process, and you can add any information you wish and even a picture of yourself.

Remember that in the event of a problem or question, you may contact us by chat, which is accessible by clicking the icon in the lower right part of the screen.

TO USE THE ICRS2022 APP:

Download the links below to the app stores, or search for "eventmagix" by Verus in your App store.

IOS: https://itunes.apple.com/app/id1497959777
Android: http://play.google.com/store/apps/details?id=us.ver.eventmagixapp

App code: **icrs2022**

Welcome to ICRS2022!

ICRS2022
GALWAY

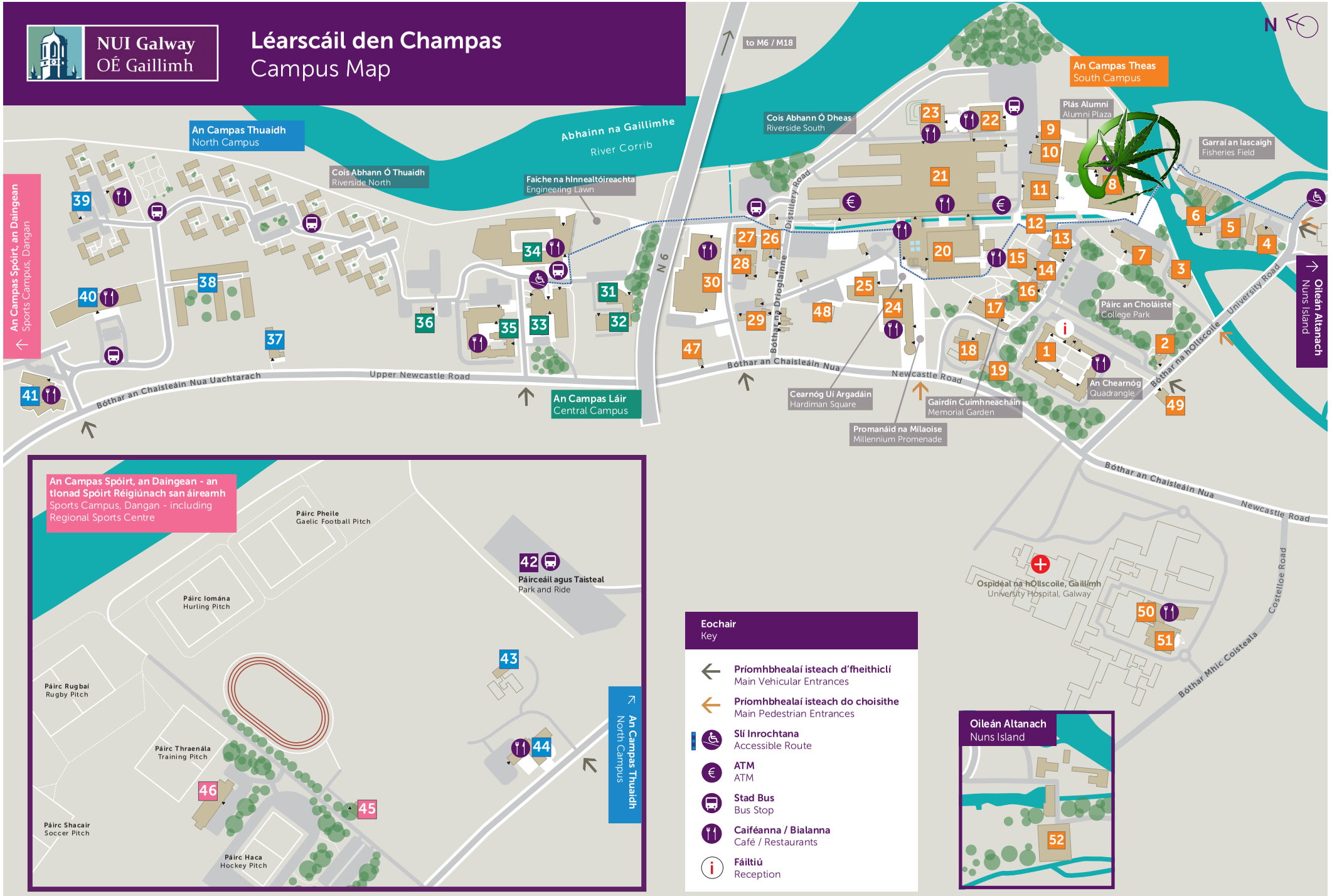
Download ICRS2022 APP

Please scan the relevent QR

App Code: icrs2022

NUI Galway
OÉ Gaillimh

Léarscáil den Champas
Campus Map



Eochair
Key

- **Príomhbhealaí isteach d'fheithiclí** — Main Vehicular Entrances
- **Príomhbhealaí isteach do choisithe** — Main Pedestrian Entrances
- **Slí Inrochtana** — Accessible Route
- **ATM** — ATM
- **Stad Bus** — Bus Stop
- **Caiféanna / Bialanna** — Café / Restaurants
- **Fáiltiú** — Reception

NUI Galway
OÉ Gaillimh

An Campas Spóirt, an Daingean - an tIonad Spóirt Réigiúnach san áireamh
Sports Campus, Dangan - including Regional Sports Centre

An tÁras Spóirt 46
Sports Pavilion

Teach Maryville 45
Maryville House

An Campas Thuaidh
North Campus

An Daingean A 43
Dangan A

Cúrsa Saoil 41
Lifecourse

Baile na Coiribe 39
Corrib Village

Baile an Chíorbhuí 38
Goldcrest Village

Eolaíochtaí Bithleighis 40
Biomedical Sciences

Institiúid na hEolaíochta Sonraí 44
Data Science Institute

Naíolann na hOllscoile 37
University Crèche

Páirceáil agus Taisteal 42
Park and Ride

An Campas Láir
Central Campus

An tIonad Nuálaíochta agus Gnó 32
Business and Innovation Centre

An tIonad Taighde agus Nuálaíochta 31
Research and Innovation Centre

An tIonad Taighde don Chothú Sláinte 36
Health Promotion Research Centre

Áras Cairnes 35
Cairnes Building

Áras Innealtóireachta Alice Perry 34
Alice Perry Engineering Building

Áras Mhaighe Seola 33
Moyola Building

An Clinic Teiripe Urlabhra agus Teanga 33
Speech and Language Therapy Clinic

An Campas Theas
South Campus

10 Bóthar an Chaisleáin Nua 47
10 Newcastle Road

14 Bóthar na hOllscoile 49
14 University Road

14 Bóthar na Drioglainne (An Oifig Slándála) 27
14 Distillery Road (Security)

An Chearnóg 1
Quadrangle

An Foirgneamh Anatamaíochta 18
Anatomy Building

An Foirgneamh IT 23
IT Building

An tIonad Spóirt 30
Sports Centre

Áras Dán na Mílaoise 24
Arts Millennium Building

Áras de Brún 17

Áras Mhairéad (Ma) Ní Éimhigh 16

Áras Mháirtín Uí Riain 7
Martin Ryan Building

Áras na Bitheolaíochta Daonna 11
Human Biology Building

Áras na Gaeilge 15

Áras na Mac Léinn 8

Áras Oirbsean 22
Orbsen Building

Áras Uí Argadáin 20
Hardiman Building

Áras Uí Chathail 10

Aula Maxima 1

Beár na Mac Léinn - Sult 8
College Bar - Sult

Bloc E 13
Block E

Bloc F 19
Block F

Bloc S 12
Block S

Bloc T 28
Block T

Bóthar na Drioglainne 26
Distillery Road

Ceoláras Emily Anderson 1
The Emily Anderson Concert Hall

Comhaltas na Mac Léinn 8
Students' Union

Deasc Eolais na Mac Léinn (SID) 10
Student Information Desk (SID)

Foirgneamh na nDán / na hEolaíochta 21
Arts / Science Building

Fortheach Institiúid Uí Riain 3
Ryan Institute Annexe

Halla Bailey Allen 8
Bailey Allen Hall

Institiúid na hEolaíochta Cliniciúla 51
Clinical Science Institute

Ionad na hÉireann do Chearta an Duine 4
Irish Centre for Human Rights

Ionad na Seirbhísí Poist 9
Mail Services Centre

Ionad Uí Dhonnchadha - An Drámaíocht, an Amharclannaíocht agus an Taibhléiriú 8
O'Donoghue Centre - Drama, Theatre and Performance

Institiúid Lambe 50
Lambe Institute

Oideachas 14
Education

Oideachas 52
Education

Réamhdhéantán Cois Abhann 29
Riverside Terrapin

Réamhdhéantán Scoil Huston 5
The Huston School Bubble

Scoil Scannán agus Meán Digiteach Huston 6
Huston School of Film and Digital Media

Séipéal Naomh Columbán 48
The Chapel of St Columbanus

Síceolaíocht 25
Psychology

Teach an Gheata 2
Gate Lodge