27TH ANNUAL

SYMPOSIUM

OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

MONTRÉAL, QC

CANADA

JUNE 22 - 27, 2017

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Registration: June 22nd, 2017 (16.00 – 18.00)

Le Centre Sheraton, Montréal, QC, Canada

Welcome Reception: 18.30 – 20.00

4th Floor Lobby

Day 1
Friday, June 23rd

7.00	**Breakfast**		
8.30	Welcome and Opening Remarks Salle De Bal Centre & Est		
Oral Session 1. Inflammation and Autoimmunity *Chairs*: Melanie Kelly and Steve Kinsey			
8.45	Jenny L. Wilkerson, Benjamin F. Cravatt and Aron H. Lichtman	DIACYLGLYCEROL LIPASE BETA KNOCKOUT MICE DISPLAY PROTECTION FROM EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS	1
9.00	Ian Burkovskiy, Juan Zhou, Melanie E. Kelly and Christian Lehmann	NEUROPROTECTION VIA CANNABINOID RECEPTOR 2 ACTIVATION AS MEANS TO PREVENT CNS INJURY-INDUCED IMMUNODEFICIENCY SYNDROME IN MICE	2
9.15	Richard Porter, Anna-Maria Szcesniak, James Toguri, Simon Gebremeskel, Brent Johnston, Christian Lehmann, Uwe Grether, Christoph Ullmer and Melanie Kelly	INVESTIGATING NOVEL SELECTIVE CANNABINOID 2 RECEPTOR AGONISTS AS POTENTIAL THERAPEUTIC DRUGS FOR THE TREATMENT OF UVEITIS	3

9.30	Natalia Murataeva, Sally Miller, Cecilia Hillard, Julian Romero and Alex Straiker	A ROLE FOR CANNABINOID CB2 RECEPTORS IN CORNEAL WOUND HEALING	4
9.45	Resat Cinar, Bernadette R. Gochuico, Malliga Iyer, Tadafumi Yokoyama, Joshua K. Park, Nathan J. Coffey, Tony Jourdan, William A. Gahl and George Kunos	IDENTIFYING CANNABINOID CB1 RECEPTOR AS A THERAPEUTIC TARGET FOR IDIOPATHIC PULMONARY FIBROSIS AND A DUAL-TARGETING PARTNER FOR IMPROVING ANTI-FIBROTIC EFFICACY	5
10.00	Daniel Couch, Jon Lund and Saoirse O'Sullivan	URB597 AND JZL184 PROTECTS THE HUMAN COLON AGAINST INFLAMMATION VIA CB1	6
10.15	Haley A. Vecchiarelli, Maria Morena, Kaitlyn Tan, Catherine M. Keenan, Martin Sticht, Kira Leitl, Winnie Ho, Min Qiao, Keith A. Sharkey and Matthew N. Hill	DYNAMIC REGULATION OF THE CENTRAL ENDOCANNABINOID SYSTEM INDUCED BY COLITIS	7
10.30	**COFFEE BREAK**		
ORAL SESSION 2. DRUG DEVELOPMENT AND MEDICINAL CHEMISTRY *CHAIRS*: MARY ABOOD AND MARIO VAN DER STELT			
11.00	Annelot C.M. van Esbroeck, Antonius P.A. Janssen, Armand B. Cognetta, Daisuke Ogasawara, Guy Shpak, Mark van der Kroeg, Vasudev Kantae, Marc P. Baggelaar, Femke M.S. de Vrij, Hui Deng, Marco Allarà, Filomena Fezza, Zhanmin Lin, Tom van der Wel, Marjolein Soethoudt, Elliot D. Mock, Hans den Dulk, Ilse L. Baak, Bogdan I. Florea, Giel Hendriks, Luciano De Petrocellis, Herman S. Overkleeft, Thomas Hankemeier, Chris I. De Zeeuw, Vincenzo Di Marzo, Mauro Maccarrone, Benjamin F. Cravatt, Steven A. Kushner and Mario van der Stelt	ACTIVITY-BASED PROTEIN PROFILING REVEALS OFF-TARGET PROTEINS OF THE FATTY ACID AMIDE HYDROLASE INHIBITOR BIA 10-2474	8

11.15	Richard C. Kevin, Alexander L. Kovach, Iain S. McGregor and Brian F. Thomas	POTENTIALLY TOXIC THERMOLYTIC DEGRADANTS OF CARBOXAMIDE SYNTHETIC CANNABINOIDS	9
11.30	Alessia Ligresti, David Woodward, Jose Martos, Sabatino Maione, Gennaro Marino and Vincenzo Di Marzo	SURPRISING MECHANISM OF FAAH INHIBITION BY NEWLY DESIGNED MULTI-TARGET ANALGESICS	10
11.45	Sergiy Tyukhtenko, Girija Rajarshi, Xiaoyu Ma, Ioannis Karageorgos, Nikolai Zvonok, Elyssia S. Gallagher, Hongwei Huang, Kiran Vemuri, Jeffrey W. Hudgens, Spiro Pavlopoulos and Alexandros Makriyannis	THE DISCOVERY OF ALLOSTERIC SITES OF HUMAN MONOACYLGLYCEROL LIPASE FOR IDENTIFICATION OF NOVEL PHARMACEUTICALS	11
12.00	**LUNCH**		
13.00 - 15.00	**POSTER SESSION 1** SALLE DE BAL OUEST		P1
15.00	**YOUNG INVESTIGATOR AWARD PRESENTATION** CARDIOVASCULAR EFFECTS OF CANNABIDIOL **SAOIRSE O'SULLIVAN** Associate Professor, Faculty of Medicine and Health Sciences University of Nottingham United Kingdom		
ORAL SESSION 3. ENDOCANNABINOIDS, RELATED LIPIDS AND CANNABINOID RECEPTORS *CHAIRS*: HEATHER BRADSHAW AND RUTH ROSS			
15.30	Diane L. Lynch, Dow P. Hurst and Patricia H. Reggio	CRYSTAL PACKING ISSUES IN THE CB1 CRYSTAL STRUCTURES	12

15.45	Attila Oláh, Majid A. Alam, Gréta Kis, Zoltán Hegyi, Johannes Lerchner, Silvia Vidali, Andreas Zimmer, Tamás Bíró and Ralf Paus	CB1 REGULATES MITOCHONDRIAL FUNCTIONS OF HUMAN EPIDERMAL KERATINOCYTES *IN SITU* AND *IN VITRO*	13
16.00	Amey Dhopeshwarkar, Zachary Osborne, Alex Straiker and Ken Mackie	1-ARACHIDONOYLGLYCEROL SIGNALING AT CANNABINOID CB1 RECEPTORS	14
16.15	Emma Leishman, Michelle Murphy, Ken Mackie and Heather B Bradshaw	REGION AND DEVELOPMENT-DEPENDENT EFFECTS OF CP 55,940 ON THE WIDER ENDOCANNABINOID LIPIDOME IN THE MOUSE BRAIN	15
16.30	Jennifer Bialecki, Alexander W. Lohman, Nicholas L. Weilinger, Haley A. Vecchiarelli, Matthew N. Hill and Roger J. Thompson	PANNEXIN-1 TRANSPORTS ANANDAMIDE RESULTING IN MODULATION OF GABA TRANSMISSION	16
16.45	**COFFEE BREAK**		
17.15	Linda Console-Bram, Sandra M Ciuciu, Pingwei Zhao, Robert E. Zipkin, Eugen Brailiou and Mary E. Abood	MODULATION OF THE ENDOGENOUS CANNABINOID SYSTEM BY NAGLY VIA GPR18 AND GPR55	17
17.30	Gregory Carbonetti, Xiaoxue Peng, Tessa Wilpshaar, Simon D'Oelsnitz, and Martin Kaczocha	TRANSPORT OF MAGL-PRODUCED LIPIDS BY FABP5 LEADS TO INCREASED PROSTATE CANCER AGGRESSION	18

17.45	Fabiana Piscitelli, Giulia Donvito, Pretal Muldoon, Asti Jackson, Rosa Maria Vitale, Enrico D'Aniello, Catia Giordano, Bogna M. Ignatowska-Jankowska, Mohammed A. Mustafa, Gavin N. Petrie, Linda Parker, Reem Smoum, Sabatino Maione, Aron H. Lichtman, M. Imad Damaj, Vincenzo Di Marzo and Raphael Mechoulam	OLEOYLGLYCINE IS PRODUCED BY BRAIN TRAUMA AS A NOVEL AND MULTI-TARGET ENDOGENOUS LIPID SIGNAL	19
18.00	**PRESIDENTIAL PLENARY SPEAKER** ACCOMPLICES TO MURDER: ENDOCANNABINOIDS DIRECT MICROGLIA TO KILL NEWBORN NEURONS **MARGARET MCCARTHY, PH.D.** Professor and Chair Department of Pharmacology School of Medicine University of Maryland United States		

NOTES:

Day 2
Saturday, June 24th

7.00	**Breakfast**		
Oral Session 4. Pain *Chairs:* Andrea Hohmann and Aron Lichtman			
8.30	Alex Hanson, Emily Kabeiseman and Brian Burrell	ENDOCANNABINOID-MEDIATED HABITUATION PERSISTENTLY REDUCES NOCICEPTIVE SIGNALING	20
8.45	Matthew Elmes, Joseph Sweeney, Olivia Joseph, Gregory Carbonetti, Simon Tong, Su Yan, Kongzhen Hu, Hao-Chi Hsu, Huilin Li, Robert Rizzo, Iwao Ojima, Martin Kaczocha and Dale Deutsch	TOWARDS A NEW CLASS OF ANTINOCICEPTIVE DRUG: DEVELOPMENT OF FATTY ACID BINDING PROTEIN INHIBITORS TO ALTER THE ENDOCANNABINOID TONE	21
9.00	Adrianne R. Wilson-Poe, Chris Trousdale and Jose Morón	INFLAMMATORY PAIN CHANGES THE EXPRESSION AND FUNCTION OF CB1 RECEPTORS IN THE PERIAQUEDUCTAL GRAY	22
9.15	Caitlin Nealon, Angela Henderson-Redmond, Rebecca LaFleur, Matt Yuill and Daniel Morgan	AGONIST-SPECIFIC MECHANISMS OF CANNABINOID TOLERANCE IN DESENSITIZATION RESISTANT MICE	23
9.30	Rebecca M. Craft, Nicholas Z. Greene and Jenny L. Wiley	CANNABIDIOL MODULATION OF ANTINOCICEPTIVE TOLERANCE TO THC	24

9.45	Richard A. Slivicki and Andrea G. Hohmann	BRAIN PERMEANT AND IMPERMEANT INHIBITORS OF FATTY-ACID AMIDE HYDROLASE SUPPRESS THE DEVELOPMENT AND MAINTENANCE OF PACLITAXEL-INDUCED NEUROPATHIC PAIN AND SYNERGIZE WITH THE OPIOID ANALGESIC MORPHINE	25
10.00	Zachary A. Curry, Jenny L. Wilkerson, Deniz Bagdas, M. Imad Damaj and Aron H. Lichtman	MONOACYLGLYCEROL LIPASE INHIBITORS: REVERSAL OF MOUSE PACLITAXEL-INDUCED NOCICEPTION WITHOUT INTRINSIC REINFORCING EFFECTS	26
10.15	David Allsop, Thomas Arkell, Jessica Driels, Jordyn Stuart, Bridin Murnion, Nicholas Lintzeris and Iain McGregor	A PILOT STUDY OF THE EFFECTS OF CHRONIC CANNABIS USE ON PAIN SENSITIVITY, PAIN TOLERANCE AND PLASMA ENDOCANNABINOID LEVELS	27
10.30	**COFFEE BREAK**		
ORAL SESSION 5. CANNABIS USE, STRESS AND PSYCHIATRY *CHAIRS*: IAIN MCGREGOR AND SACHIN PATEL			
11.00	Carrie Cuttler, Alexander Spradlin, Amy Nusbaum, Paul Whitney, John Hinson and Ryan McLaughlin	HEAVY CANNABIS USE IS ASSOCIATED WITH A BLUNTED STRESS RESPONSE AND REDUCED RELIANCE ON TOP-DOWN ATTENTIONAL CONTROL	28
11.15	Jeremy J Watts, Sina Hafizi, Tania Da Silva, Isabelle Boileau, Pablo M Rusjan, Alan A Wilson, Sylvain Houle, Ruth A Ross and Romina Mizrahi	REDUCED ENDOCANNABINOID METABOLISM IN PSYCHOSIS AND CANNABIS USE: A PILOT STUDY IMAGING FATTY ACID AMIDE HYDROLASE WITH [^{11}C]CURB PET	29
11.30	Gabriella Gobbi, Tobias Atkin, Nandini Dendukuri, Nancy Mayo, Jill Boruff, Tomasz Zytynski and Mark Ware	ADOLESCENT CANNABIS CONSUMPTION AND THE RISK OF LATER DEPRESSION: SYSTEMATIC REVIEW AND META-ANALYSIS	30

11.45	Anjali Bhardwaj, David Allsop, Kieron Rooney, Jonathon Arnold, Raimondo Bruno, Delwyn Bartlett, Mark Montebello, Thomas Arkell, Elisha Richards, Jessica Gugusheff, Sally Rooke, Wendy Kerley, Bridin Murnion, Paul Haber, Iain McGregor and Nicholas Lintzeris	RANDOMISED CONTROLLED TRIAL (RCT) OF DAILY AEROBIC EXERCISE FOR INPATIENT CANNABIS WITHDRAWAL	31
12.00	**LUNCH**		
13.00 - 15.00	**POSTER SESSION 2**		P2
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15.00	Jordan S. Farrell, Roberto Colangeli, Kwaku Addo-Osafo, Maria Morena, Matthew N. Hill and G. Campbell Teskey	COX-2 OXYGENATION OF 2-AG CAUSES A STROKE-LIKE EVENT FOLLOWING SEIZURES	32
15.15	Roberto Colangeli, Maria Vella, Massimo Pierucci and Giuseppe Di Giovanni	THE FATTY ACID AMIDE HYDROLASE INHIBITOR URB597 SUPPRESSES EPILEPTIC SEIZURES AND DOES NOT ALTER SYNAPTIC PLASTICITY AT THE PERFORANT PATH-DENTATE GYRUS SYNAPSES	33
15.30	Nephi Stella, Joshua Kaplan, William Catterall and Ruth Westenbroek	CANNABINOID-BASED THERAPEUTICS AS ANTI-EPILEPTIC STRATEGY FOR DRAVET SYNDROME	34

15.45	Kathryn Nichol and Geoffrey Guy	GW PHARMACEUTICALS' CANNABIDIOL (CBD) CLINICAL PROGRAMME IN EPILEPSY	35
16.00	**PRESIDENTIAL PLENARY SPEAKER** SEX-DEPENDENT SYNAPTIC MODULATION IN THE HIPPOCAMPUS **CATHERINE WOOLLEY, PH.D.** Professor William Deering Chair of Biological Sciences Department of Neurobiology Northwestern University United States		
17.00	**COFFEE BREAK**		
ORAL SESSION 7. FEEDING, METABOLISM AND OBESITY *CHAIRS:* PAL PACHER AND KEITH SHARKEY			
17.30	Tony Jourdan, Joshua K Park, Zoltan V Varga, Janos Paloczi, Nathan J Coffey, Avi Z Rosenberg, Grzegorz Godlewski, Resat Cinar, Ken Mackie, Pal Pacher and George Kunos	PODOCYTE-SPECIFIC DELETION OF CANNABINOID-1 RECEPTOR (CB1R) IS PROTECTIVE AGAINST HYPERGLYCEMIA-INDUCED DIABETIC NEPHROPATHY	36
17.45	Shiran Udi, Liad Hinden, Brian Earley, Adi Drori, Noa Reuveni, Rivka Hadar, Resat Cinar, Alina Nemirovski and Joseph Tam	CB1R INHIBITS AMPK-DERIVED FATTY ACID UTILIZATION IN PROXIMAL TUBULES LEADING TO OBESITY-INDUCED RENAL DYSFUNCTION	37
18.00	Stephanie Tobin, Alexandre Fisette, Horia Pribiag, Dominique Matthys, Marie-Line Peyot, Victor Ernesto Issa Garcia, David Stellwagen, Mark Prentki, Thierry Alquier and Stephanie Fulton	NEURONAL DELETION OF α/β-HYDROLASE DOMAIN 6 IN THE NUCLEUS ACCUMBENS PREVENTS DIET-INDUCED OBESITY	38

18.15	Alexander Edwards, Lindsay Hyland, Robert Aukema, Matthew Hill and Alfonso Abizaid	DINNER FOR TWO: DISENTAGLING THE INTERACTION BETWEEN GHRELIN AND ENDOCANNABINOID SYSTEMS IN MODULATING FEEDING WITHIN THE VTA	39
18.30	Martin Sticht, David Lau, Benjamin Cravatt, Keith Sharkey and Matthew Hill	ENDOCANNABINOID MODULATION OF ACUTE STRESS-INDUCED ANOREXIA IN RATS	40
18.45	Benjamin K. Lau, Min Qiao and Stephanie L. Borgland	MODULATION OF ENDOCANNABINOID-MEDIATED PLASTICITY WITHIN THE ORBITO-FRONTAL CORTEX BY A PALATABLE DIET	41
19.00	**In Memoriam**		

Notes:

Day 3
Sunday, June 25th

7.00	**Breakfast**		
Oral Session 8. Fear, Anxiety and PTSD *Chairs:* Jayme McReynolds and Maria Morena			
8.30	Anthony Berger, Hayden Wright, Janelle Lugo, Martin Sticht, Maria Morena, Matthew Hill and Ryan McLaughlin	ENDOCANNABINOID SIGNALING IN THE LATERAL HABENULA: IMPLICATIONS FOR STRESS COPING AND DOPAMINERGIC TRANSMISSION	42
8.45	Gaurav Bedse, Nolan D. Hartley, Emily Neale, Andrew Gaulden, Toni Patrick, Philip Kingsley, Md. Jashim Uddin, Niels Plath, Lawrence J. Marnett and Sachin Patel	FUNCTIONAL REDUNDANCY BETWEEN CANONICAL ENDOCANNABINOID SIGNALING SYSTEMS IN THE MODULATION OF ANXIETY	43
9.00	Mano Aliczki, Zoltan Balogh, Laszlo Szente, Zoltan K Varga, Laszlo Biro and Jozsef Haller	INTERACTIONS OF ENDOCANNABINOIDS ANANDAMIDE AND 2-ARACHIDONOYLGLYCEROL IN THE REGULATION OF BEHAVIORAL RESPONSES TO TRAUMATIC EVENTS	44
9.15	Kevin Crombie, Angelique Brellenthin, Jennifer Tinklenberg, Rachel Lange, Margaret Beatka, Kelli Koltyn and Cecilia Hillard	VOLUNTARY EXERCISE IN MICE ENHANCES THE EXTINCTION OF FEAR AND INCREASES ENDOCANNBINOID CONCENTRATIONS IN THE AMYGDALA	45
9.30	Ozge Gunduz-Cinar, Olena Bukalo, Emma Brockway, Aaron Limoges, Resat Cinar, Eric Delpire, George Kunos and Andrew Holmes	ENDOCANNABINOID SYSTEM MODULATION OF FEAR INHIBITON IN AMYGDALA INPUTS FROM INFRALIMBIC CORTEX	46

9.45	Maria Morena, Kira Leitl, Asim Rashid, Sheena Josselyn and Matthew Hill	FATTY ACID AMIDE HYDROLASE OVEREXPRESSION IN THE BASOLATERAL NUCLEUS OF THE AMYGDALA INDUCES PARADOXICAL EFFECTS ON ANXIETY AND FEAR MEMORY IN RATS	47
10.00	**COFFEE BREAK**		
ORAL SESSION 8 (CONT). FEAR, ANXIETY AND PTSD *CHAIRS:* JAYME MCREYNOLDS AND MARIA MORENA			
10.30	Christine Rabinak, Craig Peters, Farrah Elrahal, Mohammed Milad, Sheila Rauch, K. Luan Phan and Mark Greenwald	CANNABINOID FACILITATION OF FEAR EXTINCTION IN POSTTRAUMATIC STRESS DISORDER	48
10.45	Cecilia Hillard, Samantha Chesney, Olufisayo Fagbemi, Amber Brandolino and Terri deRoon-Cassini	CIRCULATING 2-ARACHIDONOYLGLYCEROL, PTSD, AND NEGATIVE MOOD 6 MONTHS AFTER TRAUMATIC INJURY	49
11.00	**KANG TSOU MEMORIAL LECTURE** THE ROLE OF GENETIC VARIATION IN THE ENDOCANNABINOID SYSTEM IN ADOLESCENT BRAIN DEVELOPMENT **FRANCIS LEE, M.D., PH.D.** Professor / Vice Chair for Research, Department of Psychiatry Professor, Department of Pharmacology Attending Psychiatrist, New York Presbyterian Hospital and Weill Cornell Medicine United States		
12.00 – 13.00	ICRS BUSINESS MEETING		
12.00 –	**FREE TIME**		

Day 4
Monday, June 26th

7.00	**Breakfast**		
Oral Session 9. Phytocannabinoids *Chairs:* Saoirse O'Sullivan and Jonathan Page			
8.30	Alyssa Laun, Patricia Reggio and Zhao-Hui Song	CANNABIDIOL, A NOVEL BIASED INVERSE AGONIST FOR GPR3	50
8.45	Douglas E. Brenneman, Dean Petkanas and William A. Kinney	EFFECT OF KLS-13019 AND CANNABIDIOL ON NEUROPROTECTION FROM OXIDATIVE STRESS IN HIPPOCAMPAL CULTURES: MECHANISM OF ACTION	51
9.00	Georgia Watt, David Cheng, Brett Garner and Tim Karl	THE THERAPEUTIC POTENTIAL OF CANNABIDIOL FOR ALZHEIMER'S DISEASE	52
9.15	Tamás Bíró, Raphael Mechoulam, Francisco S. Guimarães, Mauro Maccarrone and Ethan Russo	FLUORINATED CANNABIDIOL DERIVATIVES AS NOVEL, HIGHLY EFFECTIVE THERAPEUTIC ALTERNATIVES	53
9.30	Sally Miller and Alex Straiker	Δ^9-THC AND CBD DIFFERENTIALLY REGULATE INTRAOCULAR PRESSURE	54
9.45	Roger Pertwee, Erin Rock, Kelsey Guenther, Cheryl Limebeer, Linda Parker and Raphael Mechoulam	HU-580, A STABLE SYNTHETIC ANALOGUE OF CANNABIDIOLIC ACID, PRODUCES 5-HT1A RECEPTOR-MEDIATED SUPPRESSION OF BEHAVIOURAL SIGNS OF NAUSEA AND ANXIETY IN RATS WITH CONSIDERABLE POTENCY	55

10.00	**COFFEE BREAK**		
10.30	**ICRS LIFETIME ACHIEVEMENT AWARD** CANNABINOID / SEROTONIN INTERACTIONS IN THE REGULATION OF NAUSEA **LINDA PARKER, PH.D.** Canada Research Chair in Behavioural Neuroscience Departments of Psychology and Neuroscience University of Guelph, Guelph, Ontario Canada		
ORAL SESSION 10. HUMAN STUDIES *CHAIRS*: MARGARET HANEY AND MARK WARE			
11.00	Nicolas Schlienz, Edward Cone, Evan Herrmann, George Bigelow, John Mitchell, Ron Flegel, Charles LoDico and Ryan Vandrey	COMPARATIVE PHARMACODYNAMIC INVESTIGATION OF ORAL, SMOKED, AND VAPORIZED CANNABIS	56
11.15	Ryan Vandrey, Edward Cone, Nicolas Schlienz, Evan Herrmann, John Mitchell, Ron Flegel, George Bigelow and Charles LoDico	PHARMACOKINETICS OF CANNABIS: IMPACT OF ADMINISTRATION ROUTE AND RELATION TO DRUG EFFECTS AND PERFORMANCE	57
11.30	L. Cinnamon Bidwell, Sarah Hagerty, Sophie YorkWilliams, Raeghan Mueller, Angela Bryan and Kent Hutchison	ACUTE RESPONSES TO DIFFERENT STRAINS OF MARIJUANA: IS CBD A BUZZKILL?	58
11.45	Philippe Lucas and Nick Jikomes	MEDICAL CANNABIS PATTERNS OF USE & SUBSTITUTION FOR OPIOIDS, ALCOHOL, TOBACCO AND ILLICIT SUBSTANCES: A SURVEY OF AUTHORIZED MEDICAL CANNABIS PATIENTS	59

12.00	**LUNCH**		
12.15	**NIDA CAREER INFOSESSION** REQUIRED FOR ALL TRAINEES – LUNCH INCLUDED SALON 6 & 7		
13.00 - 15.00	**POSTER SESSION 3**		P3
ORAL SESSION 10 (CONT). HUMAN STUDIES *CHAIRS*: MARGARET HANEY AND MARK WARE			
15.00	Mark Ware, Julie Desroches, William Barakett, Pierre Beaulieu, Andrée Néron, Yola Moride and Antonio Vigano	PHARMACOVIGILANCE OF MEDICAL CANNABIS: INTERIM RESULTS FROM THE QUEBEC CANNABIS REGISTRY	60
15.15	Timna Naftali, Lihi Bar-Lev Schlieder, Jonathan Hirsch and Fred Meir Konikoff	THE EFFECT OF CANNABIS ON REFRACTORY ULCERATIVE COLITIS PATIENTS	61
15.30	Carrie Cuttler	A CONTROLLED EXAMINATION OF THE EFFECTS OF HEAVY CANNABIS USE AND ADOLESCENT ONSET CANNABIS USE ON COGNITION	62
15.45	Nehal P. Vadhan, Gill Bedi, Catherine E. Myers and Margaret Haney	SMOKED MARIJUANA ALTERS REWARD SENSITIVITY ON AN ASSOCIATIVE LEARNING TASK IN MARIJUANA USERS	63
16.00	**COFFEE BREAK**		

Oral Session 11. Reward / Addiction

Chairs: Aidan Hampson and John Mantsch

16.15	Benjamin N. Froehlich, Christopher J. Fitzpatrick, Rachel L. Atkinson, Ali Gheidi, Trevor Geary and Jonathan D. Morrow	CANNABINOID AGONIST EFFECTS ON SIGN AND GOAL TRACKING	64
16.30	Hannah Schoch, Daniele Piomelli and Stephen Mahler	ADOLESCENT CANNABINOID EXPOSURE PERSISTENTLY ALTERS NATURAL REWARD LEARNING, MOTIVATION, AND DOPAMINE CIRCUITS	65
16.45	Jibran Khokhar, Travis Todd, Wilder Doucette, David Bucci and Alan Green	LONG-LASTING IMPACT OF ADOLESCENT CANNABINOID EXPOSURE ON REWARD-RELATED BEHAVIORS: POTENTIAL INTERACTION WITH SCHIZOPHRENIA	66
17.00	Lydia Baxter-Potter, Janelle Lugo, Kennedy Bieniasz and Ryan McLaughlin	TOWARDS A TRANSLATIONALLY-RELEVANT PRECLINICAL MODEL OF CANNABIS-SEEKING BEHAVIOR	67
17.15	Carol Gianessi and Jane Taylor	AM404 REDUCES HABITUAL ALCOHOL SEEKING AND DRINKING	68
17.30	Jayme McReynolds, Colten Wolf, Dylan Starck, Cecilia Hillard and John Mantsch	CANNABINOID RECEPTOR 1 INVOLVEMENT IN COCAINE-TAKING AND COCAINE-SEEKING BEHAVIOR FOLLOWING STRESS-INDUCED ESCALATION OF COCAINE INTAKE	69

17.45	Joel Schlosburg, Leandro Vendruscolo, Benjamin Cravatt, Markus Heilig and George Koob	PROBING MECHANISMS LINKING STRESS AND OPIATE DEPENDENCE: GENERATION OF A FAAH KNOCKOUT RAT	70
20.00	**AWARDS CEREMONY AND ICRS BANQUET**		

DEPARTURE: TUESDAY, JUNE 27TH	
8.00 – 10.00	**BREAKFAST**

NOTES:

POSTER SESSION 1: TOPICS A - F
DAY 1, FRIDAY, JUNE 23RD: 13:00 - 15:00

TOPIC A. DRUG DEVELOPMENT

Jakub Mlost, Sumanta Garai, Peter Schaffer, Ganesh A. Thakur, Lesley A. Stevenson, Katarzyna Starowicz and Roger G. Pertwee	*IN VITRO* EVIDENCE THAT GAT558 IS A POSITIVE ALLOSTERIC MODULATOR OF THE HUMAN CB1 RECEPTOR	P1-1
Francesca Gado, Roger G. Pertwee and Clementina Manera	IDENTIFICATION OF A NEW LEAD COMPOUND AS AN ALLOSTERIC MODULATOR OF THE CB2 RECEPTOR	P1-2
Thuy Nguyen, Ann Decker, Thomas Gamage, Jun-Xu Li, Brian F. Thomas, Jenny L. Wiley, Terry Kenakin and Yanan Zhang	DIARYLUREA-BASED ALLOSTERIC MODULATORS OF THE CANNABINOID CB1 RECEPTOR	P1-3
Spyros Nikas, Lipin Ji, Yingpeng Liu, Marsha Eno, Anisha Korde, Shalley Kudalkar, Othman Benchama, Chandrashekhar Honrao, Srikrishnan Mallipeddi, Shu Xu, Nikolai Zvonok, Lawrence Marnett and Alexandros Makriyannis	NOVEL ENDOCANNABINOID PROBES	P1-4
Rangan Maitra, Amruta Manke, Robert Wiethe, George Amato, Rodney Snyder, Vineetha Vasukuttan, Yanan Zhang and Scott Runyon	SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF A DIPHENYL PURINE BASED PERIPHERALLY RESTRICTED CB1 RECEPTOR ANTAGONIST	P1-5
Florian Mohr, Thomas Hurrle, Stefan Braese, Hannah Jauch, Albrecht Keil and Bernd Fiebich	MODULAR SYNTHESIS OF NOVEL CANNABINOID LIGANDS BASED ON THE COUMARIN MOTIF AS CB1, CB2, GPR55 AGONISTS AND ANTAGONISTS	P1-6
C. Randall Clark, Amber Thaxton, Forrest Smith and Jack DeRuiter	STRUCTURE ACTIVITY STUDIES ON REGIOISOMERIC SUBSTITUTED INDOLES	P1-7

Melissa Wilcox, Giulia Mazzoccanti, Omar Ismail, Alessia Ciogli, Claudio Villani and Francesco Gasparrini	CHIRAL/ACHIRAL ANALYSIS OF NATURALLY OCCURRING CANNABINOIDS USING A NEW SUB-2 μM CHIRAL STATIONARY PHASE WITH ULTRA HIGH PERFORMANCE SFC-MS	P1-8
Thomas Gamage, Charlotte Farquhar, Timothy Lefever, Brian Thomas, Bruce Blough and Jenny Wiley	CHARACTERIZATION OF STRUCTURAL ANALOG OF CB1 ALLOSTERIC MODULATOR ZCZ-011 WITH ENHANCED AGONIST ACTIVITY	P1-9
Mohammed Mustafa, Lauren Moncayo, Debra Kendall, Dai Lu and Aron Lichtman	THE CB1 RECEPTOR ALLOSTERIC MODULATOR LDK 1258: *IN VIVO* PHARMACOLOGICAL EVALUATION IN MICE	P1-10
Sarah Dibble, Beatriz Rodriguez, Caleb Vogt, John Gerovac, Jordan Goddard, Huan Huang, Terrence Neumann, Friedhelm Schroeder, Cecilia Hillard and Christopher Cunningham	DISCOVERY OF LEAD INHIBITORS OF STEROL CARRIER PROTEIN-2	P1-11
Jimit Raghav, Spyros Nikas, Shashank Kulkarni, Torbjörn U. C Järbe and Alexandros Makriyannis	*IN VIVO* CHARACTERIZATION OF AM7410, A POTENT ORALLY BIOAVAILABLE CB1R AGONIST	P1-12
TOPIC B. TOXICOLOGY		
Chris Breivogel, Katlyn Nichols, Bonnie Brenseke, Khalil Eldeeb, Allyn Howlett, Sandra Leone-Kabler and Victor Pulgar	INVESTIGATING POTENTIAL CARDIOTOXICITY OF JWH-073	P1-13
Philip Wylie, Mei Wang, Mahmoud A. ElSohly, Ikhlas Khan, Chandrani Gon and Mohamed Radwan	ANALYSIS OF CANNABIS FOR PESTICIDE RESIDUES BY GC/Q-TOF	P1-14
Jonathan Belton, Berhanu Geresu, Steven Knight, Eric Catuccio and Pritesh Kumar	ANALYSIS OF PATIENTS EXPERIENCING TOXIC EFFECTS FROM SYNTHETIC CANNABINOIDS	P1-15

TOPIC C. GPR55

Rodolfo Sánchez-Zavaleta, Francisco Paz and Benjamín Florán	PRESYNAPTIC GPR55 RECEPTOR MODULATES [^{3}H]GABA RELEASE IN THE RAT SUBSTANTIA NIGRA	P1-16
Carina Hasenoehrl, Eva Sturm, Lee Stirling, Martin Pichler, Rufina Schuligoi, Johannes Haybaeck and Rudolf Schicho	G PROTEIN-COUPLED RECEPTOR GPR55 PROMOTES COLORECTAL CANCER	P1-17
Dow Hurst, Pingwei Zhao, Mary Abood and Patricia Reggio	ELUCIDATING GPR55 SPECIES STRUCTURAL DIFFERENCES IN TRANSMEMBRANE HELIX 2	P1-18

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Mauro Maccarrone, Natalia Battista, Alessandra Gambacurta and Monica Bari	POTENTIAL EXPLOITATION OF THE ENDOCANNABINOID SYSTEM TO MODULATE BONE REMODELING ABOARD THE INTERNATIONAL SPACE STATION	P1-20
Vanessa Montoya-Uribe, Kushal Gandhi, Stacy Martinez, Marcel Chuecos, Maira Carrillo, Irám Rodríguez-Sánchez and Natalia Schlabritz-Lutsevich	CHARACTERIZATION OF CB1R ISOFORMS IN FETAL AND MATERNAL TISSUES IN A BABOON (*PAPIO* SPP.) MODEL	P1-21
Toru Uyama, Zahir Hussain, Katsuhisa Kawai, Iffat Ara Sonia Rahman, Kazuhito Tsuboi, Nobukazu Araki and Natsuo Ueda	CHARACTERIZATON OF ISOFORMS OF THE CALCIUM-INDEPENDENT N-ACYLTRANSFERASE PLAAT-1 IN HUMANS AND MICE	P1-22
Nuha Anajirih, Saoirse E O'Sullivan and Steve PH Alexander	ENDOCANNABINOID HYDROLASE ACTIVITIES ARE DIFFERENTIALLY EXPRESSED IN HUMAN BLOOD FRACTIONS	P1-23

Meera Manchanda, Emma Leishman and Heather Bradshaw	INCREASES IN TEMPERATURE AND CAPSAICIN DRIVE THE PRODUCTION OF 2-AG AND RELATED LIPIDS WHILE DECREASING LEVELS OF AEA AND RELATED LIPIDS	P1-24
Ryan Kucera, Joseph Bouskila, Laurent Elkrief, Anders Fink-Jensen, Roberta Palmour, Jean-François Bouchard and Maurice Ptito	DISTRIBUTION AND LOCALIZATION OF CB1R, NAPE-PLD, AND FAAH IN THE NUCLEUS ACCUMBENS CORE AND SHELL OF VERVET MONKEYS	P1-25
Magdalena Kostrzewa, Fabiana Piscitelli, Vincenzo Di Marzo and Katarzyna Starowicz	TRPV1 AND FAAH DUAL BLOCKER MODULATES BONE MASS BY ENDOCANNABINOID/ ENDOVANILLOID INTERACTION	P1-26
Zoltan Hegyi, Klaudia Docs, Krisztina Hollo, Zoltan Meszar and Peter Szucs	INTERACTIONS BETWEEN ENDOCANNABINOID AND PROSTANOID SIGNALING PATHWAYS IN SPINAL ASTROCYTES	P1-27
Jagjeet Mnpotra, Alyssa Laun, Zhao-Hui Song, Allison Griffith, Herbert Seltzman, Dow Hurst and Patricia Reggio	CAN A LIGAND SWITCH CB1 SIGNALING FROM INHIBITORY (Gi) TO STIMULATORY (Gs) G PROTEIN?	P1-28
Israa Isawi, Paula Morales, Alyssa Laun, Dow Hurst, Zhao-Hui Song and Patricia Reggio	STRUCTURAL RELATIONSHIP OF THE CLASS A ORPHAN GPCR, GPR6 WITH THE CANNABINOID CB1 AND CB2 RECEPTORS	P1-29
Nada Mahmood, Yousra Abdul Maqsood, Sadia Shabnam, Andy Bennett and Steve Alexander	*IN VITRO* ESTERASE ASSAYS FOR HUMAN RECOMBINANT MONOACYLGLYCEROL HYDROLASES (MAGL, ABHD6, ABHD12)	P1-30
Jacob Nowatzke and Robert Zoellner	DENSITY FUNCTIONAL THEORY STUDY OF ACYL MIGRATION IN VARIOUS-CHAIN MONOACYLGLYCEROLS	P1-31
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Jiliang Zhang, Shaojuan Zhang, Xiaoxi Ling, Pin Shao, Yinghui Ge and Mingfeng Bai	COMBINED CB2 RECEPTOR AGONIST AND PHOTODYNAMIC THERAPY SYNERGISTICALLY INHIBIT TUMOR GROWTH IN TRIPLE NEGATIVE BREAST CANCER	P1-32

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B. Pflüger, M.S. Leisegang, R.P. Brandes and C. Fork	ANANDAMIDE MODULATES EPIGENETIC REGULATION OF INFLAMMATORY GENES	P1-34
Hansini Vitharanage, Adam Marentes and Nancy E. Buckley	EFFECTS OF Δ^9-THC ON CYTOKINE PRODUCTION FROM SPLENOCYTES DERIVED FROM IMMUNE COMPETENT AND IMMUNOSUPPRESSED MICE INFECTED SYSTEMICALLY WITH *CANDIDA ALBICANS*	P1-35
Nancy Buckley, Adam Marentes, Hansini Vitharanage and Elizabeth Marquez	Δ^9-THC AND *CANDIA ALBICANS* INFECTION IN MICE	P1-36
Elizabeth Marquez and Dr. Nancy E. Buckley	EFFECTS OF THC ON THE SEVERITY OF *CANDIDA ALBICANS* VULVO-VAGINAL INFECTION IN MICE	P1-37
Ashleigh Jones, Kristina Leinwand, Rick Huang, Paul Jedlicka, Soumita Ghosh, Ruin Moaddel, Jan Wehkamp, Maureen Ostaff, Jutta Bader, Carol Aherne, Edward Hoffenberg and Colm Collins	THERAPEUTIC POTENTIAL OF CANNABINOIDS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE	P1-38
Natasha Ryz, Caroline MacCallum and Robert Brooke	CANNABINOID TREATMENT INDUCES REMISSION IN DRUG-RESISTANT PEDIATRIC INFLAMMATORY BOWEL DISEASE: A CASE REPORT	P1-39
Sara Jane Ward, Devon Riggs, Ronald F. Tuma, William A. Kinney, Dean Petkanas and Douglas E. Brenneman	NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF KLS-13019 AND CANNABIDIOL IN *IN VITRO* AND *IN VIVO* MODELS OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN	P1-40
Makenzie Fulmer and Douglas Thewke	TYPE-2 CANNABINOID RECEPTOR DEFICIENCY ALTERS ATHEROSCLEROTIC PLAQUE CALCIFICATION IN HYPERLIPIDEMIC LDLR-NULL MICE	P1-41

Omayma Alshaarawy	TOTAL AND DIFFERENTIAL LEUKOCYTE COUNTS AMONG CANNABIS USERS THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY, 2005-2014	P1-42
Ephraim Brener, Adi Zuloff-Shani and Elran Haber	A NOVEL METHOD FOR POTENTIATING THE ANTIBIOTICS WITH CANNABINOID-BASED FORMULATIONS	P1-43
Daniel Couch, Jon Lund and Saoirse O'Sullivan	CANNABIDIOL AND PALMITOYLETHANOLAMIDE SHARE SIMILAR INTRACELLUAR PATHWAYS IN PREVENTING INCREASED PERMEABILITY OF INFLAMED CACO-2 MEMBRANES	P1-44

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Jenny Wiley and Timothy Lefever	OF MICE AND RATS: BEST LAID SCHEMES (TO STUDY SEX DIFFERENCES) OFTEN GO AWRY	P1-46
Erin Rock, Guillermo Moreno-Sanz, Cheryl Limebeer, Gavin Petrie, Roberto Angelini, Daniele Piomelli and Linda Parker	EFFECT OF THE FATTY ACID AMIDE HYDROLASE INHIBITOR URB937 ON RAT MODELS OF NAUSEA	P1-47
Cheryl L. Limebeer, Erin M. Rock and Linda A. Parker	NAUSEA-INDUCED 5-HT RELEASE IN THE INTEROCEPTIVE INSULAR CORTEX IS REVERSED BY SYSTEMIC MAGL INHIBITION	P1-48
Floris Luchtenburg, Marcel Schaaf and Michael Richardson	FUNCTIONAL CHARACTERIZATION OF THE CANNABINOID RECEPTOR 1 IN ZEBRAFISH LARVAE USING BEHAVIORAL READOUTS	P1-49
Thomas Hurrle, Florian Mohr, Daniel Marcato, Anjana Hariharan, Uwe Straehle, Ravindra Peravali and Stefan Bräse	NOVEL CANNABINOIDS BASED ON THE COUMARIN MOTIF AND FAST EVALUATION THROUGH PHOTOMOTOR RESPONSE STUDY ON EMBRIONIC ZEBRAFISH	P1-50

POSTER SESSION 2: TOPICS G - L
DAY 2, SATURDAY, JUNE 24TH: 13:00 - 15:00

TOPIC G. PAIN

Alex Straiker, Sally Miller, Shashank Kulkarni, Spyros Nikas, Ken Mackie and Alex Makriyannis	CONTROLLED DEACTIVATION CB1 RECEPTOR LIGANDS AS A NOVEL STRATEGY TO LOWER INTRAOCULAR PRESSURE	P2-1
Anna-Maria Szczesniak, Elizabeth Cairns, Joanna Borowska-Fielding, Melanie Kelly and Alex Straiker	HARNESSING ENDOCANNABINOIDS FOR RETINAL NEUROPROTECTION	P2-2
Pranjal Taskar, Eman Ashour, Waseem Gul, Mahmoud ElSohly and Soumyajit Majumdar	INTRAOCULAR PRESSURE LOWERING EFFICACY OF A Δ^9-TETRAHYDROCANNABINOL PRODRUG, NB1111, IN A NORMOTENSIVE RABBIT MODEL	P2-3
Dinesh Thapa and Melanie Kelly	PHYTOCANNABINOIDS, TETRAHYDROCANNABINOL (Δ^9THC) AND CANNABIDIOL (CBD), REDUCE COLD-INDUCED OCULAR PAIN IN A MOUSE MODEL OF CORNEAL HYPERALGESIA	P2-4
Stevie Britch, Jenny Wiley and Rebecca Craft	CHRONIC VERSUS ACUTE Δ^9-TETRAHYDROCANNABINOL TREATMENT OF INFLAMMATORY PAIN IN MALE VERSUS FEMALE RATS	P2-5
Hannah Gogulski, Jenny Wiley and Rebecca Craft	CANNABINOID ANTINOCICEPTION AGAINST PACLITAXEL-INDUCED NEUROPATHIC PAIN	P2-6

Sara Nass and Steven Kinsey	TARGETING THE ENDOCANNABINOID AND GLUCOCORTICOID SYSTEM TO ATTENUATE INFLAMMATORY ARTHRITIS	P2-7
Xiaohong Chen, Saadet Inan, Scott Rawls, Alan Cowan, Ronald Tallarida, Christopher Tallarida, Joseph Meissler, Toby Eisenstein and Martin Adler	DIFFERENTIAL EFFECTS OF CANNABINOID/MORPHINE COMBINATIONS IN TWO RODENT PAIN ASSAYS	P2-8
Rebecca LaFleur, Angela Henderson-Redmond and Daniel Morgan	SEX DIFFERENCES IN CANNABINOID SENSITIVITY AND THE DEVELOPMENT OF TOLERANCE IN A MOUSE MODEL OF INFLAMMATORY PAIN	P2-9
Jessica C Gaspar, Bright Okine, Alvaro Llorente-Berzal, Orla Burke, David Dinneen, Michelle Roche and David P Finn	THE EFFECTS OF PHARMACOLOGICAL BLOCKADE OF PPARs ON FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR, FEAR-CONDITIONED ANALGESIA AND CONDITIONED FEAR IN THE PRESENCE OF NOCICEPTIVE TONE IN RATS	P2-10
Lawrence Carey, Tannia Gutierrez, Liting Deng, Wan-Hung Lee, Ken Mackie and Andrea Hohmann	THE DEVELOPMENT AND MAINTENANCE OF INFLAMMATORY AND NEUROPATHIC PAIN IS PRESERVED IN GPR55 KNOCKOUT MICE	P2-11
Henry Blanton, Kelsey Donckels, Jennifer Lilley, Isabel Castro, Kevin Pruitt and Josee Guindon	CANNABINOID AGONISTS (CP55,940, ACEA AND AM1241) FOLLOWING CHRONIC ADMINISTRATION CAUSE CHANGES IN THE ESTRUS CYCLE IN AN OPTIMIZED CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN MODEL	P2-12
Iryna A Khasabova, Amy H Kim, Kalpna Gupta, Virginia S Seybold and Don A Simone	THE ROLE OF 2-AG OXIDATION IN MECHANICAL HYPERALGESIA IN A HUMANIZED MODEL OF SICKLE CELL DISEASE	P2-13
Divya Ramesh, Amy D'Agata, Leena Kader, Erin Young and Angela Starkweather	CONTRIBUTION OF *FAAH* GENOTYPE TO LOW BACK PAIN SENSITIVITY AND PAIN BURDEN	P2-14

TOPIC H. Feeding, Obesity and Metabolism

Kushal Gandhi, Cun Li, Marcel Chuecos, Maira Carrillo, Stacy Martinez, Charles Burns, Moss Hampton, Peter Nathanielsz and Natalia Schlabritz-Lutsevich	MATERNAL AND FETAL HEPATIC ENDOGENOUS CANNABINOIDS RESPONSE TO MATERNAL HIGH FAT DIET	P2-15
Shahar Azar, Shiran Udi, Adi Drori, Rivka Hadar, Kiran V Vemuri, Alexandros Makriyannis, Xiaoling Li, Jeffrey M Peters and Joseph Tam	REVERSAL OF FATTY LIVER BY PERIPHERAL CB1 RECEPTOR BLOCKADE IS SIRT1/PPARα DEPENDENT	P2-16
Georgia Balsevich, Martin A Sticht, Arashdeep Singh, Prasanth K Chelikani, Bruce S McEwen, Cecilia J Hillard, Francis S Lee, Stephanie, L Borgland and Matthew N Hill	A ROLE FOR FATTY ACID AMIDE HYDROLASE IN THE LEPTIN-MEDIATED EFFECTS ON FEEDING AND ENERGY BALANCE IN FASTED MICE	P2-17

TOPIC I. Stress, Memory and Affect

Elizabeth M. Doncheck, Jayme R. McReynolds, Evan N. Graf, Oliver Vranjkovic, Margot C. DeBaker, Laura Barron, Gage T. Liddiard, Luke A. Urbanik, Qing-song Liu, Cecilia J. Hillard and John R. Mantsch	THE PRELIMBIC CORTICAL ENDOCANNABINOID SYSTEM MEDIATES STRESS-ENHANCED COCAINE-SEEKING RELAPSE VULNERABILITY IN BOTH SEXES	P2-18
Robert Aukema, Tiffany Lee, Maria Morena, J. Megan Gray, Boris Gorzalka and Matthew Hill	RECRUITMENT OF 2-AG SIGNALING IN THE BASOLATERAL AMYGDALA MAY RELATE TO THE ONTOGENY OF STRESS HABITUATION	P2-19
Jonathan Simone, Jacqueline Leerentveld, Jennet Baumbach and Cheryl McCormick	INDEPENDENT EFFECTS OF REPEATED STRESS AND AM251 TREATMENT IN ADOLESCENCE ON ANXIETY, SOCIALITY, AND NEUROENDOCRINE STRESS RESPONSES, AND ON RELEVANT PROTEIN EXPRESSION IN THE PREFRONTAL CORTEX AND HIPPOCAMPUS, IN FEMALE RATS	P2-20

Angela Henricks, Anthony Berger, Janelle Lugo, Lydia Baxter-Potter, Kennedy Bieniasz, Rebecca Craft, Matthew Hill and Ryan McLaughlin	SEX DIFFERENCES IN ALCOHOL WITHDRAWAL-INDUCED NEGATIVE AFFECT AND CORTICOAMYGDALAR ENDOCANNABINOIDS	P2-21
Danilo De Gregorio Ryan McLaughlin, Rafael Ochoa-Sanchez, Luca Posa and Gabriella Gobbi	TARGETING THE THERAPEUTIC PROPERTIES OF CANNABIDIOL: FOCUS ON DEPRESSION AND PAIN	P2-22
Marieka DeVuono, Kiri Wills, Danielle MacPherson, Kelly Hrelja, Cheryl Limebeer and Linda Parker	EFFECTS OF STRESS ON PLACE CONDITIONING PRODUCED BY Δ^9-TETRAHYDROCANNABINOL IN SPRAGUE DAWLEY RATS	P2-23
Kiri Wills, Marieka Devuono, Cheryl Limebeer, Kiran Vemuri, Alexandros Makriyannis and Linda Parker	CB1 RECEPTOR ANTAGONISM IN THE BED NUCLEUS OF THE STRIA TERMINALIS INTERFERES WITH AN ACUTE NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL-INDUCED PLACE AVERSION	P2-24
Gavin Petrie, Kiri Willis, Cheryl Limebeer, Madeleine Shepard-Perkins, Gabiana Piscitelli, M Imad Damaj, Gielia Donvito, Aron H Lichtman, Vincenzo DiMarzo, Raphael Mechoulam and Linda Parker	REGULATION OF THE ESTABLISHMENT OF AN ACUTE MORPHINE WITHDRAWAL CONDITIONED PLACE AVERSION, BUT NOT MORPHINE PLACE PREFERENCE, BY OLEOYL GLYCINE	P2-25
Kristen Trexler and Steven Kinsey	Δ^9-THC WITHDRAWAL ACTIVATES THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AND ALTERS SOCIAL BEHAVIOR	P2-26
Christian Cayer, Rui Liu, Daria Kolmogrova, Kirtana Thirumal, Pamela Kent, Victor Cal, Zul Merali, John Arnason and Cory Harris	THE 'SACRED MAYA INCENSE,' *PROTIUM COPAL* (BURSERACEAE), ELICITS ANXIOLYTIC EFFECTS IN ANIMAL MODELS	P2-27
Lesley D. Schurman, Moriah C. Carper, Daisuke Ogasawara, Benjamin F. Cravatt and Aron H. Lichtman	CONSEQUENCES OF DAGL-α DISRUPTION ON SPATIAL LEARNING AND MEMORY PROCESSES IN C57BL6/J MICE	P2-28
TOPIC J. PSYCHIATRY		
A Boucher, D Lloyd, M Matsumoto, J Arnold, C Weickert and T Karl	IS *NEUREGULIN 1* A STRONG CANDIDATE FOR GENE-CANNABIS INTERACTIONS IN SCHIZOPHRENIA	P2-29

Veronika Borisov, Odelia Matz, Ester Fride and Sharon Anavi-Goffer	ALTERATIONS IN CB1 RECEPTOR AND IL-6 EXPRESSION IN THE PHENCYCLIDINE MOUSE MODEL OF SCHIZOPHRENIA	P2-30
Ashleigh Lauren Osborne, Nadia Solowij, Ilijana Babic, Xu-Feng Huang and Katrina Weston-Green	CANNABIDIOL IMPROVES BEHAVIOURAL AND GLUTAMATERGIC DEFICITS IN A PRENATAL INFECTION MODEL OF SCHIZOPHRENIA	P2-31

TOPIC K. Cannabis Use and Abuse

Mallory Loflin, Ryan Vandrey, Heather Jackson, John Matuszewski, Travis Hyke and Marcel Bonn-Miller	DAILY USE OF CANNABIDIOL (CBD) EXTRACTS MAY LEAD TO POSITIVE Δ^9-TETRAHYDROCANNABINOL (THC) DRUG SCREENS	P2-32
David Gorelick, Emeline Chauchard, Kenneth Levin, Marc Copersino and Stephen Heishman	CANNABIS-RELATED PROBLEMS IN ADULT, NON-TREATMENT-SEEKING RECREATIONAL CANNABIS USERS	P2-33
Alexander Spradlin, Dakota Mauzay and Carrie Cuttler	SYMPTOMS OF OBSESSIVE-COMPULSIVE DISORDER PREDICT CANNABIS MISUSE	P2-34
Alexander Spradlin and Carrie Cuttler	THE ROLES OF SEX, COPING MOTIVES, AND NEGATIVE AFFECT IN THE RELATIONSHIP BETWEEN STRESS AND CANNABIS USE	P2-35
Jonathan Martin, Stephanie Porter, Robert Roscow, Sean Conrad and Brian Reid	MARIJUANA STRAIN CHEMOTYPES ELICIT DISTINCT SUBJECTIVE FEELINGS	P2-36
Caitlin Clark and Kauyumari Sanchez	CANNABIS USE FREQUENCY AND MOOD ON CREATIVITY	P2-37
Jun Panee, Mariana Gerschenson and Linda Chang	ASSOCIATIONS BETWEEN MICROBIOTA, MITOCHONDRIAL FUNCTION, AND COGNITION IN CHRONIC MARIJUANA USERS	P2-38
Jill M. Robinson and Marvin Krank	IMPLICIT CANNABIS COGNITIONS AND EARLY USE AMONG CANADIAN ADOLESCENTS	P2-39

Kim Crosby, Michelle S. Thiessen and Zach Walsh	SUBSTITUTING CANNABIS FOR ALCOHOL: CONTEXTUAL FACTORS AND REASONS FOR PREFERENCE	P2-40
Michelle Thiessen, Zach Walsh, Kim Crosby, Ethan Russo, Tapoja Chaudhuri and Sunil Aggarwal	CANNABIS USE IN INDIA: CHARACTERISTICS, ACCESS, AND REASONS FOR USE	P2-41
TOPIC L. EPILEPSY AND NEUROLOGICAL		
Gil M Lewitus, Paula Berman, Kate Futuran, Ohad Guberman, Rony Chanoch and David Meiri	IDENTIFYING HIGH CBD CANNABIS STRAINS FOR EPILEPSY TREATMENT	P2-42
Fabricio Alano Pamplona and Ana Carolina Coan	BETTER THERAPEUTIC PROFILE OF CBD-ENRICHED EXTRACTS OVER PURIFIED CBD IN TREATMENT-RESISTANT EPILEPSY: OBSERVATIONAL DATA META-ANALYSIS	P2-43
Andre Yugo Osava Marques de Carvalho and Fabrício Alano Pamplona	HOW SAFE ARE THC AND CBD FOR EPILEPSY? INSIGHTS FROM A PRECLINICAL SYSTEMATIC REVIEW	P2-44
Anastasia Suraev, David Allsop, Jordyn Stuart, Elisha Richards, Nicholas Lintzeris and Iain McGregor	THE PELICAN STUDY: THE PERCEIVED EFFICACY AND CANNABINOID CONTENT OF ARTISANAL CANNABIS OILS USED TO TREAT CHILDHOOD EPILEPSY IN THE AUSTRALIAN COMMUNITY	P2-45
Jessica Cao, Katie Viray, Larry Zweifel and Nephi Stella	CHRONIC, BUT NOT ACUTE, INHIBITION OF ABHD6 RESCUES SELECT BEHAVIORAL SYMPTOMS IN THE HdhQ200/200 MOUSE MODEL OF HUNTINGTON'S DISEASE	P2-46
Douglas R. Smith, Christine Stanley, Theodore Foss and Kevin McKernan	RARE GENETIC VARIANTS IN ECS GENES ARE ASSOCIATED WITH NEUROLOGICAL PHENOTYPES	P2-47
Ronald F. Tuma, Soroush Assari, Dianne Langford, Kurosh Darvish and Sara Jane Ward	THE SELECTIVE CB2 AGONIST O-1966 ATTENUATES DAMAGE FOLLOWING TBI INDUCED BY BLAST INJURY	P2-48
Aurélie Stil, Lucas Paladines, Pei-Yun Tu, Jonathan Simard and Jean-François Bouchard	THE ACTIVITY OF CANNABINOID RECEPTORS MODULATES SYNAPTOGENESIS	P2-49
Ana Lago-Fernández, Laura Figuerola-Asencio, José Cumella and Nadine Jagerovic	IMPROVED SYNTHETIC APPROACH OF PM226, A SELECTIVE CB2 CANNABINOID AGONIST WITH A NEUROPROTECTIVE PROFILE	P2-50

POSTER SESSION 3: TOPICS M - Q
DAY 4, MONDAY, JUNE 26TH: 13:00 - 15:00

TOPIC M. PEPTIDE AND LIPID-DERIVED CANNABINOID LIGANDS

Allison Zarkin, Herbert Seltzman, Linda Console-Bram, Luciana Leo, Mary Abood, Dow Hurst and Patricia Reggio	NON-STEROIDAL PREGNENOLONE ANALOGS AS SIGNAL SPECIFIC ALLOSTERIC MODULATORS OF THE CB1 RECEPTOR	P3-1
Bitya Raphael, Natalya Kogan, Malka Attar-Namdar, Mukesh Chourasia, Avital Shurki, Roger G. Pertwee, Maria G. Cascio, Andreas Zimmer, Itai Bab and Yankel Gabet	THE ENDOGENOUS HORMONE H4(99-103) IS THE ONLY KNOWN PEPTIDE THAT SIGNALS VIA CANNABINOID RECEPTOR CB2	P3-2

TOPIC N. ADDICTION AND REWARD

Sherry Shu-Jung Hu, Heng-Ai Chang, Wen Dai and Mei-Ling Lai	SEX DIFFERENCE IN THE EFFECT OF RIMONABANT ON COCAINE MEMORY IN MICE	P3-3
Lindsey Friend, Jared Weed, Philip Sandoval and Jeffrey Edwards	CB1-DEPENDENT LTD IN VENTRAL TEGMENTAL AREA GABA NEURONS: A NOVEL TARGET FOR MARIJUANA	P3-4
Jacques Nguyen, Yanabel Grant, Kevin Creehan and Michael Taffe	Δ^9-TETRAHYDROCANNABINOL VAPOR INHALATION ATTENUATES OXYCODONE INTRAVENOUS SELF-ADMINISTRATION UNDER EXTENDED ACCESS CONDITIONS	P3-5

TOPIC O. PHYTOCANNABINOIDS

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Young Investigator Award Presentation

Friday, June 23, 2017
15:00 – 15:30

CARDIOVASCULAR EFFECTS OF CANNABIDIOL

Saoirse O'Sullivan, Ph.D.

Associate Professor, Faculty of Medicine and Health Sciences
University of Nottingham
United Kingdom

Cannabidiol (CBD) is a non-psychoactive phytocannabinoid already on the market as part of a licensed treatment in multiple sclerosis (Sativex® GW Pharmaceuticals, Cambridge, UK). CBD alone (Epidiolex®, GW Pharmaceuticals, Cambridge, UK) is also in clinical trials in children with intractable epilepsies and has orphan designation status in the US in neonatal hypoxia-ischaemic encephalopathy. CBD is the focus of much research because of its potential in a number of other therapeutic areas due to its anti-inflammatory, anti-convulsant, anti-oxidant, anxiolytic, anti-nausea, anti-tumoural and anti-psychotic properties. A number of preclinical studies have also shown beneficial effects of CBD in a range of disorders of the cardiovascular system (Stanley et al., 2013a), which has been the focus of my research for the last 10 years. We have shown that CBD causes both acute and time dependent vasorelaxation of rat and human arteries as measured ex vivo, and can improve endothelial function and vasodilator responses in a rat model of type 2 diabetes both ex vivo and in vivo. In healthy volunteers, we have shown that a single dose of CBD decreases resting blood pressure and the blood pressure response to stress. One cardiovascular disorder we are particularly interested in is stroke and showed through a meta-analysis that CBD significantly improves infarct size in animal models of stroke. In a cell culture model of the blood brain barrier (BBB), CBD protects against increased permeability induced by ischaemia-reperfusion damage, which is at least one of the facet by which CBD may be protective in stroke. Collectively, these data suggest that CBD is a compound of interest in the cardiovascular system and in cardiovascular disorders, which needs to be tested in relevant patient groups.

PRESIDENTIAL PLENARY SPEAKER

FRIDAY, JUNE 23, 2017
18:00 – 19:00

ACCOMPLICES TO MURDER: ENDOCANNABINOIDS DIRECT MICROGLIA TO KILL NEWBORN NEURONS

Margaret M. McCarthy,
Jonathon W. Van Ryzin and Kathryn J. Argue

Department of Pharmacology and Program in Neuroscience
University of Maryland School of Medicine, Baltimore MD
United States

Microglia are neither "micro" nor "glia" but instead innate immune cells ubiquitously but exclusively distributed throughout the brain. They both respond to and produce endocannabinoids as well as a myriad of other signaling molecules. Previously thought to serve as quiescent sentinels activated only in response to injury or inflammation, microglia are now known to be active surveyors of the healthy brain and to sculpt neural circuits both by pruning synapses and controlling cell number.

Sex differences in the brain are established early in life by a variety of steroid-induced cellular processes. Among these are differential cell death and cell genesis, both of which can impact the size and/or cellular composition of a particular brain region or nucleus. We previous identified a sex difference in cell genesis in the developing amygdala in which females show higher rates of glial and neurogenesis (Kraft-Krebs et al., PNAS 2010). The lower rates of cell genesis in the amygdala of males was attributed to a higher endocannabinoid tone compared to females, and was positively correlated with later social play behavior among juveniles, which is more frequent between males.

In an effort to understand the mechanistic origin of the sex difference in amygdala cell genesis we explored the effect of endocannabinoid receptor activation on microglia. Treatment of newborn females with CB1 and CB2 agonists ACEA and GP1a, either alone or combined, reduced the number of recently born cells in the amygdala to the level normally seen in males as determined by BrdU incorporation. Visualization of the microglia in the amygdala of the same animals revealed a significant increase in phagocytic activity and a strong negative correlation between phagocytic microglia and newborn cells. Microglia serve an important role in cleaning up cellular debris following necrotic or apoptotic cell death but can also engulf and destroy living cells, a process referred to as phagoptosis. We have now determined that the higher endocannabinoid tone in the neonatal male amygdala stimulates phagoptosis by microglia which is particularly directed towards recently born cells, thereby reducing neurogenesis. Both the mechanism and the phenotypic fate of the surviving cells in females remain active topics of investigation.

Supported by NIH R01DA039062 to MMM

Presidential Plenary Speaker

Saturday, June 24, 2017
16:00 – 17:00

Sex-Dependent Synaptic Modulation in the Hippocampus

Catherine Woolley, Ph.D.

Professor
William Deering Chair of Biological Sciences
Department of Neurobiology
Northwestern University
United States

Sex differences in the brain are misunderstood. Although commonly construed to indicate a biological basis for differences in the behavior of males and females, many sex differences evident at a mechanistic level may instead converge to similar functional outcomes in each sex. The significance of these sex differences lies in recognition that interventions that target specific molecular pathways in the brain, such as drugs, may have distinct downstream consequences for males and females.

We have investigated this idea in studies of neurosteroid estrogen modulation of synapses in the hippocampus. The hippocampus of both sexes can synthesize the key estrogen, 17β-estradiol, and 17β-estradiol acutely modulates hippocampal synapses in both sexes. We have recently discovered that seizures stimulate neurosteroid estrogen synthesis in the hippocampus of both sexes and that neurosteroid estrogens promote seizure activity similarly in both sexes. The mechanisms by which neurosteroid estrogens promote seizures differ between the sexes, however, including sex-specific endocannabinoid-mediated suppression of inhibition that occurs in females but not in males. In contrast, males show a different form of estrogen-induced suppression of inhibition that is observed only rarely in females. These and other sex differences in mechanisms of synaptic modulation point to the importance of considering sex in the translation of basic findings to the development of therapeutics that target specific molecular systems.

Kang Tsou Memorial Speaker

Sunday, June 25, 2017
11:00 – 12:00

The Role of Genetic Variation in the Endocannabinoid System in Adolescent Brain Development

Francis Lee, M.D., Ph.D.

Professor / Vice Chair for Research, Department of Psychiatry
Professor, Department of Pharmacology
Attending Psychiatrist,
New York Presbyterian Hospital and Weill Cornell Medicine
United States

During adolescence, both rodent and human studies have revealed dynamic changes in the developmental trajectories of corticolimbic structures, which are known to contribute to the regulation of fear and anxiety-related behaviors. The endocannabinoid (eCB) system critically regulates stress responsivity and anxiety throughout the lifespan. Emerging evidence suggests that during adolescence, changes in eCB signaling contribute to the maturation of local and corticolimbic circuit populations of neurons, such as mediating the balance between excitatory and inhibitory neurotransmission within the prefrontal cortex. This function of the eCB system facilitates efficient communication within and between brain regions and serves a central role in establishing complex and adaptive cognitive and behavioral processing. Although these peri-adolescent changes in eCB signaling promote brain development and plasticity, they also render this period a particularly sensitive one for environmental perturbations to these normative fluctuations in eCB signaling, such as stress, potentially leading to altered developmental trajectories of neural circuits governing emotional behaviors. In this talk, I will focus on the role of eCB signaling on the regulation of stress and anxiety-related behaviors both during and after adolescence. Moreover, I will discuss the functional implications of human genetic variation in the eCB system for the risk for anxiety and consequences of stress across development and into adulthood.

ICRS Lifetime Achievement Award

Monday, June 26, 2017
10:30 – 11:00

Cannabinoid / Serotonin Interactions in the Regulation of Nausea

Linda Parker, Ph.D.

Canada Research Chair in Behavioural Neuroscience
Departments of Psychology and Neuroscience
University of Guelph, Guelph, Ontario, Canada

One of the first recognized medical uses of Δ^9-tetrahydrocannabinol was treatment of chemotherapy-induced nausea and vomiting. Although vomiting is well controlled with the currently available non-cannabinoid anti-emetics, nausea continues to be a distressing side effect of chemotherapy and other disorders. Considerable recent evidence indicates that cannabinoids and manipulations that enhance the functioning of the natural endocannabinoid system are promising treatments for nausea.

Although the neurobiology of vomiting is known to be regulated by brainstem regions, the neurobiology of nausea is not well understood because of the lack of selective preclinical animal models to study it. The rat conditioned gaping model was developed as such a selective preclinical model of nausea, because only nausea-inducing treatments produce conditioned gaping and anti-nausea treatments prevent conditioned gaping in this species that is incapable of vomiting.

Using the rat gaping model, we have identified the interoceptive insular cortex as a central site of action of the anti-nausea effects of endocannabinoid manipulations. At this site, recent evidence from our group suggests that elevation of 2-arachidonyl glycerol (2-AG) by inhibition of monoaceyl glycerol lipase (MAGL) reduces nausea by preventing the nausea-induced elevation of serotonin (5-HT). That is, nausea-inducing treatments elevate 5-HT and MAGL inhibition reduces that elevation of 5-HT in the interoceptive insular cortex to reduce nausea. Understanding the neural mechanisms regulating nausea may result in the development of better treatments to control this distressing disorder.

This research was funded by research grants from the Natural Sciences and Engineering Research Council (NSERC 92157) and the Canadian Institutes of Health Research (CIHR 137122).

DIACYLGLYCEROL LIPASE BETA KNOCKOUT MICE DISPLAY PROTECTION FROM EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Jenny L. Wilkerson[1], Benjamin F. Cravatt[2] and Aron H. Lichtman[1]

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[2] The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA

Multiple sclerosis (MS) is a debilitating neurodegenerative disease characterized by sensory and motor impairment. Additionally, a reported 50-80% of MS patients report pathological pain symptoms during the course of the disease. Immunotherapy targeting T cells, such as Fingolimod (FTY720) and other immune cell mediators represent the most common forms of treatment for MS. Previous studies have shown that diacylglycerol lipase beta (DAGL-β) is present on such immune cells, and control the production of 2-arachidonyl glycerol. As inhibition of DAGL-β leads to a reduction of prostaglandins and other pro-inflammatory signaling molecules, we tested whether DAGL-β (-/-) mice would display a protective phenotype in the experimental autoimmune encephalomyelitis (EAE) model of MS. The use of myelin oligodendrocyte glycoprotein (MOG) to elicit a form of relapsing/remitting EAE is well characterized to produce motor and sensory deficits modeling MS, including tail, hind and fore limb paralysis, allodynia and thermal hyperalgesia. Therefore, we assessed whether DAGL-β (-/-) mice would develop the clinical symptoms, allodynia, and thermal hyperalgesia associated with EAE compared to their wildtype counterparts.

In an initial study, female C57 mice were characterized in their responses to MOG, and displayed reliable allodynia and thermal hyperalgesia beginning at approximately day 7 post MOG injection. Clinical scoring consisted of the following: 0, no symptom expression; 1, tail paralysis; 2, impairment of the righting reflex; 3, hindlimb paresis 4, hindlimb paralysis 5, partial forelimb paralysis; and euthanasia due to disease progression. Clinical signs emerged beginning on day 14 post injection, and these deficits as well as allodynia and thermal hyperalgesia persisted through the 30 day experimental period. Once daily intraperitoneal administration of the S1P receptor FTY720, given from the time of MOG immunization, through day 30 significantly reduced all the above symptoms. Strikingly, female DAGL-β (-/-) mice treated with MOG did not develop thermal hyperalgesia, allodynia or clinical scoring consistent to EAE expression at any time during the tested 30 days post MOG immunization. In contrast, their wild type littermates treated with MOG developed similar thermal hyperalgesia, allodynia and clinical scores to the C57 MOG treated female mice. These findings suggest that DAGL-β represents an attractive potential target for the treatment of MS, with the promise of additionally reducing MS associated pain.

Acknowledgements: Research was supported by NIH grants: DA009789, DA017259, DA038493

NEUROPROTECTION VIA CANNABINOID RECEPTOR 2 ACTIVATION AS MEANS TO PREVENT CNS INJURY-INDUCED IMMUNODEFICIENCY SYNDROME IN MICE

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One of the most important outcome-limiting medical risks after acute CNS injury, such as stroke or traumatic brain injury, is an increased susceptibility to infections. This dysregulation of the immune system has been termed CNS injury-induced immunodeficiency syndrome (CIDS). The underlying mechanisms that are responsible for CIDS are still not elucidated but are hypothesized to be promoted by the injured brain. The endocannabinoid system (ECS) is responsible for key homeostatic functions in both the CNS and immune system. It is suggested that local upregulation of the ECS occurs following CNS injury and represents an adaptive mechanism to limit neuroinflammation. However, CB2R expression on immune cells is immunosuppressive, suggesting that the activation of CB2R contributes to peripheral immunosuppression in CIDS. The present study investigated whether CB2R agonist pre-treatment reduces CIDS by initial reduction of CNS damage.

CNS injury was induced in C57Bl/6 mice (male, 6-8 weeks) via an intracerebral injection of the vasoconstrictor peptide, endothelin-1 (ET-1, 2µg/µl). Animals were given the CB2R agonist, HU-308 (2.5 mg/kg, i.v), before the induction of CNS injury. The immune response to endotoxin challenge was studied 24 hours later by intravital microscopy to assess leukocyte recruitment in the peripheral microcirculation (gut), a key parameter of leukocyte activation. Brain tissue was extracted and stained with triphenyl tetrazolium chloride (TTC) to evaluate the infarct volume.

Consistent with the induction of CIDS, intravital microscopy confirmed that endotoxin-challenged animals with CNS injury have a reduced number of adhering leukocytes within the intestinal microcirculation when compared to animals without CNS injury and endotoxin administration. Metabolic stain of the brain revealed that early HU-308 administration protected the brain by reducing the ischemic volume following intracerebral ET-1 injection. Intravital microscopy revealed that animals with CB2R pre-treatment showed improved immune activity compared to animals without CB2R pre-treatment.

The findings in our study suggest that early CB2R activation prevents CIDS in acute CNS injury by reducing the initial damage to the brain. Further studies should focus on investigating various time points throughout the onset of CIDS to identify the optimal treatment window for CB2R modulation therapies proposed in this and previous work by our group.

Acknowledgements: Funded by CDHA, Faculty of Medicine Bridge Funding, Canadian Foundation for Innovation, Heart & Stroke Foundation of Canada.

INVESTIGATING NOVEL SELECTIVE CANNABINOID 2 RECEPTOR AGONISTS AS POTENTIAL THERAPEUTIC DRUGS FOR THE TREATMENT OF UVEITIS

Porter, R.[1], Szczesniak, A.M.[1], Toguri, T.[1], Gebremeskel, S.[2], Johnston, B.[2], Lehmann, Ch.[1,2], Grether, U.[3], Ullmer, C.[3] and Kelly, M.E.M.[1]

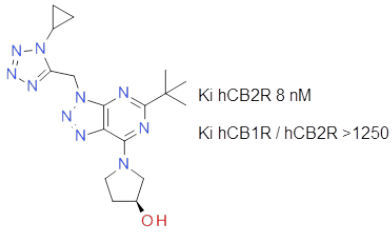
Departments of Pharmacology[1], Microbiology & Immunology[2], Dalhousie University, Halifax, Canada. Roche Innovation Center Basel[3], F. Hoffman-La Roche Ltd., 4070 Basel, Switzerland

Background and rationale: Uveitis is a heterogeneous group of ocular inflammatory diseases. Mainstay drugs used to treat uveitis, such as steroids, have many adverse effects. Identification of new non-steroidal drug targets is desirable. Activation of the cannabinoid 2 receptor (CB_2R) can decrease ocular inflammation. Therefore, drugs selectively targeting CB_2R could represent novel therapeutics for uveitis. The objective of this study was to examine the anti-inflammatory actions of the new highly potent and selective CB_2R agonists, RO6871304 and RO6871085, originating from two chemically diverse series and a novel structurally related CB_2R inverse agonist in a model of experimental endotoxin-induced uveitis (EIU).

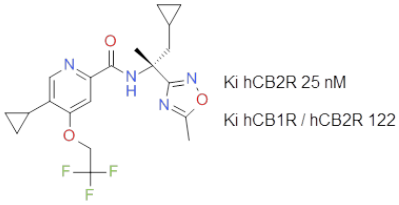
Methods: EIU was induced in mice by intravitreal injection of lipopolysaccharide (LPS). Real-time intravital microscopy was used to visualize and quantify leukocyte-endothelial interactions in the iridial microvasculature as a measure of inflammation. An *in vitro* Boyden chamber bioassay was used to determine whether the novel CB_2R agonists modulated neutrophil migration. To further examine the immune cell subtype targeted by these novel CB_2R selective agonists, neutrophils were depleted prior to induction of EIU. Leukocytes were adoptively transferred 5 hr post EIU.

Results: Topical treatment with the CB_2R agonists, RO6871304 and RO6871085 (1.5% w/v), significantly decreased LPS-induced leukocyte-endothelial adhesion compared to vehicle ($p<0.05$). Conversely, treatment with the novel CB_2R inverse agonist – Ki hCB_2R 26 nM; Ki hCB_1R / Ki hCB_2R 250 – increased LPS-induced leukocyte-endothelial adhesion ($p<0.05$). Consistent with *in vivo* inhibition of leukocyte adhesion, RO6871304 (0.01 μm) significantly decreased neutrophil migration *in vitro* compared to vehicle ($p<0.05$). Topical treatment with RO6871304 in neutrophil-depleted mice significantly decreased the LPS-induced adhesion of adoptively-transferred leukocytes compared to vehicle ($p<0.05$).

Conclusion: Treatment with the novel CB_2R agonists, RO6871304 and RO6871085, was associated with decreased inflammation in EIU and reduced neutrophil migration *in vitro* (RO6871304). Conversely, the novel CB_2R inverse agonist increased inflammation. Adoptive leukocyte transfer experiments suggest that, in addition to neutrophils, CB_2R agonists may also exert their anti-inflammatory actions through resident immune cells, such as microglia or macrophages. Taken together, these data demonstrate an anti-inflammatory role for CB_2R in the eye and suggest that drugs targeting the endocannabinoid system maybe a therapeutic target for ocular inflammation.



RO6871304



RO6871085

A ROLE FOR CANNABINOID CB2 RECEPTORS IN CORNEAL WOUND HEALING

Natalia Murataeva[1], Sally Miller[1], Cecilia Hillard[2], Julian Romero[3] and Alex Straiker[1]

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We have recently shown that cannabinoid CB_1 receptors mediate chemotaxis in corneal epithelial cells, implicating CB_1-mediated chemotaxis in corneal wound healing. We investigated a potential role of CB_2 receptors in the same model system. To determine the role of CB_2 receptors in corneal healing we examined the consequences their activation on migration and proliferation in bovine CECs (bCECs) and murine models. We now report that activation of CB_2 induces bCEC migration via chemotaxis, but appears to do so via repulsion. In mice, CB_2 receptors are upregulated in corneal epithelial cells by injury. CB_2 activation does not alter bCEC proliferation. The signaling profile of CB_2 activation is the inverse of CB_1 in the same cells, activating MAPK and, surprisingly, increasing cAMP accumulation. In vivo, CB_2 knockout mice lack the initial delay in wound closure, resulting in an enhancement of the rate of initial wound closure.

In summary, we find that CB_2 receptors are upregulated in corneal epithelium by corneal injury and that these receptors play a role in the initial delay of wound closure, possibly via chemorepulsion.

IDENTIFYING CANNABINOID CB1 RECEPTOR AS A THERAPEUTIC TARGET FOR IDIOPATHIC PULMONARY FIBROSIS AND A DUAL-TARGETING PARTNER FOR IMPROVING ANTI-FIBROTIC EFFICACY

Resat Cinar[1], Bernadette R. Gochuico[2], Malliga Iyer[1,] Tadafumi Yokoyama[2], Joshua K. Park[1], Nathan J. Coffey[1], Tony Jourdan[1], William A. Gahl[2] and George Kunos[1]

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Idiopathic pulmonary fibrosis (IPF) is a life-threatening disease with limited treatment options. Endocannabinoids (ECs) acting via the CB_1R promote fibrosis in liver, kidney, heart, and radiation-induced pulmonary fibrosis but their role in human IPF and a related mouse model has not been investigated. The pathogenesis of IPF is multifactorial and complex, and targeting multiple pathways simultaneously might improve therapeutic efficacy. Activity of the enzyme inducible nitric oxide synthase (iNOS) is also increased in fibrosis and correlates with IPF progression. iNOS inhibitors have antifibrotic activity in animal models of PF, which makes it a viable target for IPF. We assessed the status of the EC/CB_1R system and iNOS in human IPF and its mouse model, and evaluated the therapeutic potential of MRI-1867, an orally bioavailable and peripherally restricted CB_1R/iNOS hybrid inhibitor in mice. We used broncho-alveolar lavage fluid (BALF) and lung tissue samples from patients with IPF and respective controls as well as from mice with bleomycin-induced mouse PF (BL-PF) and their controls.

The EC anandamide was increased in the lung of bleomycin-treated mice and in BALF samples from IPF patients, in whom its level negatively correlated with pulmonary function tests. Decreased FAAH expression contributed to increased AEA levels in the fibrotic mouse lung. CB_1R and iNOS protein levels were significantly elevated in lung tissue from patients with severe IPF or from bleomycin-treated mice. CB_1R activation in alveolar macrophages (AM) was associated with a proinflammatory and profibrotic state, and with increased expression of the M1 marker interferon regulatory factor-5. Upregulation of CB_1R and iNOS in the fibrotic lung was independent of one another, which may underlie the additive antifibrotic efficacy of their simultaneous inhibition. Moreover, MRI-1867 treatment arrested the progression of established fibrosis in BL-PF and dramatically improved survival rate, compared to vehicle or CB_1R antagonism alone.

We identified CB_1R as a novel therapeutic target in IPF. Dual-targeting of CB_1R and iNOS for inhibition offers a novel therapeutic approach with improved efficacy.

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

URB597 AND JZL184 PROTECTS THE HUMAN COLON AGAINST INFLAMMATION VIA CB1

DG Couch, J Lund and SE O'Sullivan

School of Medicine and Health Sciences, University of Nottingham, Derby, UK

Background: Exogenous application of the endocannabinoids anandamide (AEA) and 2-Arachidonoyl-glycerol (2-AG), or inhibition of their degradation, are anti-inflammatory in experimentally induced colitis in murine models, but this has not yet been examined in human tissue. In this experiment we sought to investigate the anti-inflammatory effects of raising endogenous levels of AEA and 2-AG in inflamed human colonic samples by inhibiting their metabolising enzymes (fatty-acid aminohydrolase (FAAH) and monoacylglycerol lipase (MAGL)) using the inhibitors URB597 and JZL184.

Methods: All experiments on human tissue gained approval through local University and National Health Service Research Ethics committees. Patients undergoing planned bowel resection within Royal Derby Hospital were identified, and consented prior to procedure (n=8). Samples of normal mucosa were excised (12X12mm), and transported on ice from the operating theatre to the laboratory. Sections of tissue (2X2mm) were incubated in Minimum Essential Medium Eagle (Sigma-Aldrich), supplemented with 10% foetal bovine serum (FBS), 1% Penicillin/Streptomycin and 1% non-essential amino acids. Inflammatory conditions were simulated by adding interferon gamma (IFNγ, 10μM) to the media for 8 hours, followed by tumour necrosis factor alpha (TNFα, 10 μM) for 16 hours. URB597 (10μM) and JZL184 (10μM) were added simultaneously with IFNγ. The mechanism of action of these inhibitors were investigated by adding the CB_1 (AM251, 100nM) and $TRPV_1$ (SB366791, 500nM) inhibitors to media simultaneously with IFNγ. Control conditions were achieved as appropriate (0.01% DMSO). The anti-inflammatory effect of URB597 and JZL184 were determined by quantifying the secretion of the cytokines granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-12 (IL-12), IL-13, IL-15 and IL-1β into the media using Multiplex technology (Marck Millipore, #48-680MAG). Statistical differences between experimental groups were compared using repeated-measures one-way ANOVA.

Results: Compared to non-inflamed controls, TNFα and IFNγ treatment caused a significant rise in the secretion of all the measured cytokines ($p<0.05$). Treatment of inflamed tissue with URB597 prevented the increased secretion of all five inflammatory cytokines ($p<0.05$). This effect of URB597 was abolished when tissue was co-treated with the CB_1 antagonist AM251, but not with the $TRPV_1$ antagonist SB366791. Treatment of inflamed tissue with JZL184 also prevented the increased secretion of all five inflammatory cytokines ($p<0.05$). This effect was abolished with AM251, but not SB366791. Compared to control tissue, AM251, SB366791, URB597 and JZL184 in the absence of inflammation caused no change in the secretion of cytokines.

Conclusion: This is the first report to demonstrate the anti-inflammatory effects of FAAH and MGL inhibitors in the gut, mediated by the CB_1 receptor. FAAH and MGL inhibitors may be of significant clinical use in developing anti-inflammatory agents for gastrointestinal inflammatory disorders such as Ulcerative Colitis and Crohn's disease.

DYNAMIC REGULATION OF THE CENTRAL ENDOCANNABINOID SYSTEM INDUCED BY COLITIS

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There is a high degree of comorbidity between chronic inflammatory conditions (e.g. inflammatory bowel diseases, arthritis) and stress-associated neuropsychiatric disorders (e.g. anxiety, depression); however, the mechanisms underlying these comorbidities are not fully elucidated. The endocannabinoid system is an ideal candidate to investigate the mechanisms of these comorbidities. Our aim was to investigate how the endocannabinoid system is altered following peripheral inflammation (colitis) in order to subsequently determine its role in anxiety.

Colitis was induced by intracolonic administration of trinitrobenzene sulfonic acid (TNBS, 0.45 mL, 50 mg/mL, 50 % [vol/vol] in ethanol/water) to adult male rats. Controls received the same volume of saline. Three days after the induction of colitis, when acute inflammation is peaking in this model, we found no differences in the levels of anandamide (AEA) in any regions examined, using liquid chromatography/tandem mass spectrometry. In contrast, 2-AG levels were modestly increased in the amygdala (9.67±0.40 vs. 11.47±0.62 nmol/g tissue) and hypothalamus (10.70±0.39 vs. 12.36±0.48). In the hypothalamus, the increased levels of 2-AG were strongly correlated with the degree of macroscopic tissue damage ($r=0.53$, $p<0.01$). Previously we showed that seven days after the onset of colitis, AEA levels were reduced in the amygdala, hippocampus and medial prefrontal cortex. In contrast, 2-AG levels were increased in the hippocampus and medial prefrontal cortex. To explain these data, we have measured the activity of FAAH, the principle enzyme responsible for the metabolism of AEA, using a radioligand enzymatic activity assay. In the amygdala, the seven day decrease in AEA was accompanied by an increase in the activity of FAAH (V_{max} 749.10±97.69 vs. 1223.00±257.10 pmol/mg protein/min). Furthermore, the increase FAAH activity was strongly correlated with the degree of macroscopic tissue damage ($r=0.61$, $p<0.05$). We also investigated CB_1 receptor binding using a competitive radioligand binding assay in rats seven days after the onset of colitis. In the hippocampus, CB_1 receptor binding was reduced (B_{max} 0.40±0.01 vs. 0.30±0.02 pmol/mg protein) and this reduction was very strongly correlated with macroscopic tissue damage score ($r=-0.89$, $p<0.02$).

We show that changes in corticolimbic endocannabinoid levels are dynamically regulated in response to colitis, with the anxiety-related reduction in AEA levels occurring following the peak of disease activity. We are currently exploring the role of the CRH receptor 1 in these effects and assessing the activity of MAGL. This work shows that chronic inflammation alters endocannabinoid levels in corticolimbic brain regions that are important for the regulation of stress and anxiety. Seven days after the onset of colitis, the changes we observe resemble chronic stress exposure with regard to endocannabinoid levels and anxiety behaviour (data previously presented at ICRS 2016), which is not seen at three days following colitis exposure. Together these findings increase our understanding of the mechanisms underlying anxiety behaviors in chronic inflammatory states. They suggest that similar to stress-induced anxiety, inflammation-induced decreases in AEA signaling are likely relevant for the change in emotional behaviors associated with chronic inflammatory states.

ACTIVITY-BASED PROTEIN PROFILING REVEALS OFF-TARGET PROTEINS OF THE FATTY ACID AMIDE HYDROLASE INHIBITOR BIA 10-2474

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A recent phase I clinical trial of the fatty acid amide hydrolase (FAAH) inhibitor BIA 10-2474 led to the death of one volunteer and produced mild-to-severe neurological symptoms in four others. Although the cause of the clinical neurotoxicity is unknown, it has been postulated that off-target activities of BIA 10-2474 might have played a role. To evaluate this hypothesis, we determined the protein interaction landscape of the drug in human cerebral cortex and differentiated cortical neurons using activity-based proteomics assays. We found that BIA 10-2474 inhibits FAAH, as well as several other hydrolases that are not targeted by PF04457845, another clinically tested FAAH inhibitor. BIA 10-2474 produced substantial alterations in lipid networks in human neurons that affected both FAAH and non-FAAH substrates. These lipid changes correlated with the off-targets inhibited by BIA 10-2474 as well as with the BIA 10-2474-induced cytotoxicity. Our results thus reveal a distinctively poor selectivity profile for BIA 10-2474 compared to another clinically tested FAAH inhibitor.

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POTENTIALLY TOXIC THERMOLYTIC DEGRADANTS OF CARBOXAMIDE SYNTHETIC CANNABINOIDS

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The use of novel synthetic cannabinoids as intoxicants continues in spite of the health risks associated with the use of chemicals of largely uncharacterised toxicology. These compounds are typically used via smoking and inhalation, yet many synthetic cannabinoids contain chemical moieties with questionable thermal stability. For example, UR-144 forms ring-opened thermal degradants that retain efficacy at cannabinoid receptors[1], while other compounds, like JWH-018, may also produce potentially toxic degradants such as naphthalene. Recently, large numbers of synthetic cannabinoids containing a carboxamide moiety have been detected in recreational products, some of which have produced toxicity in humans[2]. However to our knowledge, the thermal stability and thermolytic degradants of these compounds have not been characterised.

In this study, six carboxamide synthetic cannabinoids (CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18) were heated sequentially to 200, 400, 600 and 800 °C using a thermolysis/pyrolysis probe. Heating was conducted under an ambient airflow which was passed through a charcoal trap held at 50 °C. The trap was then rapidly heated to 300 °C under helium flow and analysed via GC-MS.

Generally, each synthetic cannabinoid degraded to its indole or indazole amide when heated to 400 °C (shown below), which was then dehydrated to the corresponding nitrile. At 600 °C, the nitrile-free indole or indazole compound was observed. Some synthetic cannabinoids formed specific thermal degradants; methylbenzene (toluene) and α-methylstyrene were liberated from CUMYL-PICA and 5F-CUMYL-PICA, 1-napthylamine was produced from NNEI, and napthalene was formed from both NNEI and MN-18. These degradants may contribute to the toxicity of carboxamide synthetic cannabinoids. It is also possible that thermolytic liberation of a cyanide anion (and subsequent production of hydrogen cyanide) could occur if the indole/indazole compound forms directly from the nitrile. Further studies are underway to directly address this hypothesis.

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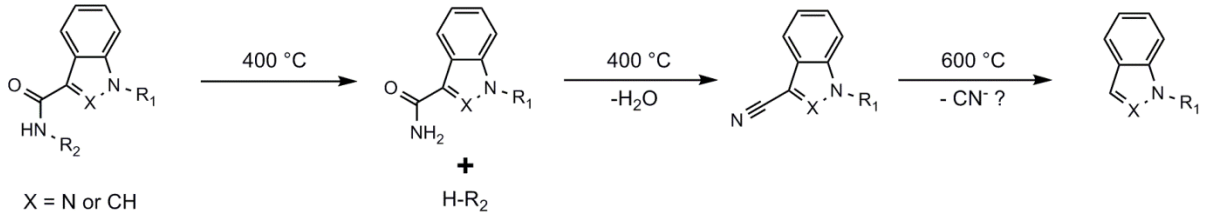

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SURPRISING MECHANISM OF FAAH INHIBITION BY NEWLY DESIGNED MULTI-TARGET ANALGESICS

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Pain remains the most prominent target in drug research, reflecting a need for more effective therapeutic interventions. Regardless of its origin, pain almost invariably accompanies inflammatory diseases. Hence, anti-inflammatory medicaments generally ameliorate the associated pain. However, blockade of prostanoid biosynthesis by cyclo-oxygenase (COX) inhibitors is often insufficient to combat pain. The endocannabinoid-CB_1 receptor pathway offers a separate analgesic option exploitable for additional analgesic efficacy. Exogenous CB_1 receptor agonists cause psychotropic side effects but this may be avoided by preventing endocannabinoid breakdown by inhibiting the enzyme principally involved in endocannabinoid inactivation, the serine hydrolase fatty acid amide hydrolase (FAAH). Clinical studies with a potent and bioavailable FAAH inhibitor have already demonstrated no meaningful psychotropic side effects. However, no analgesic effect has been reported in humans either. Recent analyses of the prostanoid and endocannabinoid analgesic/pro-algesic pathways provide an explanation for the lack of therapeutic effect of FAAH inhibitors *versus* the high efficacy of "putative" selective COX-2 inhibitors. Prostanoids on the one hand are involved in inflammation via certain receptors, and on the other hand, they limit the extent of the inflammatory and/or immune reaction via other receptors. The receptors described as pro-inflammatory are DP_1 and EP_4, while activation of other receptors, such as EP_1, mediate nociceptive pain or, in the case of TP receptors, may produce unwanted cardiovascular side effects.

Based on these considerations, we chemically modified prostanoid receptor pan-antagonists to host the inhibition of fatty acid amide hydrolase (FAAH), and increase their analgesic activity. Structurally, the compounds are unique showing an oxabicycloheptane scaffold, an upper chain terminating in an anionic acyl sulfonamide, and a lower chain incorporating an amide moiety. The optimised lead AGN220653 showed efficacy against acute/inflammatory pain via the expected prostaglandin and endocannabinoid receptors and behaved as a non-competitive, slowly reversible FAAH inhibitor. Molecular docking suggested a nucleophilic attack by Ser241 only on the carboamide carbonyl group. Autoradiography of the pure enzyme incubated with radiolabelled AGN220653 indicated covalent binding and fragmentation of the inhibitor. Combined with docking results, it also suggested the oxazole-containing moiety as the only leaving group following the formation of the covalent complex, thus pointing to a surprising non-amidase mechanism. Quantum mechanics studies and MRM-MS analyses confirmed the cleavage of the highly stable carbonyl-4-oxazole bond by Ser241, with formation of a slowly hydrolysable carbamate. Our results exemplify how achieving drug multi-targeting may lead to the discovery of unexpected mechanisms of amidase action and inhibition.

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THE DISCOVERY OF ALLOSTERIC SITES OF HUMAN MONOACYLGLYCEROL LIPASE FOR IDENTIFICATION OF NOVEL PHARMACEUTICALS

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Monoacylglycerol lipase (hMGL) plays an important role in lipid metabolism by catalyzing specifically the hydrolysis of monoacylglycerol into free fatty acid and glycerol. It is an important pharmaceutical target implicated in inflammation, pain sensation and has been identified as a regulator of a fatty acid network that promotes cancer pathogenesis. Understanding the molecular basis of hMGL function is critical for development of novel, selective inhibitors.

We have identified intramolecular communication between residue pairs of hMGL and characterized allosteric coupling between distal sites that are related to hMGL function. Using a multimethod approach, we demonstrate that the effects of a single point mutation at a specific distal site of hMGL are transduced across 18 Å or more to the active site and to other remote regions, suggesting allosteric regulation of hMGL function. For the present study, we used a mutational structure perturbation approach to reveal long-range communications and the presence of conformational switches in the interior of the hMGL enzyme. Structural, dynamic, and functional responses to conservative and nonconservative mutations were observed and characterized. We found that structural excitations at several sites distant to the catalytic triad lead to conformational changes and concerted motions of structurally distinct regions. Experimental insight into the mechanism of inter-residue interactions within a communication network was achieved by combination of site-directed mutagenesis, kinetics, NMR spectroscopy, hydrogen deuterium exchange mass spectrometry (HDX-MS) and molecular dynamic (MD) simulations. Identification of these sites may provide novel opportunities for development of new strategies toward modulating hMGL function and identification of novel pharmaceuticals.

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CRYSTAL PACKING ISSUES IN THE CB1 CRYSTAL STRUCTURES

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Two crystal structures of CB1 were reported in 2016 [1,2]. Such structures are expected to accelerate the progress in understanding and manipulating the endocannabinoid system and should provide an exceptional starting point for structure-based drug discovery. However, both of these structures have undergone modifications to assist in their crystallization and both display crystal packing issues on the extracellular side that has resulted in flattening of all EC loops and intrusion of portions of the N terminus into the binding pocket. As a result, the structures fail to be consistent with much of the CB1 SAR developed over the years via mutation studies and ligand development. The issue of crystal packing is not a new one. The first Rhodopsin crystal structure had issues that impacted intracellular loop conformations [3]. Lastly, it is generally accepted that GPCRs are highly flexible proteins, with a rich and varied energy landscape [4]. Therefore, the question arises as to the extent of structural distortion introduced into the extracellular side of CB1 due to the crystal environment and what role dynamics plays on the structural ensemble of the CB1 receptor.

In order to investigate such perturbations of the CB1 structure, we employed all atom molecular dynamics (MD) simulations to relax each crystal structure in a realistic hydrated POPC membrane environment. We then used the Collective Variables MD technique to move the N-terminus up and out of the binding pocket. As the N-terminus was raised, all EC loops came up and key residues like K3.28 became accessible from within the binding pocket. Subsequent all atom MD (400ns) was conducted on the modified structure to be certain it was a stable structure. The modified structure was found to be consistent with the CB1 SAR literature. [Support: RO1 DA003934 and KO5 DA021358 PHR]

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CB_1 REGULATES MITOCHONDRIAL FUNCTIONS OF HUMAN EPIDERMAL KERATINOCYTES *IN SITU* AND *IN VITRO*

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It has recently been shown that functional CB_1 is expressed not only in the cell membrane ($cmCB_1$), but also in the outer mitochondrial (mt) membrane ($mtCB_1$), where it can directly suppress mt activity, and it is responsible for e.g. cannabinoid-induced memory impairment. It has been demonstrated that mt activity plays a role in the regulation of proliferation and differentiation, as well as of immunological responses of human epidermal keratinocytes. While the cutaneous endocannabinoid system (cECS) is now recognized to play a key role in human skin physiology and pathology, we lack data about the role of cECS in controlling mt functions in the epidermis. Therefore, the current study aimed to clarify whether CB_1-mediated signaling impacts on mt biology of human epidermal keratinocytes.

First, we treated organ cultured full-thickness human skin of 4 donors with the selective CB_1 inverse agonist AM251 (1 μM) w/wo the CB_1 agonist ACEA (30 μM) or appropriate vehicles for 24 hrs. As revealed by *in situ* enzyme activity assays, AM251 significantly increased activities of respiratory complexes (RC) I, II and IV in the epidermis in a CB_1-dependent manner, whereas ACEA was found to CB_1-dependently increase heat production, as measured by chip-calorimetry. Next, we asked if keratinocytes express CB_1 not only on their surface, but also in their mitochondria. Immunoelectron microscopic analysis of wild-type C57BL/6 mice revealed that ~20% of the mitochondria of epidermal keratinocytes were CB_1 positive, whereas $CB_1^{-/-}$ littermates showed only negligible nonspecific labeling. Importantly, immunoelectron microscopy of human epidermis yielded similar CB_1 positivity rate of keratinocyte mitochondria (i.e. ~20%). Interestingly, $mtCB_1$ immunoreactivity was maximal in basal layer keratinocytes, and significantly lower in the spinous and granular layers, indicating that $mtCB_1$ may play a role in human keratinocyte terminal differentiation. Finally, by using extracellularly restricted (hemopressin) and cell-permeable (AM251) inverse agonists, we intended to dissect differential roles of $cmCB_1$ and $mtCB_1$ mediated signaling. We found that $mtCB_1$ (but not $cmCB_1$) regulates mtROS production (MitoSOX™ Red assay) of cultured primary human epidermal keratinocytes. Moreover, our preliminary data suggest that anti-inflammatory actions of CB_1 may be coupled to $cmCB_1$, whereas differentiation-inhibitory effects appear to be mediated by $mtCB_1$.

Collectively, our data indicate that CB_1 negatively regulates epidermal mt functions, and highlight the possibility that $cmCB_1$ and $mtCB_1$ may play differential roles in regulating epidermal biology.

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1-ARACHIDONOYLGLYCEROL SIGNALING AT CANNABINOID CB1 RECEPTORS

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2-arachidonoylglycerol (2-AG) is the most abundant endogenous cannabinoid in the brain and an agonist at both cannabinoid receptors (CB_1 and CB_2). The synthesis, degradation and signaling of 2-AG have been investigated in detail but its relationship to its isomer 1-arachidonoylglycerol (1-AG) is still poorly understood. 2-AG is produced enzymatically in neurons 'on demand' but is known to spontaneously isomerize to 1-AG over tens of minutes, ultimately stabilizing at a 2-AG/1-AG ratio of 1:9. Two schools of thought regarding 1-AG contend either that 1-AG is an inactive byproduct of 2-AG signaling or that 1-AG is an active signaling molecule in its own right. Because of the centrality of 2-AG to cannabinoid signaling, it is important to clarify the disposition of 1-AG signaling. We therefore tested 1-AG in several signaling pathways.

We now report that 1-AG broadly mimics the 2-AG signaling profile but with notable exceptions, indicating that 1-AG exhibits pathway selectivity relative to its isomer. 1-AG signaling is comparable to that for 2-AG in internalization, pERK and forskolin stimulated cAMP formation assays. However 1-AG signals poorly in arrestin recruitment, and also in a neuronal model of endogenous 2-AG mediated cannabinoid signaling.

In summary, our results show that in contrast to the perception of 1-AG as an 'inactive isomer' of 2-AG, 1-AG is indeed active and that it exhibits functional selectivity in its signaling.

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REGION AND DEVELOPMENT-DEPENDENT EFFECTS OF CP 55,940 ON THE WIDER ENDOCANNABINOID LIPIDOME IN THE MOUSE BRAIN

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The synthetic cannabinoid CP 55,940 (CP) is a significantly more potent agonist at CB_1 and CB_2 than the phytocannabinoid, Δ^9-tetrahydrocannabinol (THC). Recreational use of synthetic cannabinoids like CP is on the rise, especially amongst adolescents and young adults, with potentially detrimental consequences for brain development. At the 2016 ICRS meeting, we showed data that acute THC has broad effects on the mouse brain lipidome that are age and region dependent, wherein there were significantly greater changes in the adult versus the adolescent brain. Here, we test the hypothesis that CP, likewise, has effects on the brain lipidome in both an age and region dependent manner. The wider endogenous cannabinoid (eCB) lipidome of ~80 lipids was screened in 8 regions of the adult and adolescent (post-natal day (PND) 50) mouse brain. Groups of 8 mice treated with a single dose of 3mg/kg CP (the same dose used in our THC studies) were each compared to 8 age and sex (all female) matched vehicle treated mice from the same CD1 genetic background. Animals were sacrificed 2 hours post injection, brains were removed and 8 targeted areas were dissected and stored at -80C. Methanolic extracts were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS.

Opposite to the effects of THC, CP has a more robust effect on the adolescent lipidome compared to the adult. Overall, there were 141 changes in lipid levels found across all 8 brain regions in the PND50 mice and there were only 97 changes in the adult mice. In both age groups, the majority of the changes in lipid levels were decreases in a lipid's concentration in the CP group relative to vehicle. Results here highlight those of eCBs, 2-AG and AEA, and prostaglandins (PGs); however, many differences were observed in other lipids. In both age groups, eCB levels were **reduced** in a region-dependent manner. CP lowered levels of 2-AG in the striatum, and thalamus in both adult and PND50 mice; however, whereas only the adult mice had lower 2-AG levels in the cerebellum, midbrain, and brainstem, only PND50 mice had lower 2-AG levels in the hypothalamus. CP decreased levels of AEA in both adolescents and adults in the midbrain, cerebellum and thalamus, but decreases in the hippocampus and cortex were only measured in PND50 mice and decreased levels of AEA in the striatum were only detected in adult mice. Linking the eCB system to broader lipid signaling, eCBs are derived from arachidonic acid, which is a substrate for PGs. We measured 3 PGs in these mouse brain samples: PGE_2, $PGF_{2\alpha}$, and 6-keto$PGF_{1\alpha}$. In both age groups, the effects of CP on PGs were highly region dependent, with each PG showing a pattern of both increases and decreases in different brain areas. Overall, there were more changes in PG levels in the adolescent brain. For example, in the PND50 hippocampus, levels of all 3 PGs increased. In contrast, acute CP decreased levels of all 3 PGs in the PND50 brainstem. Here, we show that in contrast to acute administration of the lower potency CB_1 agonist, THC, acute administration of the higher potency, synthetic CB_1 agonist, CP, has significantly more effects on the brain lipidome of the adolescent versus the adult. These data provide a novel insight into how different CB_1 agonists can drive different cellular signaling effects and that these effects are also age dependent.

PANNEXIN-1 TRANSPORTS ANANDAMIDE RESULTING IN MODULATION OF GABA TRANSMISSION

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Anandamide (AEA) is a dual signaling molecule capable of modulating synaptic transmission by acting as an agonist at CB1 receptors and TRPV1 channels. In order to terminate anandamide signaling, it must move into the post-synaptic neuron and be degraded by fatty acid amide hydrolase (FAAH). It is unknown how AEA clearance from the synapse occurs but mechanisms for its transport have been proposed. AEA is a lipid that could be removed from the cleft by simple diffusion along its concentration gradient. However, it is known that anandamide transport is saturable and can be blocked by structural anandamide analogs. It has also been noted that anandamide transport does not require ATP and is moderately dependent on temperature. These characteristic point towards facilitated diffusion via a membrane channel as opposed to simple diffusion or an energy dependent transporter. If the anandamide transporter is a membrane channel, it should be large for AEA and possibly other lipids such as HETE or arachidonic acid to permeate. The transporter should be present throughout the brain since AEA signals in various brain regions.

Pannexin-1 (Panx1) is a large pore channel with the ability to pass molecules up to 1kDa in size including ions, signaling molecules, lipids such as HETE, as well as arachidonic acid. Panx1 is expressed ubiquitously in the brain and could readily allow passage of the 0.35kDa AEA, thus fulfilling the criteria for an anandamide transporter. Using cell-attached patch clamp recordings and dye flux imaging, we show that Panx1 transports AEA in cultured hippocampal neurons and it transports fluorescent AEA (CAY10455) in transfected HEK293 cells. Whole-cell patch clamp recordings of CA1 pyramidal neurons from acute rat hippocampal slices show depressed evoked GABA neurotransmission when Panx1 is blocked. This was mimicked with the FAAH antagonist URB597 and prevented by the CB1 receptor reverse agonist, AM-251. Spontaneous GABAergic transmission was not affected by Panx1 block. Lastly, AEA concentrations were elevated in hippocampal slices when Panx1 channels were inhibited as quantified by mass spectrometry analysis, suggesting that Panx1 is important for clearance of AEA. We conclude that Panx1 can transport AEA into hippocampal neurons and that interruption of AEA transport leads to depression of GABA signaling.

MODULATION OF THE ENDOGENOUS CANNABINOID SYSTEM BY NAGLY VIA GPR18 AND GPR55

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Support for a role of endogenous lipoamino acids in tempering G-protein coupled receptor signaling is increasingly strengthened due to the association of these endogenous mediators with numerous physiologic processes. The impact of these lipid-conjugated amino acids on the endocannabinoid system is escalating as a consequence of the involvement of endocannabinoids in the physiology of a broad range of cellular systems. The investigations reported here considered the effects of four N-arachidonoyl amino acids (NAAA); N-arachidonoyl serine (NASer), N-arachidonoyl tyrosine (NATyr), N-arachidonoyl glutamic acid (NAGlu), and N-arachidonoyl glycine (NAGly), on intracellular signaling mediated by the candidate cannabinoid receptors, GPR18 and GPR55. Several transduction pathways; ß-arrestin, MAPK and calcium mobilization, utilizing CHO cell lines with stable receptor expression, were explored.

Interestingly, results indicate that in the presence of NAAA differential patterns of GPR18- and GPR55-mediated intracellular signaling exist. Whereas increases in GPR55-mediated ß-arrestin activity were moderate for all of the NAAA, there were no appreciable increases of ß-arrestin activity in GPR18 expressing cells in the presence of NAAA. Unlike ß-arrestin signaling, enhanced levels of MAPK activity were similarly induced amongst the NAAA in both GPR18 and GPR55 cell lines. Noting the similarity of results in the MAPK assay, we explored receptor activation by one of the NAAA, NAGly, in calcium mobilization studies. Surprisingly, increases in calcium mobilization in the presence of NAGly, were not evoked in the GPR18/ and GPR55/ß-arrestin cell lines. However, in CHO cell lines expressing only the receptor (GPR18 or GPR55) and not ß-arrestin, NAGly induced increases in intracellular calcium. It is interesting to speculate that manipulations to the C-terminal of the receptors, necessary for the PathHunter enzyme complementation assay, preclude the ability of the receptor to signal through calcium mobilization.

The specificity of receptor mediated, NAGly-induced, intracellular signaling events were verified by pre-incubation with receptor specific antagonists (PSCB5, GPR18; ML193, GPR55). Since NAGly is an active metabolite of anandamide, and both endogenous lipids activate GPR18, signaling via cannabinoid receptors may be strengthened, and or prolonged. GPR55 may serve as a switch to alter cannabinoid receptor signaling as anandamide is a partial agonist/antagonist and NAGly is an agonist at GPR55. Results from this study suggest that both GPR18 and GPR55 have the potential to modulate endocannabinoid signaling via NAGly, in calcium and MAPK mediated pathways, and GPR55 alone in ß-arrestin mediated cellular events.

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TRANSPORT OF MAGL-PRODUCED LIPIDS BY FABP5 LEADS TO INCREASED PROSTATE CANCER AGGRESSION

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The 5-year relative survival rate of men in the United States diagnosed with distantly metastasized prostate cancer is 28%. Fatty acid binding proteins promiscuously bind long-chain fatty acids and facilitate their transport to diverse cellular compartments, including the nucleus. Upregulation of FABP5 increases the migratory and invasive potential (aggression) of prostate carcinomas and is associated with shorter survival time of prostate cancer patients. Previous work indicates that overexpression of monoacylglycerol lipase (MAGL), the enzyme that hydrolyzes monoglycerides into fatty acids, increases prostate cancer aggression. Because FABP5 translocates free fatty acids into the nucleus and MAGL activity produces endogenous fatty acids, it is possible that FABP5 binds to MAGL-generated fatty acids and facilitates their nuclear entry to exert pro-tumorigenic effects.

Here we test the central hypothesis that FABP5 is required to facilitate the transport of MAGL generated lipids to promote an aggressive phenotype in human prostate carcinomas. Lentiviral vectors were constructed to manipulate the expression of FABP5 and/or MAGL in LNCaP and PC3 prostate cancer cell-lines. Overexpression of MAGL in the weakly aggressive LNCaP cells (which do not express FABP5) does not promote an aggressive phenotype *in vitro*, whereas concomitant overexpression of FABP5 and MAGL significantly increases aggression (greater than overexpression of FABP5 alone). This effect was dependent upon nuclear entry of FABP5 because a cytoplasmically-restricted FABP5 was unable to increase cancer cell aggression regardless of MAGL expression. Overexpression of MAGL in highly aggressive PC3 cells (which express FABP5) promotes an aggressive phenotype, whereas knockdown of FABP5 expression in these cells results in a significant decrease in aggression both *in vitro* and *in vivo*.

In conclusion, the ability of MAGL to increase prostate cancer aggression depends upon the presence of FABP5.

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OLEOYLGLYCINE IS PRODUCED BY BRAIN TRAUMA AS A NOVEL AND MULTI-TARGET ENDOGENOUS LIPID SIGNAL

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Cigarette smokers presenting with traumatic brain injury (TBI)-induced damage of the insula cortex display cessation of nicotine addiction. In the present study, we asked whether experimental TBI in mice would lead to the elevation of an endogenous anti-addiction mediator within the insula that counteracts the rewarding effects and dependence liability of nicotine. We used a targeted lipidomics approach aimed at investigating changes in the brain levels of endocannabinoids, *N*-acylethanolamines and lipoaminoacids. We show that one day after inducing experimental TBI in mice using the weight drop model, a largely uncharacterized *N*-acyl-glycine, *N*-oleoyl-glycine (OlGly), is produced in the insular cortex. Exogenous administration of synthetic OlGly (30 mg/kg i.p.) reduced precipitated withdrawal responses in nicotine-dependent mice and nicotine conditioned place preference (CPP), which was mediated through the activation of peroxisome proliferator-activated receptor alpha (PPAR-α) receptors. Accordingly, using in silico modeling approaches and luciferase functional assays that OlGly was activates PPAR-α. OlGly was inactive against morphine-induced CPP. These findings identify the endogenous molecule OlGly as a PPAR-α agonist that reduces the rewarding effects of nicotine as well as nicotine dependence in mice.

Next, we tested the possible neuroprotective effects of OlGly in this model of TBI. Animals were treated with OlGly (50 and 100 mg/kg, i.p.) for 14 days once a day, starting one day after injury and then submitted to several behavioural tests to assess pain and depression. At the end of the treatment, the animals were decapitated and brains dissected for endocannabinoid analysis. Interestingly, treatment with OlGly normalized motor impairment and reckless behavior; reduced thermal hyperalgesia and mechanical allodynia; and normalized aggressiveness and depression induced by TBI, as well as normalizing TBI-induced alterations of endocannabinoid levels. Work is in progress to determine whether PPAR-α is involved in these protective effects of OlGly.

Finally, using the lateral fluid percussion model (LFP) of TBI in rats, we found that OlGly is also produced in this species. In particular, OlGly was found only in the ipsilateral side of injured rats in the hippocampus and prefrontal cortex, and administration of synthetic OlGly (5 mg/kg, ip) inhibited the aversive effects of morphine withdrawal in a place aversion paradigm. Preliminary results indicate that these effects are mediated by CB_1 receptors, possibly owing to the inhibitory actions observed for OlGly against FAAH from rat brain membranes (IC_{50}=8.65±0.17 μM).

In summary, OlGly is a novel TBI-inducible lipid mediator in both mice and rats, where it plays species-dependent pharmacological actions against either nicotine or morphine-induced withdrawal and TBI-induced behavioural disturbances, seemingly via different molecular targets.

ENDOCANNABINOID-MEDIATED HABITUATION PERSISTENTLY REDUCES NOCICEPTIVE SIGNALING

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Repetitive stimulation of non-nociceptive afferents can reduce nociceptive signaling and this modulatory process has been the basis for transcutaneous electrical nerve stimulation and (TENS) and spinal cord stimulation (SCS) therapies to treat pain. Recent studies in both *Hirudo verbana* (the medicinal leech; *J Neurophysiol* (2013) 110: 2607) and in rodents (*Pain* (2016) 157: 2582) have found that endocannabinoid-mediated long-term depression (LTD) of nociceptive synapses plays an important role in this anti-nociceptive effect. It is not clear, however, why repetitive stimulation of non-nociceptive afferents should persistently reduce nociceptive afferent signaling. One possibility is that this process represents a form of habituation, a simple non-associative form of learning. Specifically, the observed analgesic effect may represent a form of generalization or transfer of habituation in which repetitive (habituating) stimuli in one stimulus-response pathway also produces habituation in a related, but unactivated pathway.

Studies using *Hirudo* were conducted to investigate whether habituation to non-nociceptive stimuli can generalize to reduce responses to nociceptive stimuli and whether endocannabinoid signaling plays a role. The endocannabinoids 2-AG and anandamide are known to be present in the *Hirudo* central nervous system (CNS) as are the 2-AG synthesizing (DAG lipase) and metabolizing (MAG lipase) enzymes. Furthermore, the *Hirudo* CNS is well-characterized, especially in terms of the identities of the nociceptive vs. non-nociceptive afferents and their postsynaptic targets. This facilitates linking changes at the synaptic level to changes at the behavioral level. Repetitive non-nociceptive stimulation elicited habituation of a localize withdrawal reflex. These habituating stimuli (20 stimuli at 1 min intervals) produced a parallel decrease in responses to nociceptive thermal stimulus that elicited a global withdrawal reflex. This transfer of habituation to the nociceptive pathway was blocked when animals were injected with either the DAG lipase inhibitor tetrahydrolipstatin (THL) or the TRPV channel inhibitor SB366791. Neither drug affected habituation to the non-nociceptive stimulus-response pathway. Surprisingly, a single block of 20 habituating stimuli reduced responses to nociceptive stimuli for approximately four days and this long-term effect was also blocked by THL or SB366791 when applied during, but not after, habituation training. However, when habituation training consisted of four, spaced training blocks (30 mins between each block) the reduced response to nociceptive stimuli only lasted 1 day.

The implications of this study are as follows. First, that the analgesic effects of repetitive non-nociceptive stimulation may represent a type of habituation and that utilizing what is known about this simple form of learning may lead to more effective use of TENS or SCS types of therapies. Second, that it is possible to diminish the duration of this analgesic effect by essentially overstimulating the system. Third, it may be possible to utilize endocannabinoid signaling mechanisms to enhance TENS and SCS therapies.

TOWARDS A NEW CLASS OF ANTINOCICEPTIVE DRUG: DEVELOPMENT OF FATTY ACID BINDING PROTEIN INHIBITORS TO ALTER THE ENDOCANNABINOID TONE

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The fatty acid binding proteins (FABPs) have been recognized as critical modulators of endocannabinoid signaling by facilitating the intracellular transport, and subsequent inactivation, of anandamide. The FABP isoforms expressed in the mammalian central and peripheral nervous systems have subsequently emerged as potential therapeutic targets for the treatment of pain. Previous work by our group has led to the identification of SBFI-26, a novel small molecule competitive FABP inhibitor that has shown efficacy as an antinociceptive and anti-inflammatory agent in diverse rodent models. In an effort to increase the efficacy of our lead drug, we used the truxillic acid core chemical structure of SBFI-26 as a scaffold to design and synthesize a multitude of derivatives based upon this compound. The principal goal was to develop compounds that display increased affinity for target FABPs (i.e. FABP5 and FABP7) compared to SBFI-26, with secondary aims of designing compounds that exhibit higher aqueous solubility, prolonged *in vivo* half-life, and increased selectivity against FABP3.

The functional groups to be incorporated onto the SBFI-26 scaffold structure were chosen based on computationally predicted binding free energies to the target FABPs. These *in silico* predictions heavily utilized the recently resolved co-crystal structures from our group of FABP5 and FABP7 in complex with SBFI-26 (2.2 Å and 1.9 Å, respectively). High scoring compounds were subsequently synthesized and subjected to fluorescence displacement binding assays to assess their *in vitro* affinity towards purified human FABPs. Select compounds were further assessed for biological efficacy in the Hargreaves test of inflammatory hyperalgesia.

This multi-collaborative drug discovery effort has resulted in the synthesis and *in vitro* analysis of over fifty novel SBFI compounds. Most notable was the SBFI-81 derivative, which displayed a four-fold increase in affinity compared to SBFI-26 towards FABP5 while maintaining modest selectivity against FABP3. Administration of SBFI-81 to mice resulted in significant analgesic effects in the complete Freund's adjuvant model of thermal hyperalgesia. To investigate the possibility that one chiral species may be more biologically active than others, four optically pure stereoisomers were resolved from racemic SBFI-81. No significant differences in FABP affinities were observed between the racemic mixture and any of these stereoisomers. The data obtained from this study has allowed us to gain considerable insight into the mechanisms of inhibitor binding and selectivity among the three FABP isoforms expressed in the mammalian nervous system. Future efforts will focus on improving the selectivity and aqueous solubility of SBFI-81, our new lead drug candidate.

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INFLAMMATORY PAIN CHANGES THE EXPRESSION AND FUNCTION OF CB1 RECEPTORS IN THE PERIAQUEDUCTAL GRAY

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The midbrain periaqueductal gray (PAG) mediates the antinociceptive properties of analgesics, including opioids and cannabinoids. Administration of either opioids or cannabinoids directly into the PAG induces antinociception, and the presumed mechanism of action is the disinhibition of GABAergic neurons. However, most studies characterizing the antinociceptive properties of cannabinoids in the PAG have been conducted in naive animals, using evoked pain models as behavioral outputs. Few studies have reported on the role of CB1 receptors in the PAG during conditions which would prompt the administration of analgesics; namely, in chronic pain. In this study, we used the Complete Freund's adjuvant model to characterize CB1 receptor expression and function during persistent inflammatory pain. Using cellular fractionation and western blot analysis, we demonstrate that 48 hours after CFA injection, there is a significant upregulation in the expression of synaptic CB1 receptors. To assess whether this protein upregulation induces a functional change, we measured the anti-hyperalgesic action of the pan-cannabinoid receptor agonist WIN 55,212-2 on the Hargreaves test of thermal hyperalgesia. We also measured the potency of WIN to inhibit GABAergic transmission in the PAG using whole cell patch clamp electrophysiology. We were able to isolate the role of PAG interneurons using 470 nm light-evoked GABA release, after microinjecting AAVDJ-EF1a-DIO hChR2(H134R)-mCherry virus into the PAG of GAD-cre rats. By studying the potency of WIN during conditions in which analgesics would normally be administered, we have unveiled a novel compensatory change in the descending pain pathway during persistent pain. Opioids are still the most commonly prescribed analgesics for chronic pain. Because there are neuroanatomical and functional interactions between PAG CB1 and mu-opioid systems, these pain-induced changes provide critical insight into the analgesic efficacy of these drugs during persistent pain, and may aid the development of novel pharmaco-therapies.

AGONIST-SPECIFIC MECHANISMS OF CANNABINOID TOLERANCE IN DESENSITIZATION RESISTANT MICE

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The focus of this study was to better understand the mechanisms responsible for tolerance to cannabinoids such as delta-9-tetrahydrocannabinol (Δ^9-THC). We used a newly developed knock-in mouse line which expresses a desensitization-resistant form of the cannabinoid receptor 1 (CB_1) to investigate tolerance to WIN55,212-2, and CP55,940. These mutant mice (S426A/S430A) lack two serine residues that are phosphorylated by G protein-coupled receptor kinases (GRKs) and recruit β-arrestin2. Antinociceptive tolerance to repeated daily administration of WIN55,212-2 and CP55,940 was assessed in wild-type and S426A/S430A mice using the hot plate, tail flick, and formalin tests. S426A/S430A mutant mice exhibit a modest delay in tolerance for Δ^9-THC (Morgan et al., 2014); interestingly, tolerance to the synthetic cannabinoid agonist WIN55,212-2 is profoundly delayed in S426A/S430A mutant males while tolerance to CP55,940, another synthetic full cannabinoid agonist, is only modestly affected. This finding suggests the likelihood of agonist-specific mechanisms of cannabinoid tolerance where tolerance to the antinociceptive effects of WIN55,212-2 are mediated entirely through a classic GRK/β-arrestin2 mechanism while tolerance to Δ^9-THC and CP55,940 are mediated, in part, by other signaling pathways.

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CANNABIDIOL MODULATION OF ANTINOCICEPTIVE TOLERANCE TO THC

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Cannabidiol (CBD) has been shown to enhance some effects of delta-9-tetrahydrocannabinol (THC), and attenuate other effects of THC. In humans, these two cannabinoids are often chronically self-administered in combination (either in the form of cannabis, cannabis extract, or Sativex) to relieve pain, and it has been suggested that one benefit of the combination is decreased development of tolerance. Thus, the purpose of the present study was to compare the development of tolerance to the antinociceptive effects of THC alone and in combination with CBD in animal models of pain. Because we have previously found differential tolerance development to THC in female vs. male rats, both sexes were included in this study. THC dose-effect curves on the warm water tail withdrawal and paw pressure tests were obtained before and after 4 days of twice-daily vehicle, CBD (10 mg/kg), THC (3.6 mg/kg in females, 9.3 mg/kg in males, to adjust for greater initial THC potency in females), or CBD+THC administration. On the pre-chronic day, THC was more potent in females than in males on both nociceptive tests. THC potency on the tail withdrawal test decreased more in females than males from the pre- to post-chronic days. CBD significantly enhanced the development of tolerance to THC on both nociceptive tests, as evidenced by greater rightward/downward shifts of the THC dose-effect curves in chronic CBD+THC-treated rats compared to chronic vehicle+THC-treated rats. Only minor sex differences were observed in CBD enhancement of THC tolerance. Serum cannabinoid analysis conducted on blood samples taken immediately after completion of behavioral testing on the post-chronic day showed that serum THC levels were higher in CBD+THC-treated females than in vehicle+THC-treated females. Conversely, serum levels of THC's active metabolite 11-OH-THC and its inactive metabolite THC-COOH were lower in CBD+THC-treated rats than in vehicle+THC-treated rats of both sexes; this effect was greater in females than in males. CBD also increased serum levels of the active metabolite cannabinol in both sexes. These results suggest that CBD-induced enhancement of antinociceptive tolerance to THC in the rat is at least partly due to CBD-induced shifts in THC metabolism.

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BRAIN PERMEANT AND IMPERMEANT INHIBITORS OF FATTY-ACID AMIDE HYDROLASE SUPPRESS THE DEVELOPMENT AND MAINTENANCE OF PACLITAXEL-INDUCED NEUROPATHIC PAIN AND SYNERGIZE WITH THE OPIOID ANALGESIC MORPHINE

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Inhibitors of endocannabinoid deactivation have been developed as an alternative analgesic strategy aimed at harnessing the therapeutic potential of endocannabinoid signaling while bypassing unwanted cannabimimetic effects associated with direct binding to CB1 receptors. Both brain permeant and impermeant inhibitors of the anandamide-hydrolyzing enzyme fatty-acid amide hydrolase (FAAH) suppress established pathological pain in rodent models (Guindon et. al (2013) *Pharmacol Res.* 67: 94-109), Clapper et al. (2010) *Nat. Neuro.* (10):1265-70). However, FAAH inhibition was shown to be ineffective in a clinical trial for osteoarthritis (Huggins et al. (2012) *Pain*. 153(9):1837–1846) and genetic deletion of FAAH can produce pro-nociceptive effects in certain pathological pain states (Carey et al. *Mol. Pain* (2016) 12: 1-23). We compared the efficacy of brain permeant (URB597) and impermeant (URB937) inhibitors of FAAH in reducing the development and maintenance of neuropathic pain induced by the chemotherapeutic agent paclitaxel. Prophylactic treatments with URB937 and URB597 were equally efficacious in blocking the development of paclitaxel-induced mechanical and cold allodynia throughout the entire 20 day dosing period. Similarly, therapeutic treatments with URB937 and URB597 were also equally efficacious in blocking the maintenance of established paclitaxel-induced allodynia, under conditions in which dosing with FAAH inhibitors was initiated when paclitaxel-induced allodynia was maximal and stable. Isobolographic analysis revealed a synergistic interaction between morphine and either URB937 or URB597 in suppressing paclitaxel-induced mechanical and cold allodynia. However, anti-allodynic efficacy of URB597 (1 mg/kg i.p.) was blocked by either a CB_1 (AM251; 5 mg/kg i.p.) or a CB_2 (AM630; 5 mg/kg i.p.) antagonist, whereas anti-allodynic efficacy of URB937 (1 mg/kg i.p.) was blocked by the CB1 antagonist (AM251; 5 mg/kg i.p.) only. Finally, we ascertained whether long term FAAH inhibition could be associated with signs of CB_1-dependent precipitated withdrawal by challenging paclitaxel-treated mice receiving chronic URB937 or URB597 treatment with the CB_1 antagonist rimonabant (10 mg/kg i.p.). Rimonabant challenge did not elicit CB_1-dependent withdrawal behaviors in either treatment condition. Our data suggest that either peripheral or central FAAH inhibition represents a valid potential therapeutic strategy for suppressing both development and maintenance of chemotherapy-induced neuropathic pain. Moreover, inhibition of FAAH outside the CNS is sufficient to fully block both the development and maintenance of paclitaxel-induced neuropathic pain. Furthermore, the therapeutic efficacy of both brain permeant and impermeant FAAH inhibitors synergizes with the opioid analgesic morphine. Our studies suggest that FAAH inhibition represents a promising therapeutic approach that may be employed to maximize opioid analgesic efficacy and limit unwanted side effects that accompany opioid administration.

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MONOACYLGLYCEROL LIPASE INHIBITORS: REVERSAL OF MOUSE PACLITAXEL-INDUCED NOCICEPTION WITHOUT INTRINSIC REINFORCING EFFECTS

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Chemotherapy-induced peripheral neuropathy (CIPN), manifesting as burning, tingling or numbness in the hands and feet, is a serious side effect associated with a variety of chemotherapeutics, including paclitaxel. Due to functional impairment, CIPN often requires a reduction or cessation of paclitaxel use, deviating from established anti-neoplastic protocols. At present, there are no effective preventative or analgesic strategies available for the clinical management of paclitaxel-induced peripheral neuropathies. Thus, a serious need exists for the identification of novel antinociceptive agents. Here, we tested whether inhibitors of monoacylglycerol lipase (MAGL), the primary degradative enzyme of the endogenous cannabinoid 2-arachidonoylglycerol, will reverse paclitaxel-induced mechanical allodynia in mice. Four intraperitoneal (i.p.) injections of paclitaxel (8 mg/kg) given every other day to C57BL/6J mice produced significant mechanical allodynia as assessed using von Frey filaments. The selective MAGL inhibitors, JZL184 (1.6-40 mg/kg) and MJN110 (1-5 mg/kg; i.p.), reversed mechanical allodynia in paclitaxel-treated mice to pre-paclitaxel thresholds in dose-dependent manners. Maximum anti-allodynic effects occurred 2-3 h post-administration, with respective ED_{50} values (95% confidence limits) of 12.2 (7.5-19.9) and 2.0 (1.1-3.5) mg/kg. Furthermore, complementary pharmacologic and genetic approaches demonstrated that the anti-allodynic effects of both inhibitors required both CB_1 and CB_2 receptors. Whereas high dose JZL184 (40 mg/kg) completely reversed paclitaxel-induced allodynia, these antinociceptive effects underwent tolerance following repeated daily injections for six days, a treatment regimen known to cause CB_1 receptor down-regulation and desensitization in the brain. In contrast, acute administration of low dose JZL184 (4 mg/kg) did not significantly produce antinociceptive effects, but six days of daily administration fully reversed paclitaxel-induced allodynia. Importantly, MAGL inhibition alone does not elicit a conditioned place preference in control mice, suggesting that this drug lacks rewarding effects. Ongoing studies are testing whether MAGL inhibition produces a conditioned place preference in paclitaxel-treated mice, which would be indicative of "pain relief". As clinical analgesic use would likely require repeated treatment, the results of the present study strongly suggest that MAGL represents a viable pharmacologic target for the relief of CIPN.

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A PILOT STUDY OF THE EFFECTS OF CHRONIC CANNABIS USE ON PAIN SENSITIVITY, PAIN TOLERANCE AND PLASMA ENDOCANNABINOID LEVELS

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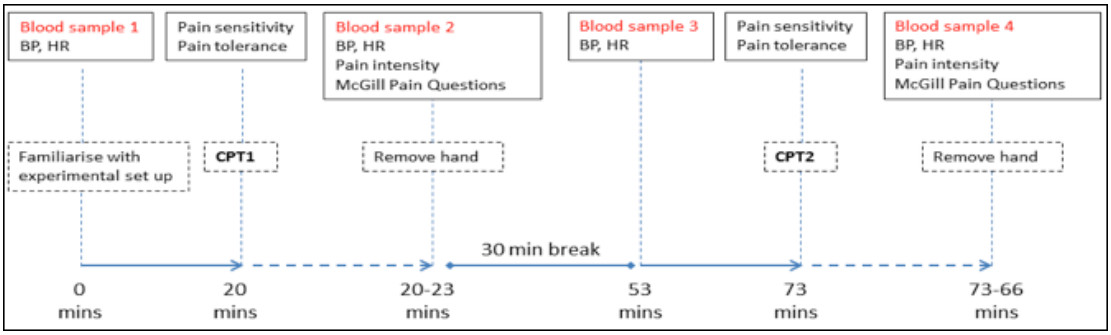
Chronic opiate users often exhibit hypersensitivity to painful stimuli, most likely due to downregulation of endogenous opioid pain-control mechanisms. Cannabis is frequently used for chronic pain and it is possible that chronic cannabis use might downregulate endocannabinoid pain control systems leading to pain hypersensitivity. To address this hypothesis, we conducted a small pilot study to examine if regular cannabis users exhibit differential sensitivity and tolerance pain relative to non-cannabis using controls on the Cold Pressor Test (CPT). We also examined plasma cannabinoid, endocannabinoid and cortisol levels.



Twenty-one males were recruited to the study (10 cannabis, 11 control). The cannabis users smoked a $\overline{x}$ of 6.3 grams per week and smoked for a $\overline{x}$ of 13 years. All gave negative saliva test results for THC on arrival at the experimental session, indicating abstinence from cannabis for approximately 12 hours prior to entering the experiment, and therefor a lack of acute cannabis intoxication. The sequence of experimental events are shown in Figure 1, and involved two CPTs with blood samples taken before and after each test.

Results showed that cannabis users had greater pain tolerance than controls on the second CPT (p=0.044), but not the first (p=0.182). Similarly, cannabis users showed a trend towards longer time to reporting first pain than controls at CPT2 (p=0.057), but not at CPT1 (p=0.89). There were no significant differences in ratings of the intensity of pain between groups or between CPTs. Interestingly, cannabis users displayed elevated plasma levels of anandamide (p=0.016), oleoylethanolamide (p=0.008), linoleyl-ethanolamide (0.011), and palmitoylethanolamide (p=0.037) relative to controls. There were no group differences in 2-arachidonoylglyceraol levels. Some of the endocannabinoids increased over time, possibly in response to CPT stress. Anandamide was significantly elevated over controls at bloods 3 and 4 (flanking CPT2, post hoc p=0.007 and 0.003 respectively). Palmitoylethanolamide was elevated compared to controls at bloods 2 and 4 (immediately after both CPT immersions, post hoc p = 0.041 and 0.037 respectively). Plasma levels of THC and THC-COOH in cannabis users did not change with CPT exposure. Cannabis users had lower plasma cortisol than controls (p=0.042), and only controls showed significantly increased cortisol in response to the two CPTs (p=0.041).

These pilot results, somewhat unexpectedly, show that cannabis users had *greater* pain tolerance and reduced pain sensitivity than controls, blunted basal and stress-induced cortisol responses, as well as elevated basal levels of some endocannabinoids with a possible endocannabinoid response to pain.

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HEAVY CANNABIS USE IS ASSOCIATED WITH A BLUNTED STRESS RESPONSE AND REDUCED RELIANCE ON TOP-DOWN ATTENTIONAL CONTROL

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One of the most common reasons people report using cannabis is to cope with stress. Furthermore, elevated levels of stress can be associated with cognitive dysfunction. Yet to our knowledge no previous researchers have compared the stress response of cannabis users and non-users under conditions of acute stress or examined the cognitive test performance of cannabis users under conditions of acute stress. We used a 2 (stress, no stress) x 2 (cannabis users, non-users) between-groups factorial design to determine whether cannabis use is associated with an altered stress response and to determine whether stress exacerbates cognitive impairments in cannabis users. We sampled 40 non-users (used cannabis < 10 times in life, no use in past year, and negative urine THC test) and 39 heavy cannabis users (used cannabis daily/near daily for > 1 year and positive THC test) from the community. Participants were screened for medical, psychiatric, and neurological conditions; learning and intellectual disabilities; heavy use of alcohol; and use of illicit substances in the past 6 months. Further, cannabis users were required to refrain from using cannabis on the day of testing. Half of the non-users and approximately half of the cannabis users were randomly assigned to a stress condition which required participants to place their hand in ice cold water for varying intervals of time. Between these trials, participants were required to perform difficult math under conditions of social evaluation while observing themselves on a monitor. The remaining participants completed a no stress condition in which they were required to place their hand in lukewarm water for the same time intervals, and, in between trials, they were required to count from 1 to 20 without social evaluation or self-monitoring. Immediately following the stress manipulation, two computerized tests of cognitive flexibility were administered. Salivary cortisol and subjective stress ratings were collected before, during, and after the stress manipulation.

Results revealed a significant stress x cannabis use interaction on salivary cortisol and subjective stress ratings. Specifically, non-users in the stress condition demonstrated a significant increase in subjective stress ratings and salivary cortisol relative to those in the no stress condition. In contrast, the stress manipulation had no effect on cannabis users' cortisol levels, and cannabis users showed a significantly smaller increase in subjective stress ratings relative to non-users. Moreover, in the no stress condition, non-users showed a reliance on top-down attentional control when it was advantageous, while users did not. In contrast, in the stress condition the use of top-down attentional control was low for both users and non-users. While the cannabis users exhibited an overall decreased reliance on top-down attentional control, their overall performance did not suffer. Rather, cannabis users appear to utilize a different strategy that allows them to perform comparably without relying on top-down attentional control. In conclusion, the results suggest that chronic cannabis use is associated with a blunted stress response and a reduced reliance on top-down attentional control that does not cause overall cognitive performance to suffer.

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REDUCED ENDOCANNABINOID METABOLISM IN PSYCHOSIS AND CANNABIS USE: A PILOT STUDY IMAGING FATTY ACID AMIDE HYDROLASE WITH [^{11}C]CURB PET

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Objective: The endocannabinoid (eCB) system modulates brain responses to factors related to psychosis risk and relapse including cannabis and stress. To date, in vivo imaging of the eCB system in psychosis is limited to the CB1 receptor, which is altered both in psychosis and in cannabis use. No other component of the eCB system has been investigated in the brain in vivo in psychosis. We aim to investigate the eCB system in untreated first episode psychosis (FEP) patients using positron emission tomography (PET) imaging with [^{11}C]CURB, a ligand for Fatty Acid amide Hydrolase (FAAH), the enzyme responsible for setting brain levels of anandamide.

Methods: In this pilot study, we recruited antipsychotic-naïve FEP and demographically-matched healthy control (HC) participants, and healthy cannabis users. Diagnosis of FEP participants was confirmed by a psychiatrist (RM) and active symptoms were confirmed by clinical interview. HV had no current or past DSM IV axis I diagnosis (except cannabis-use disorder in cannabis users) and no family history of psychotic disorders.

Each participant underwent a 60-minute [^{11}C]CURB PET scan on a high resolution PET scanner and a structural MRI scan. [^{11}C]CURB binding (λk_3) was calculated using an irreversible 2-tissue compartment model with a metabolite-corrected arterial plasma input function. The FAAH rs324420 variant affects FAAH protein levels: carriers of the minor A-allele exhibit lower levels of FAAH. Thus we determined the FAAH rs324420 genotype for all participants. Preliminary data were analyzed using an analysis of covariance design, controlling for rs324420 genotype.

Results: Preliminary analysis revealed main effects of group on [^{11}C]CURB binding such that binding was lower in the FEP and in cannabis users than in HC. Reductions of FAAH in FEP and cannabis users were observed in overlapping (but not identical) limbic, basal ganglia and cortical regions. Across all groups, carriers of the rs324420 A-allele exhibited lower [^{11}C]CURB binding than in individuals with the rs324420 C/C genotype.

Discussion: Data from this pilot sample provide the first *in vivo* evidence that FAAH is altered in early psychosis. These results suggest that reductions of FAAH are present early in psychosis, and that chronic cannabis users also exhibit broad reductions of FAAH. An expanded sample is required to better understand the involvement of eCB-metabolizing enzymes in psychosis and cannabis use.

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ADOLESCENT CANNABIS CONSUMPTION AND THE RISK OF LATER DEPRESSION: SYSTEMATIC REVIEW AND META-ANALYSIS

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Preclinical studies in animals have demonstrated that chronic exposure to cannabinoids during adolescence but not during adulthood produces an increase in anhedonia. depressive-like behavior and anxiety in adulthood, paralleled by a decrease of the firing rate of serotonergic neurons (Bambico et al., *Neurobiology of Disease*, 2010). Though several epidemiological studies have explored the link between adolescence cannabis consumption and mental health, no systematic reviews or meta-analyses to date have examined longitudinal epidemiological studies of human adolescents in order to discern whether cannabis consumption during adolescence is related to an increase in depressive symptoms during adulthood.

A systematic search, conducted by a librarian following the IOM standards (Institute of Medicine, U.S., 2011) identified 3188 articles. Preliminary data analysis has focused on 22 papers with N ranging from 554 to 85,088, published between 1977 and 2013. Fourteen studies are positive and demonstrate a significant correlation between increased cannabis consumption during adolescence and increased later depressive symptoms. Seven studies are negative, and two are equivocal. Two studies found opposite effects in men and women.

We carried out a random effects meta-analysis of these data. All analyses were conducted in Stata statistical software version 11.2 (StataCorp LP, USA). Preliminary data suggests that adolescent consumption of cannabis is a significant predictor of later depressive symptoms. The effects of adolescent cannabis consumption on suicidality are less consistent and more research will be necessary in order to elucidate a connection.

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RANDOMISED CONTROLLED TRIAL (RCT) OF DAILY AEROBIC EXERCISE FOR INPATIENT CANNABIS WITHDRAWAL

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Introduction: Increasing evidence exists for the benefits of regular exercise on mood, wellbeing and general health. The effects on brain structure and function include boosting proliferation and neurogenesis and increased volume of brain regions involved in mood and cognition. Emerging literature indicates that exercise assists in the clinical management of brain disorders and to have equivalent efficacy to antidepressants in improving mood for mild to moderate depression, and improve the management of drug dependence. Cannabis withdrawal is thought to be a major contributor in relapse to cannabis use and is now included in the DSM-5. Aerobic exercise relieves withdrawal symptoms from tobacco and other drugs, but has yet to be tested in cannabis users. To address this, a RCT was developed to examine whether aerobic exercise can ameliorate the symptoms of cannabis withdrawal in a cannabis-dependent population undergoing inpatient detoxification, and improve treatment outcomes for cannabis dependence.

Method: A single blind, parallel two-group RCT compared a structured daily aerobic exercise intervention to a control stretching intervention during a seven-day inpatient hospital admission, with follow-up at 28-days post-discharge. Participants in the intervention group underwent 35 minutes of aerobic exercise daily, at 60% of their VO_2 Max. Control group engaged in a structured non-aerobic stretching routine for 35 minutes. The primary outcome measure is the severity of cannabis withdrawal symptoms assessed daily using the Cannabis Withdrawal Scale and Marijuana Cravings Questionnaire, across the week. Mechanisms by which exercise may affect cannabis withdrawal were assessed by analyzing endogenous cannabinoids, plasma and urine cannabinoid levels.

Results: Forty-six cannabis dependent users, used $\overline{x}$ 1.62 (1.07SD) grams/day over a $\overline{x}$ of 19 years completed the 7-day inpatient detox. Mean age of participants was 35.49 (11.82SD) years, with BMI 23.93 (4.11SD). Twenty-five were randomized to daily aerobic exercise. Overall, patients who underwent the intervention arm (exercise) reported lower cannabis withdrawal symptoms over the 7-days compared to the control group $F_{1,53.20}=3.74$, $P<0.05$, reporting significant improvements in irritability [$F_{1,56.13}=6.16$, $P<0.05$], anxiety [$F_{1,51.58}=4.32$, $P<0.05$], sleep difficulty [$F_{1,53.24}=6.60$, $P<0.01$] and appetite [$F_{1,52.85}=4.00$, $P<0.05$].

Conclusion: The findings of this pioneering RCT of aerobic exercise for cannabis dependence has important implications for the treatment of cannabis and other drug withdrawals as an effective, inexpensive and accessible treatment approach.

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COX-2 OXYGENATION OF 2-AG CAUSES A STROKE-LIKE EVENT FOLLOWING SEIZURES

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A growing literature implicates cyclooxygenase-2 (COX-2) as an important metabolic enzyme for endocannabinoids. Unlike the conventional endocannabinoid metabolic pathways that liberate arachadonic acid (AA), oxygenation of AEA and 2-AG by COX-2 generates the novel lipid signalling molecules prostaglandin-ethanolamides (PG-EAs) and prostaglandin–glycerol esters (PG-Gs), respectively. Conditions for brain production of PG-EAs and PG-Gs remain elusive, as well as their potential roles in downstream signalling. We hypothesize that seizures recruit the activity-dependent processes of 2-AG synthesis and COX-2 activation to promote the production of PG-Gs. The resulting PG-Gs may play a central role in a mediating a novel post-seizure stroke-like event.

Our lab previously identified that following seizures, there is a local hypoperfusion/hypoxia event in brain that lasts for over an hour in animal models and people with epilepsy. This phenomenon was blocked by pharmacological and genetic COX-2 inhibition. The degree to which COX-2 oxygenates endocannabinoids or its conventional substrate, AA, is unknown. We tested this by using substrate-selective COX-2 inhibitors that prevent endocannabinoid, but not AA, oxygenation. These inhibitors prevented hypoxia after hippocampal seizures and this suggests a central role for endocannabinoid oxygenation. To understand which endocannabinoid was responsible for post-seizure hypoxia, we inhibited the well-known metabolic pathways of AEA (FAAH) or 2-AG (MAGL or ABHD6) to boost AEA or 2-AG levels prior to seizure induction. Since only ABHD6 inhibition exacerbated hypoxia after seizures, 2-AG was identified as the candidate COX-2 substrate. To further investigate this, we measured hippocampal AEA and 2-AG immediately after single and four consecutive seizures. Seizures elevated levels of AEA in both paradigms, but did not increase 2-AG levels after the fourth seizure. Though not statistically significant, it is possible that less 2-AG is available to COX-2 after the fourth seizure. Relative to a single seizure, post-seizure hypoxia was less severe after a fourth, which warrants further investigation into changes in 2-AG substrate availability with repeated seizures. Together, these data suggest that 2-AG is oxygenated by COX-2 during seizure activity leading to the production of PG-Gs, which act on local blood vessels to cause vasoconstriction. These data advance our growing understanding of COX-2 and endocannabinoid interaction and could lead to more specialized treatments for epilepsy.

THE FATTY ACID AMIDE HYDROLASE INHIBITOR URB597 SUPPRESSES EPILEPTIC SEIZURES AND DOES NOT ALTER SYNAPTIC PLASTICITY AT THE PERFORANT PATH-DENTATE GYRUS SYNAPSES

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Temporal lobe epilepsy is frequently associated with learning and memory deficits. Antiepileptic drugs mainly act to control seizures, with little impact on comorbid cognitive impairments. Cannabinoids have been implicated for the treatment of several neurological disorders associated with abnormal neuronal excitability, such as epilepsy. Synthetic cannabinoids and phytocannabinoids have been shown to suppress seizures in both humans and experimental models of epilepsy. However, they generally have detrimental effects on memory. Endogenous cannabinoids (eCBs) act as a key regulators of glutamate and GABA release providing protection against excessive neuronal activity, for example during epileptic seizures, and modulate different forms of short- and long-term synaptic plasticity of excitatory and inhibitory neurotransmission. Here, we examined the antiseizure effect of the fatty acid amide hydrolase inhibitor, URB597 in the maximal dentate activation model of limbic seizures in anesthetised rats. Then, we investigated the effect of URB597 on short and long-term plasticity at perforant path–dentate granule cell synapses in normal condition and after repeated seizures. Finally, we contrasted these effects with the non-selective direct cannabinoid agonist WIN 55,212-2 (WIN). The involvement of the CB1 receptor was evaluated by blocking URB597 and WIN effects by using AM251.

We found that URB597 and WIN showed anti-seizure effects. WIN impaired long-term potentiation and affected paired-pulse facilitation at perforant path-dentate gyrus synapses when tested with the dose that showed anti-seizure effect. Interestingly, the anti-seizure dose of URB597 did not affect either neuronal excitability or short and long term synaptic plasticity. Strikingly, URB597 administered during the maximal dentate activation, prevented seizure-induced alterations of paired pulse facilitation and the seizure-induced impairment of long-term potentiation in its early phase. These data suggest that boosting the eCB tone rather than a general CB1 activation might represent a potential target for the development a new class of drugs for treatment of seizures and the comorbid memory alterations associated with epilepsy.

CANNABINOID-BASED THERAPEUTICS AS ANTI-EPILEPTIC STRATEGY FOR DRAVET SYNDROME

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The life-threatening pediatric encephalopathy Dravet Syndrome (DS) affects 1:15,700 individuals and is unresponsive to standard-of-care treatment. This severe childhood disorder is caused by loss-of-function mutations in the *SCN1A* gene the encodes for voltage-gated brain sodium channels $Na_V1.1$. A large body of scientific evidence has accumulated since the 1980_s indicating that cannabinoids exert remarkable anti-seizure properties in preclinical rodent models and more recently in human patients with epilepsy including DS.

We studied the anti-seizure activity of cannabidiol (CBD) in *Scn1a*$^{+/-}$ mice, a validated preclinical genetic mouse model of DS, and found that acute treatments with CBD (100 or 200 mg/Kg, i.p.) significantly reduced both the frequency of spontaneous seizures and duration and severity of thermally-induced seizures. Twice daily treatment with similar doses of CBD over the period of maximum sudden expected death in epilepsy (SUDEP) risk, 21-28 days, diminished lethality by 75%. Electrophysiological studies show that phenotypic rescue of seizures was associated with restoration of inhibitory-interneuron excitability in the hippocampal dentate gyrus through antagonism of GPR55 receptors. Remarkably, treatment with acute lower doses of CBD (10 mg/kg, i.p.) improved autism-like social interaction deficits in DS mice as measured by the proportion of time spent interacting with the stranger mouse in the 3-Chamber Test. These results provide critical pre-clinical support for the efficacy of CBD in DS. These results also introduce inhibition of GPR55 as a possible therapeutic approach to provide relief from DS.

α/β-hydrolase domain 6 (ABHD6) hydrolyses 2-arachidonoylglycerol (2-AG) and as such controls the levels and efficacy of this endocannabinoid at CB_1, CB_2 and $GABA_A$ receptors. We previously reported that ABHD6 inhibitors decrease seizure incidence and severity in the pentylenetetrazole (PTZ)-induced generalized tonic-clonic and myoclonic seizure mouse model without producing overt side effects or losing efficacy resulting from acquired tolerance. Our most recent preliminary results obtained with the newest generation of orally bioavailable ABHD6 inhibitor, KT-182 (Hsu et al. 2013 J. Med Chem. 56: 8270-9), show that this therapeutic approach also reduces seizures in *Scn1a*$^{+/-}$ mice. Current efforts are dedicated to establish an optimal dosing regimen for KT-182 to reduce seizures and possibly behavioral impairments, as well as determine the cellular mechanism of action and molecular target involved in the anti-seizure properties of ABHD6 inhibition. Together our studies on CBD and ABHD6 inhibitors highlight the promises of cannabinoid-based therapeutics for the safe and durable treatment of DS.

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GW PHARMACEUTICALS' CANNABIDIOL (CBD) CLINICAL PROGRAMME IN EPILEPSY

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The purpose of this talk is to present an update on GW clinical trials, with a focus on data from the epilepsy programme with cannabidiol (CBD).

GW has partnered with leading paediatric and adult epilepsy clinicians to provide CBD through an extensive, FDA authorised, Expanded Access Programme (EAP) that includes >20 physician-sponsored Investigational New Drug applications (INDs) and 6 state-sponsored INDs. On the basis of initial open-label data from the EAP, GW initiated the GWPCARE Phase III clinical trial programme to assess CBD's safety and efficacy for the treatment of seizures associated with childhood onset epilepsy disorders that are not adequately controlled with current antiepileptic drugs (AEDs).

The GWPCARE Phase III clinical trial programme includes six double-blind, placebo-controlled studies evaluating the safety and efficacy of Epidiolex as adjunctive therapy across four epilepsy indications. In addition, patients who complete the pivotal trials may participate in open-label extension studies to evaluate long-term outcomes with CBD. The GWPCARE trials are as follows:

– GWPCARE1 and GWPCARE2: Dravet syndrome (DS)
– GWPCARE3 and GWPCARE4: Lennox-Gastaut syndrome (LGS)
– GWPCARE5: Open-label extension (OLE) for patients in GWPCARE1,2,3,4
– GWPCARE6: Tuberous sclerosis complex (TSC); includes OLE
– GWPCARE7: Infantile spasms (IS Pilot); includes OLE

GWPCARE1, GWPCARE3, and GWPCARE4 have been completed. Data from these trials suggest that adjunctive cannabidiol may lead to significant seizure reductions in patients with treatment-resistant epilepsy, with more adverse events than placebo but generally well tolerated.

Acknowledgements: GWPCARE studies were funded by GW Research, Ltd.

PODOCYTE-SPECIFIC DELETION OF CANNABINOID-1 RECEPTOR (CB1R) IS PROTECTIVE AGAINST HYPERGLYCEMIA-INDUCED DIABETIC NEPHROPATHY

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Diabetic nephropathy, a highly prevalent and serious complication of type-2 diabetes (T2D) and a leading cause of renal failure, is characterized by albuminuria, decreased glomerular filtration rate (GFR), mesangial expansion, thickening of the glomerular basement membrane and glomerular sclerosis. Multiple mechanisms have been implicated in the development of diabetic nephropathy including activation of the renin-angiotensin system (RAS), increase in oxidative and nitrosative/nitrative stress, as well as an increase in local inflammation. We previously reported that CB1R activation in podocytes mediates the deleterious effects of both hyperglycemia and increased RAS activity on podocyte functions in a rat model of T2D, which could be reversed by treatment with the peripherally-restricted CB1R antagonist JD5037. Here we report that mice with podocyte-specific deletion of CB1R (pCB1Rko) are partially protected against streptozotocin (STZ)-induced diabetic nephropathy.

pCB1Rko mice were generated by breeding the B6.Cg-Tg$^{(NPHS2\text{-}cre)295Lbh/J}$ mouse with a CB1R$^{flx/flx}$ mouse. STZ-induced hyperglycemia was similar in pCB1Rko mice and their wild-type littermates (WT), but by 12 weeks of chronic hyperglycemia, pCB1Rko mice displayed less proteinuria and albuminuria and higher glomerular filtration rate (GFR) than STZ-treated WT mice, suggesting better glomerular and podocyte function. No difference in these parameters was observed in the absence of STZ treatment. In parallel, diabetic pCB1Rko mice were protected from podocyte loss, as demonstrated by preservation of Wilms' tumor-1 (WT-1) and podocalyxin immunostaining, compared to reduced levels in diabetic control mice. In addition, the tuft area was increased in diabetic control but not pCB1Rko mice. These histological observations were confirmed by parallel changes in podocyte-specific gene expression. Indeed, the diabetes-induced decrease in the expression of genes encoding for podocin, nephrin and zonula occludens and increase in desmin expression in control mice was absent in pCB1Rko mice. Unexpectedly, pCB1Rko mice were also protected from diabetes-induced tubular dysfunction, as manifested in less severe polyuria, glycosuria, and elevated urinary N-acetylglucosamine (NAG) activity and increased expression of the proximal tubular cell marker megalin in renal cortices. We conclude that specific targeting of CB1R in podocytes could represent a therapeutic approach for diabetic nephropathy.

CB_1R INHIBITS AMPK-DERIVED FATTY ACID UTILIZATION IN PROXIMAL TUBULES LEADING TO OBESITY-INDUCED RENAL DYSFUNCTION

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Obesity-related renal structural and functional changes develop early in the course of obesity, independently of its association with diabetes, hypertension, and dyslipidemia, justifying the search for novel regulators that could then be targeted for therapy. Over-activation of the endocannabinoid (eCB)/CB_1R system in obesity plays an important role in the onset of nephropathy, whereas blockade of CB_1R by synthetic antagonists are known to improve renal function.

To specifically define the involvement of CB_1R in obesity-induced renal dysfunction, we generated a mouse strain that lacks CB_1R in the renal proximal tubular cells (RPTCs), which are critically important for normal kidney function. These cells are responsible for the active reabsorption made in the kidney by a mechanism requiring a large amount of energy mainly derived from fatty acid β-oxidation. The latter is regulated by a molecular mechanism that involves the cellular energy and redox sensor, AMP-activated protein kinase (AMPK).

Here, we fully identify the role of the proximal tubular CB_1R in the development of obesity-induced nephropathy by demonstrating that proximal tubule CB_1R plays a crucial role in renal lipotoxicity by inhibiting the AMPK signaling pathway, leading to decreased fatty acid β-oxidation and increased intracellular fat accumulation in the RPTCs. This, in turn, produces impairment in renal structure, function, and homeostasis. All of these effects were not associated with the metabolic role of RPTC-CB_1R in the development of obesity and its comorbidities.

Collectively, our work could provide a novel therapeutic approach to slow down the development of renal injury through chronic blockade of peripheral CB_1R, and would further support strategies aimed at reducing the activity of the eCB system, specifically in the treatment of obesity-induced nephropathy.

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NEURONAL DELETION OF α/β-HYDROLASE DOMAIN 6 IN THE NUCLEUS ACCUMBENS PREVENTS DIET-INDUCED OBESITY

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Nucleus accumbens (NAc) GABAergic medium spiny neurons (MSNs) figure prominently in the control of appetite and body weight regulation. The activity of NAc MSNs is strongly modulated by endocannabinoid (eCB) signals and manipulations of eCB in the NAc are well-established to affect food-motivated behaviour. The accumulation of 2-arachidonoyl-glycerol (2-AG) and its signalling actions are modulated in part by serine hydrolase α/β-hydrolase domain 6 (ABHD6) which degrades 2-AG at the site of synthesis to act as a gatekeeper of 2-AG pools. Complete ABHD6 loss-of-function protects against diet–induced obesity in mice; however, if and how central ABHD6 contributes to these catabolic actions is not known. In the present study we investigated the effects of prolonged (4 weeks) central infusion of the ABHD6 inhibitor WWL70 on food intake, energy expenditure and body weight gain in high-fat mice. In addition, we studied the impact of ABHD6 knockout in NAc neurons of adult mice (Cre-Lox: viral injections of AAV.Syn.GFP.Cre into ABHD6$^{lox/lox}$ mice) on the same parameters and operant responding for food rewards, and we recorded the activity of inhibitory and excitatory input onto NAc MSNs in slice preparations.

Central pharmacological inhibition of central ABHD6 significantly reduced weight gain in high-fat fed male mice via a reducing food intake and increasing expenditure. In addition, knockout of ABHD6 in NAc neurons of adult mice (viral injections of AAV-Cre-GFP into ABHD6-LoxP mice), that increased NAc 2-AG levels, blunted inhibitory postsynaptic potentials, suppressed food reward, increased energy expenditure and prevented diet-induced obesity. Together, our data suggest that central ABHD6 inhibition has catabolic actions mediated by ABHD6 in the NAc to control feeding, energy expenditure and food-motivated behaviour via increased NAc 2-AG, GABA-CB1R signalling and suppression of GABAergic tone.

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DINNER FOR TWO: DISENTAGLING THE INTERACTION BETWEEN GHRELIN AND ENDOCANNABINOID SYSTEMS IN MODULATING FEEDING WITHIN THE VTA

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Ghrelin targets growth hormone secretagogue receptors (GHSR) in the brain to stimulate feeding behaviours. Its ability to induce feeding circuits in the hypothalamus (HYP), a brain region integral to the modulation of feeding, is well understood. Ghrelin induced activation of GHSRs in the ventral tegmental area (VTA), a reward related brain region, also stimulates food seeking and consumption. Endocannabinoids, like ghrelin, stimulate appetite upon binding cannabinoid 1 receptors (CB-1Rs) within the HYP. Interestingly, a functional endocannabinoid system (ECS) is also necessary for the orexigenic effects elicited following ghrelin's infusion into the HYP. Given that cannabinoid receptors (CB-1Rs) are likewise expressed within the VTA and that independent modulation of the ECS within this region influences feeding behaviours, we set out to elucidate if and how ghrelin and endocannabinoid systems might interact within the VTA and the role that this putative interaction may have in regulating feeding behaviours. To this end, we first examined if there were inherent differences in endocannabinoids and/or mRNA of important ECS proteins between WT and GHSR KO rats. Indeed, 2-AG levels were found to be significantly lower in GHSR KO relative to WTs rats as were the transcripts for CB-1R, DAGL-α, and FAAH. Second, similar to the experiments conducted in the HYP, we were interested in whether modulation of the endocannabinoid system would influence the ability of ghrelin to stimulate feeding when infused into the VTA. Interestingly, peripheral injection of rimonabant (i.e. CB-1R antagonists) efficiently blocked intra-VTA ghrelin induced feeding. Given the aforementioned data, we hypothesized that ghrelin and endocannabinoid systems may work collaboratively within the VTA to stimulate feeding behaviours. To examine the relationship between these two systems specifically within the VTA we have recently conducted experiments where we pharmacologically blocked CB-1Rs in the VTA before infusing ghrelin within this region. Preliminary data, does not appear to support our original hypothesis as intra-VTA rimonabant unexpectedly enhanced the short term feeding effects of intra-VTA ghrelin. Due to small sample sizes, efforts are underway to confirm these findings.

ENDOCANNABINOID MODULATION OF ACUTE STRESS-INDUCED ANOREXIA IN RATS

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Acute stress is known to cause a reduction in food intake. This effect is modulated by factors such as stressor intensity, as well as motivation to feed, i.e. hunger/satiety. Many aspects of the stress response are regulated by the endocannabinoid (eCB) system, as is the control of food intake, but it is unclear whether the eCB system regulates stress-induced anorexia. We investigated food intake and body weight in stressed and unstressed male Sprague-Dawley rats (400-450g) by blocking the degradation of 2-AG/anandamide with specific monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) inhibitors.

Rats were administered the MAGL inhibitor, MJN110, or the FAAH inhibitor, PF04457845, by intraperitoneal injection (ip) or intracerebroventricular (icv) infusion prior to an acute psychological stressor (restraint stress). Post-stress feeding coincided with the onset of the dark cycle, and intake of regular lab diet (Prolab RMH 2500) was measured throughout the post-stress and post-treatment periods (22hr). Body weights were measured for 3 consecutive days in either condition.

Following 2hr restraint stress, animals consumed significantly less chow (35% of unstressed controls) within the first hour of re-feeding. Food intake was unaffected by systemic PF04457845 (10 mg/kg, ip); in contrast, MJN110 (10 mg/kg, ip) administration reduced feeding in both stressed (81% of controls) and unstressed animals (71% of controls) through the 22hr post-stress/treatment period. Remarkably, despite reducing feeding, MAGL inhibition paradoxically increased body weight gain among animals exposed to stress (+4.7g for MJN110; -2.1g for vehicle). Central (icv) administration of MJN110 (5 μg) or PF04457845 (3 μg) alone did not modulate feeding or weight gain in either stressed or unstressed conditions. However, dual FAAH/MAGL inhibition increased feeding in unstressed rats (157% of controls). Interestingly, a higher dose of PF04457845 (30μg, icv) attenuated stress-induced anorexia through a CB_1-dependent mechanism, yet reduced feeding in unstressed rats independent of CB_1-signaling.

Taken together, these results suggest that: 1) FAAH/MAGL inhibition modulates food intake and stress-induced changes in feeding through distinct central/peripheral mechanisms; and 2) central administration of a high dose of a FAAH inhibitor can significantly attenuate the anorectic effect of acute stress through a CB_1 receptor dependent mechanism, while in unstressed animals can reduce food intake through a CB_1 receptor-independent pathway. Current experiments are exploring the mechanism(s) of action underlying these paradoxical effects of FAAH/MAGL inhibition on stress-induced and baseline feeding behaviour.

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

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The orbitofrontal cortex (OFC) plays a key role in the cognitive and emotional processing of decision-making. Dysfunction of the OFC is thought to underlie compulsive behaviours, including obsessive-compulsive disorder, drug and behavioural addictions. Prior evidence suggests that the endogenous cannabinoid (endocannabinoid) system is involved in regulating cognitive/emotional flexibility in the prefrontal cortex; however, its specific role in the OFC has yet to be confirmed. Given that obesity is linked to an overactive endocannabinoid system, we investigated whether it mediates compulsive-like overeating via actions within the OFC.
Using *in-vitro* patch clamp electrophysiology, we show that CCK-expressing, inhibitory GABAergic synaptic inputs onto layer II/III OFC pyramidal neurons are sensitive to endocannabinoids. Specifically, they exhibit depolarization-induced suppression of inhibition (DSI) that is blocked by the cannabinoid CB1 receptor inverse agonist, AM251, and the diacylglycerol lipase inhibitor, tetrahydrolipstatin (Orlistat). Furthermore, OFC pyramidal neurons exhibit endocannabinoid-mediated long-term depression; however, there is a switch to long-term potentiation following blockade of CB1 receptors.

Since cannabinoids are associated with increased hunger for palatable food, we examined if extended (24 hr) or restricted (1 hr) access to a cafeteria "junk food" diet altered endocannabinoid signaling in the OFC. Interestingly, we found there was a reduction of inhibitory GABAergic transmission onto OFC pyramidal neurons in animals with extended, but not restricted access to a cafeteria diet. This suppression of inhibition was partly reversed by the neutral CB1 receptor antagonist, NESS-0327, indicating the presence of an endocannabinoid tone. This was associated with alterations in endocannabinoid-mediated short- and long-term synaptic plasticity. Together, our results suggest that enhanced endocannabinoid signalling and basal tone partially mediate a GABAergic disinhibition of OFC pyramidal neurons following prolonged exposure to a palatable diet. The resulting hyperexcitability of the OFC is thought to drive compulsive-like overeating.

ENDOCANNABINOID SIGNALING IN THE LATERAL HABENULA: IMPLICATIONS FOR STRESS COPING AND DOPAMINERGIC TRANSMISSION

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The ability to effectively cope with stress is a critical determinant of disease susceptibility. The lateral habenula (LHb) fundamentally guides the selection of motor strategies in anticipation of aversive stimuli by suppressing the activity of midbrain monoamine neurons, most notably dopamine (DA) neurons in the ventral tegmental area (VTA). Notably, the LHb is hyperactive in depressed patients and rodents subjected to chronic stress, while deep brain stimulation of the LHb alleviates depression-related behaviors. Although the precise mechanisms responsible for LHb hyperexcitability remain unknown, one intriguing possibility is through stress-induced alterations in the endocannabinoid (ECB) system within the LHb.

We systematically examined the effects of acute and chronic stress on the ECB system in the LHb of male and female rats, and tested whether site-specific pharmacological manipulations of CB1R signaling in the LHb alters stress coping, anxiety-like behavior, and the firing activity of VTA DA neurons *in vivo*. Electron microscopy analyses point to a diverse expression profile of CB1Rs in the LHb, the *ex vivo* activation of which suppresses both excitatory and inhibitory postsynaptic currents. We further show that acute immobilization stress preferentially increases the extracellular concentration of 2-AG (but not AEA) in the LHb, and accordingly, exposure to chronic unpredictable stress (CUS) increases LHb 2-AG content in both male and female rats. This 2-AG enhancement is likely driven by CUS-induced adaptations in the activity of monoacylglycerol lipase (MGL), as we found a significant increase in the K_m of MGL for its substrate (i.e., decrease in affinity) compared to control rats. Intra-LHb CB1R activation robustly increased immobility time in the forced swim test, while local blockade reduced immobility and increased selection of escape-directed coping strategies. Moreover, intra-LHb CB1R blockade increased open arm exploration in the elevated plus maze and decreased the latency to consume a palatable treat (without affecting general consummatory behavior) in the novelty-suppressed feeding test in males only. Intra-LHb CB1R blockade also significantly increased the latency to be attacked in the resident-intruder paradigm, and increased and decreased approach and avoidance behavior, respectively, in both stressed and non-stressed rats. Finally, intra-LHb CB1R activation modestly decreased the firing rate of a subset of VTA DA neurons, whereas intra-LHb CB1R blockade led to a pronounced reduction in VTA DA firing *in vivo*.

Overall, these data indicate that stress exposure increases 2-AG/CB1R signaling in the LHb, which is likely due to stress-induced alterations in the affinity of MGL for 2-AG. Local CB1R blockade increases selection of proactive stress coping strategies, reduces social avoidance and anxiety-like behavior, and increases VTA DA firing. Follow-up studies are now examining whether local manipulations of 2-AG/CB1R signaling in the LHb are necessary and/or sufficient to produce CUS-induced deficits in stress coping, anxiety-like behavior, and VTA DA activity.

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FUNCTIONAL REDUNDANCY BETWEEN CANONICAL ENDOCANNABINOID SIGNALING SYSTEMS IN THE MODULATION OF ANXIETY

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Increasing the available repertoire of effective treatments for mood and anxiety disorders represents a critical unmet need. Pharmacological augmentation of endogenous cannabinoid (eCB) signaling has been suggested to represent a novel approach to the treatment of anxiety disorders; however, the functional interactions between two canonical eCB pathways mediated via anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in the regulation of anxiety are not well understood. We utilized pharmacological augmentation and depletion combined with behavioral and electrophysiological approaches to probe the role of 2-AG signaling in the modulation of stress-induced anxiety, and the functional redundancy between AEA and 2-AG signaling in the modulation of anxiety-like behaviors in mice. Selective 2-AG augmentation reduced anxiety in the light-dark box assay, and prevented stress-induced increases in anxiety associated with limbic AEA deficiency. In contrast, acute 2-AG depletion increased anxiety-like behaviors, which was normalized by selective pharmacological augmentation of AEA signaling and via direct CB1 receptor stimulation with tetrahydrocannabinol (THC). Electrophysiological studies revealed 2-AG modulation of amygdala glutamatergic transmission as a key synaptic correlate of the anxiolytic effects of 2-AG augmentation. Although AEA and 2-AG likely subserve distinct physiological roles, a pharmacological and functional redundancy between these canonical eCB signaling pathways exists in the modulation of anxiety-like behaviors. These data support development of eCB-based treatment approaches for mood and anxiety disorders and suggest a potentially wider therapeutic overlap between AEA and 2-AG augmentation approaches than previously appreciated.

INTERACTIONS OF ENDOCANNABINOIDS ANANDAMIDE AND 2-ARACHIDONOYLGLYCEROL IN THE REGULATION OF BEHAVIORAL RESPONSES TO TRAUMATIC EVENTS

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Endocannabinoid (eCB) signaling is a major contributor in the regulation of responses to traumatic events. The specific role of eCBs anandamide (AEA) and 2-arachidonoilglycerol (2-AG), however, are still to be clarified, particularly as recent data suggest that i.) they differentially affect particular aspects of behavior and ii.) their signaling show functional interactions. Here we aimed to describe specific eCB involvement and possible interactions in the regulation of acute responses to trauma and dynamics of traumatic memory.

Rats received a series of traumatic footshocks during which acute fear responses were measured, then they were reintroduced to the footshock context daily for seven days for assessment of acquisition and extinction of traumatic memories. Firstly, eCB impact on acute fear and traumatic memory acquisition was studied by systemic pharmacological enhancement of AEA and/or 2-AG signaling prior to traumatization *via* the blockade of their respective degrading enzymes fatty acid amide hydrolase and monoacylglycerol lipase. The involvement of ventral hippocampal (vHC), prelimbic (PrL) and basolateral amygdalar (BLA) eCB signaling was specifically studied as well employing local pharmacological treatments. Secondly, eCB effects on traumatic memory extinction were assessed similarly except the time of treatments was prior to the first contextual reminder.

Acute fear responses were dampened by systemically enhanced 2-AG signaling which effect was abolished by AEA. Interestingly, enhanced AEA, but not 2-AG signaling in the vHC had similar effects while no eCB effects on acute fear responses occurred at the PRL or BLA. Acquisition of traumatic memories were strengthened by systemically enhanced AEA signaling, an effect abolished by 2-AG. AEA enhancement in the vHC and PRL led to similar effects, while interestingly BLA AEA enhancement prevented traumatic memory formation. Extinction of traumatic memories was facilitated by enhanced AEA and 2-AG signaling as well.

Our findings are the first to show that AEA and 2-AG functionally interact in the regulation of behavioral processes, particularly, behavioral responses to trauma. While acute fear responses to trauma are possibly regulated by a complex interaction between vHC, PRL and BLA eCB signaling mechanisms, acquisition of traumatic memories are predominantly regulated by vHC and PRL AEA signaling processes under the control of 2-AG signaling mechanisms.

VOLUNTARY EXERCISE IN MICE ENHANCES THE EXTINCTION OF FEAR AND INCREASES ENDOCANNBINOID CONCENTRATIONS IN THE AMYGDALA

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Introduction: Treatments that facilitate extinction learning may help to enhance extinction-based exposure therapies for the treatment of stress and anxiety disorders, such as Post-Traumatic Stress Disorder (PTSD). Pharmacological augmentation of the endocannabinoid (eCB) system has been shown to improve extinction learning in animals and humans (Mariscano et al., 2002; Rabinak et al., 2014). Although previous investigations have used exogenous pharmacological approaches, there is a strong rationale to investigate non-pharmacological approaches (e.g., exercise) that have been shown to activate the eCB system (Koltyn et al., 2014; Galdino et al., 2014). Therefore, the purpose of the current study was twofold: 1) to examine the beneficial effects of voluntary exercise on the extinction of conditioned fear, and 2) to investigate exercise-induced eCB system adaptations in brain regions involved in fear-extinction.

Methods: Eight-week old male ICR/CD1 mice were subjected to a fear-conditioning and extinction protocol. Animals were individually housed for a ten-day acclimation period following transfer to the behavioral core. On day 1, all animals were conditioned to an auditory conditioned stimulus (CS) in a novel context using an electric foot shock. After fear conditioning on day 1, half of the mice were assigned to caging that contained unlimited access to an unlocked running wheel (VEx) for the entire duration of the experiment (days 1 through 5), while the other half were assigned to caging that contained unlimited access to a locked running wheel (SED) for the entire duration of the experiment (days 1 through 5). Extinction to the auditory CS took place every twenty-four hours on days 2 through 5. Freezing behavior was used to index the acquisition and extinction of learned fear. VEx and SED control animals were sacrificed 48 hours (between 0900 and 1100h) after the last day of testing in order to quantify eCB content using a previously described lipid extraction process (Patel et al., 2003). The contents of eCBs (AEA and 2AG) along with related biogenic congeners (OEA, PEA, 2OG) within lipid extracts in methanol from brain tissues were determined using isotope-dilution liquid chromatography-mass spectrometry as described previously (Patel et al., 2005). Cohen's *D* between-group effect size calculations were used for data analyses, given that this was a preliminary study with a relatively small sample (n = 12).

Results: There were no group differences in the acquisition of fear on day 1. VEx animals demonstrated an enhanced freezing response (d = 0.93) on day 2 compared to the SED animals. However, the VEx animals demonstrated less freezing on days 3 through 5 compared to the SED animals which exhibited very robust levels of freezing on days 3 through 5. Specifically, there was a small effect size difference in average freezing time between VEx and SED animals on day 3 (d = 0.23), and moderate to large effect size differences between groups at day 4 (d = 0.90) and day 5 (d = 0.72). Effect size calculations also revealed that the VEx animals had greater amygdalar AEA (d = 1.08), 2AG (d = 1.22), OEA (d = 0.44), PEA (d = 0.81), and 2OG (d = 0.19) content in comparison to the SED animals. There were no group differences for prefrontal cortex AEA (d = 0.06) and 2AG (d = 0.10) content, although VEx animals exhibited somewhat greater PEA (d = 0.20), OEA (d= 0.26), and 2OG (d = 0.58) content.

Conclusion: These preliminary results suggest that voluntary exercise enhances the extinction of fear, possibly due to augmentation of the eCB system in brain regions (especially the amygdala) responsible for fear-extinction.

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ENDOCANNABINOID SYSTEM MODULATION OF FEAR INHIBITON IN AMYGDALA INPUTS FROM INFRALIMBIC CORTEX

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Extinction of fearful memories is interrupted in anxiety disorders such as posttraumatic stress disorder. The endocannabinoid anandamide plays a crucial role to facilitate the learned inhibition of fear (i.e. extinction) in mice. Here, we extend our previous findings to investigate the neurocircuitry that influences this behavior. First, we focused on the infralimbic subregion (IL) of the ventromedial prefrontal cortex (vmPFC) inputs to amygdala, as a canonical pathway that is crucial for promoting extinction learning. Using optogenetics as a tool we showed that activating the IL→BLA pathway facilitates extinction and increases anandamide but not 2-arachidonoylglycerol levels in amygdala. Next, we showed that pharmacological blockage of CB1Rs systemically prior to the extinction impairs extinction learning while the IL→BLA pathway is activated. Currently we are investigating the effects of blocking CB1Rs only from the IL→BLA projections. For this, we used site specific viral infusions as an approach to selectively target the IL→BLA pathway with restricted expression of Cre recombinase in CNR1floxed mice. Our results will be useful to understand the role of the endocannabinoid system in the neurocircuitry for extinction facilitation.

FATTY ACID AMIDE HYDROLASE OVEREXPRESSION IN THE BASOLATERAL NUCLEUS OF THE AMYGDALA INDUCES PARADOXICAL EFFECTS ON ANXIETY AND FEAR MEMORY IN RATS

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

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

Seventy-two hours following HSV administration, a time point in which protein transfection is maximal, we found increased FAAH-mediated AEA hydrolysis together with decreased AEA levels within the BLA, confirming that the virus did successfully increase FAAH expression. At this same time point, a separate cohort of rats was tested for anxiety behaviour in an elevated plus maze, a light/dark box and an open field task. Quite surprisingly, we found that the overexpression of FAAH induced consistent anxiolytic effects in all three behavioural tasks we performed, relative to HSV-GFP rats. An additional cohort of animals was tested for fear memory in an auditory fear conditioning paradigm. Animals were bilaterally cannulated in the BLA, a week later were fear conditioned and 24 h after conditioning, the animals were injected with HSV-FAAH-GFP or its control vector. Seventy-two hours following HSV administration rats were re-exposed to the tone alone for 4 consecutive days to examine fear extinction dynamics. Unexpectedly, rats infused with the HSV-FAAH-GFP vector exhibited a dramatic reduction in fear expression during the extinction training and first extinction retrieval sessions when exposed to the tone, as compared to their HSV-GFP control rats. Moreover, both HSV-GFP and HSV-FAAH-GFP animals showed reinstatement of fear memory, when given unsignaled foot shocks after the extinction had occurred in both groups and presented 24 h later the tone alone, thus demonstrating that the FAAH overexpression did not interfere with the ability to correctly acquire the conditioned-unconditioned stimulus association and to express freezing behaviour. Furthermore, the effects of FAAH overexpression on fear memory were completely blocked by intra-BLA injections of the FAAH inhibitor URB597.

These findings suggest that the exact modes of action of AEA within the amygdala in the regulation of emotional states and memory are still far from being clear, thus, opening the avenue to investigate new potential mechanisms by which these processes may occur.

CANNABINOID FACILITATION OF FEAR EXTINCTION IN POSTTRAUMATIC STRESS DISORDER

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Exaggerated fear responses are the hallmark of posttraumatic stress disorder (PTSD). Empirically-supported psychotherapy for PTSD, Prolonged Exposure (PE), involves repeated exposure to fear-linked cues to produce "extinction" of fear. PE is generally effective, but many patients have incomplete extinction or fail to sustain extinction learning-related improvements over time. Recall of extinction learning depends upon limbic-frontal brain networks (hippocampus [HPC], ventromedial prefrontal cortex [vmPFC]) and PTSD patients show decreased activity in these regions and poor extinction recall. Our prior work suggests that an acute oral dose of Δ^9-tetrahydrocannibinol (THC), prior to experimental fear extinction procedures in healthy volunteers, facilitates recall of extinction learning via increased activation and functional connectivity of vmPFC-HPC. As extinction recall deficits and vmPFC-HPC dysfunction have been observed in PTSD, our preliminary findings indicate the cannabinoid system is a promising target to improve the efficacy and durability of learning during PE in treating PTSD (e.g., shortening treatment while strengthening and prolonging gains). However, direct tests of cannabinoid effects on recall of extinction learning and associated neural circuits have not yet been conducted in PTSD patients. In an ongoing randomized, double-blind, placebo-controlled, between-subjects study we are coupling a standard Pavlovian fear extinction paradigm in functional magnetic resonance imaging (fMRI) and skin conductance recordings (SCR) to compare the effects of THC (vs. placebo [PBO]) administered *prior to extinction learning* in in 41 trauma-exposed individuals with (n=12; PTSD) and without (n =29; TEC) PTSD and 20 non-exposed healthy adult volunteers (HCs), testing extinction recall 24 hours after extinction learning.

PTSD patients who received PBO during fear extinction exhibited, as expected, poor extinction recall as evidenced by increased peripheral measures of fear (SCR and US expectancy ratings) to a previously conditioned stimulus (CS) that was previously extinguished (CS+E). In contrast, PTSD patients who received THC during fear extinction exhibited good extinction recall (significantly lower peripheral measures of fear compared to PBO) and increased HPC activation to the CS+E during recall of extinction learning. There was no drug effect on extinction recall in either control group (TEC, HC). Together these findings provide the first evidence that pharmacological enhancement of recall of extinction learning is feasible in PTSD patients using cannabinoid system modulators. Ultimately, the cannabinoid system may serve as a promising target for innovative intervention strategies in PTSD and other fear learning-related disorders.

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CIRCULATING 2-ARACHIDONOYLGLYCEROL, PTSD, AND NEGATIVE MOOD 6 MONTHS AFTER TRAUMATIC INJURY

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Posttraumatic stress disorder (PTSD) is one of the most debilitating and costly psychiatric conditions. A significant portion of those with PTSD experienced a traumatic injury that precipitated the onset of the disorder. Traumatically injured patients experience lower quality of life compared to the general population, and PTSD significantly contributes to this poor quality of life. Research on emotion regulation shows that those individuals who suppress their emotional reactions tend to report more mental health problems. While there are treatments for PTSD that are efficacious, approximately 1-in-3 patients drop out of exposure based interventions, suggesting a need for further treatments. Pre-clinical and other studies in humans strongly suggest the hypothesis that endocannabinoid signaling is suppressed in those with PTSD. Therefore, the purpose of this study was to test this hypothesis and to investigate the relationship between circulating 2-AG levels, PTSD symptoms, and negative mood.

Two hundred seventy-six participants who experienced a traumatic injury requiring hospitalization underwent a baseline evaluation, including a blood draw. At 6-months post-injury, another blood draw was completed, marijuana use was assessed, and participants underwent evaluation of PTSD symptoms [with the Clinician Administered PTSD Scale (CAPS)] that also includes an evaluation of negative alterations in mood and cognitions (NACM). As a diagnostic instrument, the CAPS was used to dichotomize individuals according to whether they met diagnostic criteria for PTSD as outlined in the Diagnostic and Statistical Manual of Mental Disorders – 5th edition.

Analyses indicated a significant negative relationship between 2-AG levels and overall PTSD symptom severity (r = -.23). Those individuals meeting diagnostic criteria for PTSD at 6-months post-injury had significantly lower 2-AG levels (t(109.97) = 3.04, p = 0.003, mean difference = 18.17). Those individuals who reported marijuana use had significantly lower levels of 2-AG (t(101) = 2.46, p = 0.02) and greater severity of PTSD symptoms (t(159) = 2.46, p = 0.02) than those who did not use. Although there was not a relationship between marijuana use and emotion suppression, the symptom cluster showing the largest differences between individuals using marijuana and those who were not was the NACM cluster (t(74.61) = 2.52, p = 0.01).

Taken together, these results demonstrate the meaningful intersection between an individual's biological reactions after trauma, behavioral management of posttraumatic emotions, and their overall level of PTSD symptom severity. The role of the endogenous cannabinoid system to reduce PTSD symptom severity after traumatic injury should be explored further, particularly for those who may be more likely to not tolerate exposure based interventions.

CANNABIDIOL, A NOVEL BIASED INVERSE AGONIST FOR GPR3

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GPR3 is a constitutively activated, Gs protein-coupled receptor that is phylogenetically related to the cannabinoid receptors. GPR3 has been shown to be involved in neurological disorders such as Alzheimer's disease and physiological processes such as maintenance of oocyte meiotic arrest. Our screen of cannabinoids on GPR3 revealed that cannabidiol (CBD), a major non-psychoactive component of marijuana, is a novel inverse agonist for GPR3. In the current work, using a cAMP accumulation assay and a β-arrestin2 recruitment assay, the activity of CBD on GPR3 was compared to a known GPR3 inverse agonist AF64394. In addition, the involvement both G protein-dependent and G protein-independent components of GPR3 signaling were examined.

Our results demonstrate that GPR3 is constitutively active in both the cAMP accumulation and the β-arrestin recruitment assays. AF64394 concentration-dependently decreased both GPR3-mediated cAMP accumulation and β-arrestin2 recruitment. In contrast, CBD concentration-dependently reduced β-arrestin2 recruitment to GPR3 but had no effect on GPR3-mediated cAMP accumulation. After cholera toxin pretreatment, AF64394 lost its ability to inhibit GPR3-mediated cAMP accumulation, but was still effective at reducing β-arrestin2 recruitment. Furthermore, cholera toxin had no effect on the inverse agonistic effects CBD on β-arrestin2 recruitment to GPR3.

These data demonstrate for the first time that CBD, an important ingredient of marijuana, is an inverse agonist for the GPR3, with a bias toward the G protein-independent β-arrestin2 recruitment. In addition, our data show that AF64394 is an unbiased inverse agonist for GPR3 that works on both the G protein-dependent and G protein-independent pathways. Our discovery of GPR3 as a novel target for CBD indicates that some of the therapeutic effects of CBD may be mediated through this important receptor. In addition, as a biased inverse agonist for the G protein-independent, β-arrestin2 recruitment, CBD may be effective in inhibiting some of the deleterious effects of GPR3 (e.g. β-amyloid production) while keeping the beneficial actions of GPR3 (e.g. neuroprotection) intact.

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EFFECT OF KLS-13019 AND CANNABIDIOL ON NEUROPROTECTION FROM OXIDATIVE STRESS IN HIPPOCAMPAL CULTURES: MECHANISM OF ACTION

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Cannabidiol (CBD) has been reported to exhibit neuroprotective properties in many experimental systems. However, several properties have confounded the implementation of CBD as a drug candidate: 1) low potency for neuroprotection; 2) the large number of targets that mediate CBD actions; 3) marginal pharmacokinetic (PK) properties characterized by low oral bioavailability; and 4) the designation of CBD as a schedule 1 controlled substance. The current work has compared the neuroprotective protective actions of CBD with a novel small molecule (KLS-13019) that possesses structural similarities to CBD, while exhibiting marked increases in potency, decreases in toxicity and improved PK properties from CBD. The pharmacological strategy in synthesizing and identifying the new compound focused on enhancing two properties: increasing hydrophilicity and increasing neuroprotective potency against oxidative stress.

The pharmacological responses of CBD and KLS-13019 were compared in dissociated E18 rat hippocampal cultures co-treated with 30 mM ethanol to produce oxidative stress and acute toxicity; however, other oxidative stressors also were tested including hydrogen peroxide and ammonium acetate. The responses of cultures were assessed with standard fluorescent assays (neuronal viability with carboxyfluorescein diacetate and cell death with propidium iodide) after 5 hour treatment period. A comparison of potencies between CBD and KLS-13019 revealed an average 50-fold increase with the new compound, as shown with multiple assays and multiple toxins. Both compounds were completely effective in preventing ethanol-induced toxicity, as well as toxicities from other oxidative stressors. Treatment with KLS-13019 alone was 10-fold less toxic than CBD after 5 hours treatment of hippocampal cultures.

A challenging aspect in this area is the many known targets for CBD, including at least 4 that have been associated with neuroprotection. Our selection of a target candidate was highly influenced by the previous work of Ryan et al (J. Neurosci. 29:2053, 2009), which suggested CBD targeted the Na^+ /Ca^{2+} exchanger in mitochondria (mNCX), an important regulator of calcium homeostasis. This property is particularly relevant because of the known increases in intracellular calcium that occur during oxidative stress. Utilizing a mNCX inhibitor (CGP-37157), complete prevention of both CBD- and KLS-13019-mediated neuroprotection was observed with 10 μM treatment (IC50 0.7 μM). This complete block of neuroprotection was observed against multiple oxidative toxins. CGP-37157 pre-treatment was for 10 min. Importantly, treatment with 0.1-10 μM CGP-37157 alone had no effect on neuronal viability. Treatment with other known calcium regulators had no effect on CBD-mediated protection. In summary, a novel compound with structural similarities to CBD has been explored for neuroprotective properties and mechanism of action. Our studies suggest that neuroprotection from oxidative stress mediated by both compounds can act through the mitochondrial NCX exchanger. KLS-13019 may provide an alternative to CBD has a therapeutic candidate to treat disease associated with oxidative stress-related toxicity.

THE THERAPEUTIC POTENTIAL OF CANNABIDIOL FOR ALZHEIMER'S DISEASE

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Background: Alzheimer's disease (AD) is characterised by beta-amyloid (Aβ) plaques and neurofibrillary tangles as well as neurodegeneration, neuroinflammation, and oxidative damage. AD patients exhibit various behavioural and cognitive symptoms including social withdrawal and memory loss. The phytocannabinoid cannabidiol (CBD) possesses antioxidant, anti-inflammatory and neuroprotective properties and prevents Aβ-induced neuroinflammation, and tau hyperphosphorylation *in vitro*. CBD also reverses cognitive deficits of pharmacological Aβ models. Thus, CBD may offer therapeutic value for AD.

Methods: We determined the remedial as well as preventative effects of CBD in a double transgenic mouse model of AD (APPxPS1 transgenic mice). Animals were treated with CBD (20 mg/kg) either a) orally for 8 months before the onset of AD symptoms or b) i.p. for 3 weeks post development of AD symptoms. Control and APPxPS1 transgenic mice were then tested in various cognitive domains and their brains analysed for AD-relevant pathology.

Results: CBD a) prevented the development of a range of cognitive deficits typically seen in AD transgenic mice (e.g. spatial and recognition memory deficits) and b) was also able to reverse those impairments once established in transgenic mice. Brain analyses suggest that CBD's impact on neuroinflammation might be involved in this beneficial effect.

Conclusions: CBD might have therapeutic potential for AD, which is highly relevant, as it has already been tested in clinical trials. Future research will have to clarify the mechanisms involved further.

FLUORINATED CANNABIDIOL DERIVATIVES AS NOVEL, HIGHLY EFFECTIVE THERAPEUTIC ALTERNATIVES

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Cannabidiol (CBD), a non-psychotropic phytocannabinoid, is being evaluated in Phase 3 clinical trials in the US in connection with rare forms of childhood epilepsy and is available on a limited basis via an FDA-authorized Expanded Access program (Epidiolex®), and is also a principal component in a drug currently available commercially in 16 countries (and has received regulatory approval in an additional 12 countries) to treat spasticity associated with multiple sclerosis (Sativex®). Moreover, CBD as well as other phytocannabinoids are subjects of ongoing clinical trials for other indications. Notably, one of the major pitfalls of CBD applications is that, most probably due to its relatively poor bioavailability, the therapeutic efficacy could only be reached with very high daily doses (e.g. 25-50 mg per body kg for childhood epilepsy). This shortcoming, on the one hand, requires extremely high production of GMP-grade CBD; on the other hand, it makes the protocol very expensive and difficult to ensure likelihood of patient compliance with the treatment regimen. Therefore, there is an emerging and significant demand to identify/synthesize such agents which exhibit similar (if not better) therapeutic and safety profiles to those of CBD but, of greatest importance, with (preferably much) better efficacies/potencies.

It is common wisdom in pharmacology that fluorination can significantly increase the efficacy of the active components in pharmaceuticals – actually, ca. 30% of the best-selling drugs worldwide contain fluorinated compounds. By keeping the above in mind, the laboratory of Prof. Mechoulam has synthesized a series of fluorinated CBD derivatives (F-CBDs) which were then out-licensed to Phytecs Inc. These F-CBDs have been then systematically assessed in a wide-range of experimental systems which model multiple human diseases.

In this presentation, we provide compelling evidence that several F-CBDs investigated fully mimic the cellular and multicellular actions of CBD. Of greatest significance, we further show that certain F-CBDs exhibit 3-10-20 times greater efficacies/potencies when compared to CBD, depending on the actual model used. Indeed, F-CBDs were highly effective in:

- *in vivo* animal models predictive of anxiolytic, antidepressant, antipsychotic, and anti-compulsive activities (PLoS One. 2016 14;11(7):e0158779)
- multiple *in vivo* pain assays (hot-plate, abdominal writhing, carrageenan-induced hyperalgesia)
- *in vitro* human epidermal keratinocyte systems that model various inflammatory skin diseases such as contact, allergic, pruritic, solar (UVB-induced) and chemical-induced dermatitis; bacterial and viral skin inflammation; and atopic eczema

Taken together, these intriguing pre-clinical data strongly argue for that fluorination can indeed increase the efficacy/potency of CBD. Moreover, our novel findings also invite further pre-clinical and clinical studies to exploit the therapeutic potential of selected F-CBDs in the management of multiple human diseases.

Δ^9-THC AND CBD DIFFERENTIALLY REGULATE INTRAOCULAR PRESSURE

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It has been known for nearly 50 years that marijuana and its chief psychoactive constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) lower intraocular pressure (IOP). Elevated IOP remains the chief hallmark and therapeutic target for glaucoma, a condition that places millions at risk of blindness. Despite considerable early interest in (and continued public perception of) Δ^9-THC as a potential glaucoma therapeutic, we do not know how Δ^9-THC regulates IOP. It is commonly assumed that Δ^9-THC acts via cannabinoid receptors CB_1 and/or CB_2, but Δ^9-THC also activates other receptors, including GPR18, a receptor we have shown to regulate IOP. Cannabidiol (CBD) is a second major constituent of marijuana that has mostly been found to be without effect in studies of intraocular pressure. Using a mouse model to explore Δ^9-THC and CBD actions in the eye, we now report 1) Δ^9-THC acts via a combination of two receptors and in a sex-dependent manner and, 2) CBD has two opposing actions on IOP.

In our model a single topical application of Δ^9-THC lowers IOP substantially (~28%) for eight hours. This effect is partly ablated in CB_1 knockout mice, but fully blocked by topical pretreatment with the GPR18 antagonist O1918 in CB1 knockouts, indicating that Δ^9-THC acts through a combination of CB_1 and GPR18. In a second notable finding, we found that the observed effect in males was sex-dependent. In female mice the same treatment was slower to take effect and lowered pressure only half as much as in males. Surprisingly, topical CBD raised IOP, but even more surprisingly, CBD *lowered* IOP in CB_1 knockout mice. This indicates that CBD has two opposing actions, one CB_1-dependent, the other CB_1-independent. CBD raises IOP in a CB_1-dependent manner, an action consistent with the considerable endocannabinoid tone we have recently reported (Miller et al., 2016) and with the allosteric antagonism of CB_1 recently assigned to CBD. The mechanism of the IOP reduction by CBD remains unknown.

Our results indicate that the mechanism by which Δ^9-THC regulates intraocular pressure is complex, involving two receptors - CB_1 and GPR18. Development of dual CB_1/GPR18 ligands may therefore represent a useful therapeutic strategy for treatment of glaucoma. The finding of sex-dependence of Δ^9-THC action is significant both for interpreting the existing literature on cannabinoid regulation of IOP and in the design of potential drug trials in animals and humans. Lastly, our CBD findings raise the intriguing possibility that CBD interferes with the actions of Δ^9-THC while the second mechanism of CBD action, once identified, may yield a novel therapeutic target for treatment of glaucoma.

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HU-580, A STABLE SYNTHETIC ANALOGUE OF CANNABIDIOLIC ACID, PRODUCES 5-HT_{1A} RECEPTOR-MEDIATED SUPPRESSION OF BEHAVIOURAL SIGNS OF NAUSEA AND ANXIETY IN RATS WITH CONSIDERABLE POTENCY

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We have reported previously (Bolognini *et al*., 2013) that cannabidiolic acid (CBDA), the immediate precursor of cannabidiol (CBD) in cannabis, is more potent than CBD at enhancing the activation of 5-HT_{1A} receptors and at inducing 5-HT_{1A} receptor-mediated suppression of nausea-induced behaviour in rats. We have now synthesized CBDA methyl ester (HU-580), because this is more stable than CBDA, and investigated its ability to enhance 5-HT_{1A} receptor activation *in vitro*, and to induce 5-HT_{1A} receptor-mediated suppression of signs of nausea and anxiety in rats.

First, we compared the ability of HU-580 and CBDA to enhance stimulation by the selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), of [^{35}S]GTPγS binding to membranes obtained from Chinese hamster ovary (CHO) cells stably transfected with human 5-HT_{1A} receptors and kindly provided by Dr Keith Parker. Then we compared the abilities of HU-580 and CBDA (i.p.) to suppress LiCl-induced conditioned gaping (nausea-like behaviour) in rats in the absence or presence of the selective 5-HT_{1A} antagonist, WAY100635 (0.1 mg kg^{-1} i.p at -15 min). We also investigated whether HU-580 and CBDA could reduce signs of anticipatory nausea and of stress-enhanced anxiety in rats. For more detailed descriptions of most of the methods we used, see Bolognini *et al*. (2013), Cascio *et al*. (2015) and Rock *et al*. (2017).

Significant increases ($P < 0.05$) in the maximal effect of 8-OH-DPAT for its stimulation of [^{35}S]GTPγS binding to CHO cell membranes were induced by HU-580 at 0.01, 0.1, 1.0 and 10 nM but not at 0.001or 100 nM, and by CBDA at 0.1, 1 and 10 nM, but not at 0.01 or 100 nM. Thus HU-580 was even more potent than CBDA, but like CBDA, displayed a bell-shaped concentration-response curve. None of the above concentrations of HU-580 or CBDA affected [^{35}S]GTPγS binding in the absence of 8-OH-DPAT. LiCl-induced conditioned gaping in rats was significantly reduced ($P < 0.05$) by HU-580 at 0.1 and 1.0, but not 0.01, μg kg^{-1}, and by CBDA at 1.0, but not 0.01 or 0.1 μg kg^{-1}. The above *in vivo* effect of 0.1 μg kg^{-1} HU-580 was prevented by WAY100635.
We also found first, that signs of anticipatory nausea in rats were significantly reduced by HU-580 (0.01 & 0.1 μg kg^{-1}) and CBDA (0.1 but not 0.01 μg kg^{-1}), and second, that at 0.01 μg kg^{-1}. HU-580, but not CBDA, significantly reduced signs of stress-enhanced anxiety in rats.

These findings suggest that (i), HU-580 is even more potent than CBDA as an enhancer of 5-HT_{1A} receptor activation and as an inhibitor of nausea-like behaviour, (ii), as postulated previously for CBD and CBDA, the suppression by HU-580 of LiCl-induced nausea-like behaviour is mediated by 5-HT_{1A} receptors, and (iii), HU-580 is also a potent inhibitor of stress-enhanced anxiety. We conclude that HU-580 merits investigation as a potential medicine for the treatment of nausea, anxiety and/or other disorders that might be ameliorated by enhancing 5-HT_{1A} receptor activation.

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COMPARATIVE PHARMACODYNAMIC INVESTIGATION OF ORAL, SMOKED, AND VAPORIZED CANNABIS

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Background: Changes in the legality of medicinal and non-medicinal use of cannabis has become common among developed countries. In the U.S., these changes have occurred alongside a burgeoning cannabis marketplace that offers a growing number of consumable goods and products intended for use via a variety of methods of administration. Novel products and alternative routes of administration were not developed in traditional pharmaceutical pathways and there are few controlled studies that have been conducted using administration methods other than smoking. We conducted a series of studies to directly compare the dose effects of cannabis following oral, smoked, and vaporized routes of administration.

Methods: The comparative pharmacodynamic effects of cannabis were assessed in healthy adults across 2 studies. One study was conducted to examine the dose effects of oral cannabis (0, 10mg, 25mg, and 50mg THC doses), and a separate study evaluated smoked and vaporized cannabis (0, 10mg, and 25mg THC doses for both routes). Both studies used a within-subjects crossover design; 17 participants completed each study, 8 of whom completed both studies. Participants had a history of cannabis use, but had not used cannabis for at least one month prior to randomization. Outpatient drug administration sessions were conducted 1 week apart until study completion. Subjective effects ratings, cardiovascular measures (heart rate, blood pressure), and a cognitive performance battery (Digit Symbol Search Test [DSST], Divided Attention Test [DAT], Paced Auditory Serial Addition Test [PASAT]) were assessed at baseline and for 8 hours following drug exposure. Data from the 10mg and 25mg dose conditions were compared with placebo for each route of administration to detect significant drug effects. Similarly, the same doses were compared across routes of administration to probe for differences in the magnitude and time course of drug effects.

Results: Relative to placebo, significant dose-dependent changes were observed for subjective drug effect ratings, cardiovascular measures, and cognitive performance across routes of administration. Notable differences were observed in the time course of effects. Onset of peak drug effects was observed fifteen minutes post-exposure for smoked and vaporized cannabis followed by a gradual return to baseline over the observation period. In contrast, oral cannabis drug effect onset was evident 60-90min post-administration and remained at peak levels for a greater duration. The time course of subjective, cardiovascular, and cognitive performance effects greatly differed; cardiovascular effects occurred more immediately (peak effect at +90min) compared with subjective drug effects (peak effect at +180min) and cognitive performance impairment (peak effect at +300min). Across outcomes, the magnitude of peak drug effects was comparable across the smoked and oral routes of administration, but greater following vaporized cannabis administration.

Conclusion: Significant variability in the pharmacodynamic effects of cannabis was observed across doses and routes of administration. Oral administration was associated with a slower onset and longer duration of sustained peak effects compared to the two inhaled routes of administration. At the same doses, the magnitude of peak drug effects was comparable for both oral and smoked cannabis. This is in contrast to prior reports suggesting poor bioavailability of cannabis/THC via the oral route. Data suggest that vaporization is a more efficient route of cannabis delivery compared to smoked and oral routes of administration. This may be due to a lower rate of drug loss due to combustion and/or first pass metabolism.

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PHARMACOKINETICS OF CANNABIS: IMPACT OF ADMINISTRATION ROUTE AND RELATION TO DRUG EFFECTS AND PERFORMANCE

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Background: Cannabis use is increasing and regulatory changes regarding the legality of cannabis use in many developed countries have led to an increased interest in the identification of biomarkers that can be used to determine recency of use and/or impairment. This interest has been complicated by the emergence of novel cannabinoid products and variety in the methods by which cannabis is administered (e.g. edibles). Most data on the pharmacokinetics of cannabis have been conducted with frequent cannabis users self-administering smoked cannabis. We conducted a series of studies to evaluate quantitative cannabinoid levels in whole blood and oral fluid, subjective drug effects, and cognitive performance following oral, smoked, and vaporized cannabis administration.

Methods: Healthy adults were administered cannabis in 2 laboratory studies. In one study, oral cannabis (0, 10mg, 25mg, and 50mg THC doses) was administered, and in the other study smoked and vaporized cannabis (0, 10mg, and 25mg THC doses for both routes) was administered. Both studies used a within-subjects crossover design; 17 participants completed each study, 8 of whom completed both studies. Participants had a history of cannabis use, but had not used cannabis for at least one month prior to randomization. Outpatient drug administration sessions were conducted 1 week apart until study completion. Whole blood and oral fluid specimens were collected prior to and following cannabis administration at multiple time points for up to 8 hours post-drug administration. Subjective effect ratings and performance on a cognitive test battery were obtained at the same nominal time points as blood and oral fluid specimen collection for comparison. Specimens were analyzed for quantitative levels of THC, 11-OH-THC, and THCCOOH via LC/MS/MS (LOQ = 0.5ng/mL for blood; 1.0 ng/mL for oral fluid). Here we present comparative data within studies and across routes of administration for the 25mg THC dose.

Results: Blood and oral fluid specimens for all participants were cannabinoid-free during the baseline period of each drug administration session. Post-drug administration, cannabinoid concentrations in whole blood were dose dependent and were highest following vaporization, followed by smoked, and then oral administration. The maximum window of detection for THC in blood at the 25mg THC dose was 6hr, 4hr, and 3hr following oral, smoked, and vaporized routes of administration respectively; the window of detection for 11-OH-THC at the 25mg THC dose was 8hr, 2hr, and 3hr following oral, smoked, and vaporized routes of administration respectively; and the window of detection for THCCOOH was >8hr for all three routes of administration. Significant correlations of comparable magnitude were observed between blood THC ($r = 0.51 – 0.54$), 11-OH-THC ($r = 0.47 – 0.66$) and THCCOOH ($r = 0.56 – 0.65$), and subjective ratings of Drug Effect across routes of administration. Blood THC concentration was inversely correlated with performance on a psychomotor performance task (computerized Digit Symbol Substitution Task) following all 3 routes of administration ($r = -0.18$ - -0.36), but correlations between blood 11-OH-THC ($r = -0.24$) and THCCOOH ($r = -0.40$) were significant only after cannabis vaporization on this task. Following vaporization, significant inverse correlations were observed between blood THC ($r = -0.19$), 11-OH-THC ($r = -0.20$), and THCCOOH ($r = -0.38$) concentrations and working memory performance (computerized Paced Auditory Serial Addition Task), and blood THCCOOH ($r = -0.26$) was inversely correlated with divided attention performance. Correlations between blood cannabinoids and divided attention and working memory performance following oral and smoked cannabis were not significant.

There was no difference between routes of administration for mean Cmax of THC in oral fluid. Peak levels were observed at the first time point of collection (+10min) across routes. The window of detection was slightly longer for participants after the smoked and vaporized routes of administration; most participants had oral fluid THC levels near LOQ at the 8-hour post-administration time point (mean 4ng/mL and 1ng/mL for smoked and vaporized respectively). The maximum window of detection for oral fluid THC following oral cannabis was 6hr. Oral fluid THC was significantly correlated with self-reported Drug Effect following smoked ($r = 0.21$) and vaporized ($r = 0.26$) cannabis, but not oral cannabis. Oral fluid THC was not significantly correlated with psychomotor, divided attention, or working memory performance following any route of administration.

Conclusions: Following acute administration of cannabis containing 25mg THC, the highest concentration of cannabinoids in blood were observed following vaporization. This, combined with the observation of stronger drug effects, suggest that vaporization is a more efficient route of cannabis delivery compared with smoked and oral routes of administration. Significant correlations were observed between blood cannabinoids and self-reported drug effects across routes of administration. Significant correlations were observed between blood cannabinoids and performance on psychomotor, divided attention, and working memory tasks after vaporization, but were not as strong or as consistent as the correlation between blood cannabinoids and self-reported drug effects. Blood cannabinoids were poorly correlated with performance assessments following oral and smoked cannabis despite significant impairment of the user on these tasks. Oral fluid THC was significantly correlated with self-reported drug effects only following smoked and vaporized cannabis was poorly correlated with performance tasks. Blood cannabinoids are better predictors of acute drug effects than oral fluid THC, but differences observed across routes of administration highlight the challenges in attempts to make inferences about cannabis impairment based on biomarkers alone.

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ACUTE RESPONSES TO DIFFERENT STRAINS OF MARIJUANA: IS CBD A BUZZKILL?

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Over the last several years, there have been enormous changes concerning the public acceptance of marijuana around the world. In the U.S, there is legalized medical use in twenty-eight states and the District of Columbia and legalized recreational use in eight states and D.C. Use is increasing and legally available marijuana is becoming more potent and more diverse in terms of cannabinoid content. Thus, the scientific data that can inform marijuana public policy and consumer decisions are critically needed. We sought to study the effects of commonly used marijuana strains, as they are used in everyday life, as opposed to relying solely on testing the effects of U.S. government grown, lower potency marijuana in controlled laboratory experiments, which may underestimate effects of tetrahydrocannabinol (THC) and may ignore the effects of other major cannabinoids, such as cannabidiol (CBD). We examined the effects of two common commercially available strains, one with the average potency of THC in Colorado and one strain that had lower THC and higher CBD on measures commonly associated with the potential for harm (subjective effects, greater reward, acute deficits in cognitive function) as well as a measure associated with the potential health effects of marijuana (peripheral inflammatory markers). **Methods:** 23 regular marijuana users were recruited for this observational study [Mean age = 24.5(SD=4.2) years old; Mean days of marijuana use in the last 30 days was 23.0 (SD=8.2)]. Regular marijuana users were asked to switch from their normal high THC (~18%) strain to a common strain with high THC similar to what they normally use (THC ~ 18%; CBD <= 1%) or to a strain with lower THC but high CBD (THC ~ 9%; CBD ~12%). With 15 minutes of marijuana self-administration, users in the two strain groups were given a blood draw for an objective measure of intoxication [average blood levels of THC and its primary metabolites, THC-COOH and THC-OH (THC level)], followed by measures relevant to harm reduction following cannabis use: 1. desire to smoke more cannabis; 2. feeling mentally stoned; and 3. verbal memory impairment. We also tested the inflammatory effects of the two strains via circulating cytokine levels (TNFa, IL6, IL1beta) in the blood. **Results:** Results revealed significant interactions between strain type and our harm reduction measures. At the average THC level, use of high CBD strain is associated with a lower desire to smoke more (p<.05; η_p^2 = .194), with a feeling less mentally stoned (p<.005; η_p^2 = .394), and, at trend level, with fewer verbal memory errors (η_p^2 = .105), than use of the high THC strain. In addition, although the groups did not differ at baseline, the CBD strain group had significantly lower average circulating cytokine expression than the THC strain group (p = .017). **Conclusions:** Although this pilot study is not adequately powered to provide a comprehensive test of the effect of different strains of marijuana, the data suggest strong effect sizes and provide preliminary evidence that strains with higher CBD may mitigate some of the harmful effects of marijuana. Clearly, more research on commercially available strains is needed to inform the public policy makers about strains that may have less potential for harm.

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MEDICAL CANNABIS PATTERNS OF USE & SUBSTITUTION FOR OPIOIDS, ALCOHOL, TOBACCO AND ILLICIT SUBSTANCES: A SURVEY OF AUTHORIZED MEDICAL CANNABIS PATIENTS

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Background
Canada has one of the longest standing federally-regulated medical cannabis programs in the world. The Access to Cannabis for Medical Purposes Regulations (ACMPR) present a unique opportunity to enhance scientific understanding of patient experiences with well characterized cannabis and cannabis-based extract products. This study presents findings from the largest cross-sectional survey of authorized medical cannabis patients ever conducted in Canada.

Methods
These are results are from the Tilray Patient Survey, a 239 question online survey conducted in January 2017 that gathered responses from 3405 patients throughout Canada, 2032 of which provided validated patient IDs and were included in analysis. The survey collected demographic data, health-related information, and significant details about the rates of cannabis use, methods of administration, and the impact of cannabis on the use of prescription drugs, alcohol, tobacco and illicit substances.

Findings
Mental Health conditions account for 40% (n=813) of Primary Illnesses cited by patients, followed by Pain Conditions (37%; n=761). Over 74% (n=1515) of respondents use cannabis daily and >78% (n=1595) use 2 grams or less per day. Vaporization is the primary method of ingestion (31%; n=628), and >38% (n=782) report using extract products (oral "drops"), with 49% (n=376) preferring higher CBD products when using this form of ingestion. Over 69% (n=943) of patients who regularly use prescription drugs report using cannabis instead of prescription drugs, with >37% (n=610) of these substituting for pharmaceutical opioids, 22% (n=371) for antidepressants, and 13% (n=215) for benzodiazepines/muscle relaxants/sleep aids. Interestingly, women and lower income patients report higher rates of substitution overall ($p<.05$). Additionally, 44% (n=515) of lifetime regular users of alcohol, 31% (n=406) of regular users of tobacco, and 26% (n=136) of regular users of illicit substances report substituting cannabis, with over half reporting they cut down by 75% or 100%.

Discussion
This broad survey gathered highly detailed & previously unreported data on patient experiences with medical cannabis. This includes specifying the actual prescription and illicit drugs patients report reducing/replacing due to cannabis use, and the amount (%) by which they reduced use. The high rate of cannabis use for pain and mental health conditions and subsequent substitution for opioids and benzodiazepines/muscle relaxants/sleep aids adds to research suggesting that cannabis may play a harm reduction role in the current prescription drug/opioid dependence and overdose crisis, and the significant rate of substitution for tobacco is a novel finding that has public health implications for both patient and non-patient populations.

PHARMACOVIGILANCE OF MEDICAL CANNABIS: INTERIM RESULTS FROM THE QUEBEC CANNABIS REGISTRY

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Since April 1st 2014, legal access to dried cannabis in Canada has been regulated by the *Marihuana for Medical Purposes Regulations* (MMPR). The MMPR obliges the medical profession to authorise cannabis outside the usual framework of prescription drugs, as there is a lack of scientific evidence required for good medical practice. The Quebec Cannabis Registry (QCR) was launched in May 2015 as a pharmacovigilance initiative to allow adult patients in Quebec to access cannabis for medical purposes, with the support of their physician, as part of a research framework. The main objectives of the QCR are to: (1) systematically collect data on indications, dosages, benefits or side effects of the products used; (2) stimulate future research; and (3) support the ongoing development of a drug monitoring program.

We performed an interim extraction of key variables to present the structure and content of the QCR. As of March 20th 2017, with 39 physician-collaborators actively recruiting, 1158 patients have been enrolled to date. The average age of participants is 49.1 years (range 18 to 96 years), with a higher proportion of men (55.3%). Almost half of the participants reported symptoms of neuropathic, somatic, visceral or mixed pain (39.5%), whereas almost one fifth suffer from HIV, cancer or chronic pain (18.6%). Pain represents almost three quarter (72.0%) of all reported primary symptoms. Primary diagnoses were also collected and can be classified into pain disorders (57.0%), oncology (9.2%) as well as mood disorders (8.2%), among others. A detailed analysis of primary outcomes is planned for May 2017 to represent 2 years of the Quebec Cannabis Registry which will be presented at the meeting.

The QCR gathers valuable data on the use of medical marijuana in Quebec. We are beginning to see reductions in pain severity and interference, and opioid doses appear to be reducing. The analysis of the data collected over time will identify new research questions and will increase knowledge about the clinical effects, including safety and effectiveness, of cannabis used for medical purposes.

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THE EFFECT OF CANNABIS ON REFRACTORY ULCERATIVE COLITIS PATIENTS

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Objective: We aimed to assess the effect of cannabis on refractory Ulcerative colitis patients.

Methods: In a double blind, randomized placebo-controlled trial patients received either 1-gram of cannabis cigarettes containing 23 mg THC per day or placebo for eight weeks. Parameters of disease including disease activity index (DAI), inflammatory markers and quality of life (QOL) were assessed before, during and after treatment.

Results: The cannabis group had 14 patients (8 females) and the placebo group had 13 patients (2 females), with an average age of 33.5±9.9. In the cannabis group, the DAI score improved from 10.2 ±3.3 before the treatment to 3.9 ±3.3 after eight weeks of cannabis treatment ($p<0.01$), whereas in the placebo group the score changed from 10.6 ±2.8 to 8.2 ±2.1 (p NS). Quality of life score in the cannabis group improved from 76.0 ±21.0 to 99.6 ±19.2 ($p<0.01$). In the placebo group, quality of life was 71.6 ±13.7 before and 80.8 ±14.0 after treatment (p NS).

Conclusions: This eight weeks trial shows that cannabis has a positive effect on the quality of life and on the DAI of Ulcerative colitis patients. In light of these findings, cannabis should be further explored as a possible therapy alongside conventional modalities. Larger and longer follow up trials should be conducted.

A CONTROLLED EXAMINATION OF THE EFFECTS OF HEAVY CANNABIS USE AND ADOLESCENT ONSET CANNABIS USE ON COGNITION

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Despite the common assumptions that chronic cannabis use has detrimental effects on cognition and that use of cannabis during adolescence exacerbates the negative effects of cannabis on cognition, the results of research on humans are equivocal. The contradictory nature of the results of these literatures appears to result from an overwhelming number of poorly controlled studies muddying the literature. Therefore, the purpose of the present study was to examine and compare adolescent onset cannabis users', non-adolescent onset cannabis users', and non-cannabis users' (controls) performance on tests of retrospective memory, prospective memory, executive functioning, and attention whilst controlling for potentially confounding factors (e.g., age, sex, income, education, cigarette use, alcohol use, other substance use, impulsivity, verbal IQ). Further, participants were screened for medical, psychiatric, and neurological conditions; learning and intellectual disabilities; heavy use of alcohol; and use of any illicit substance other than cannabis in past 6 months. To date, 100 control participants (who have used cannabis < 10 times in their life, have not used cannabis in the past year, and tested negative for THC in urine) and 88 heavy cannabis users (who have used cannabis on daily/ near daily basis for at least one year and tested positive for THC in urine) have passed all screenings and been tested. All participants completed a survey to assess their demographic characteristics, cannabis use history, and other potentially confounding variables (e.g., use of other substances, impulsivity). Further, participants completed a battery of neuropsychological tests, including tests of verbal IQ (Wechsler Test of Adult Reading), retrospective verbal free recall (Rey Auditory Verbal Learning Test II), retrospective visuospatial memory (Brief Visuospatial Memory Test-Revised), working memory (Digit Span Backwards), source memory, temporal order memory, prospective memory (Reminder Lab Test, Email Field Test), executive functioning (e.g., Tower Test, Zoo Maps Tests, Stroop Test), and attention (Ruff 2s and 7s, Digit Symbol Substitution).

The results revealed small effects of cannabis use on retrospective verbal free recall, prospective memory, and attention. However, only the effects on verbal free recall and prospective memory remained significant after controlling for confounding variables. No significant effects of cannabis use were detected on any other measures of memory or executive functioning. Moreover, while significant differences in adolescent onset vs. non-adolescent onset cannabis users' verbal free recall, executive functioning, and attention were initially discovered, these effects were no longer significant after controlling for confounding variables. Overall the results indicate that heavy, chronic cannabis use, initiated at any age, is associated with modest problems with verbal free recall (i.e., remembering lists of items) and prospective memory (i.e., remembering to do things in the future). As such cannabis users are advised to adopt memory-aiding strategies. Moreover, the results underscore the importance of controlling for confounding variables when comparing cannabis users to non-users and adolescent onset users to non-adolescent onset users.

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SMOKED MARIJUANA ALTERS REWARD SENSITIVITY ON AN ASSOCIATIVE LEARNING TASK IN MARIJUANA USERS

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Marijuana (MJ) is thought to increase the enjoyment of nondrug rewards. The purpose of this exploratory analysis was to examine the acute effects of smoked MJ on sensitivity to a laboratory measure of reward and punishment in MJ users. Six MJ smokers (4 male, 2 female) not seeking treatment for their MJ use participated in a 3-session outpatient study as controls in a broader study of marijuana and psychosis. They reported currently using MJ on 3.8 days (SD=1.7) per week ($48.8/wk, SD=50.1), and MJ use was confirmed by urine toxicology. Participants completed a computerized stimulus classification task at 105 min following controlled administration of smoked MJ cigarettes (0.0, 2.02 or 5.5% Δ^9-THC); Δ^9-THC concentration and task stimulus order were randomized and double-blind. On some task trials, correct responses were followed by a gain of 25 points but incorrect responses were not followed by any feedback (reward trials). For other task trials, incorrect responses were followed by a loss of 25 points, but correct responses were not followed by any feedback (punishment trials). Each point was worth 1 cent in real money. The outcome measures were percent optimal responses (primary) and reaction time (secondary) for reward and punishment trials (160 trials total).

On the reward trials, 2.02% Δ^9-THC increased optimal responding (by about 19 percent) but 5.05% Δ^9-THC decreased optimal responding (by about 14 percent), relative to placebo ($p>0.05$). In contrast, both active MJ cigarettes increased rewarding subjective effects (e.g., "Good MJ Effect"), relative to placebo, at a similar timepoint. Active MJ did not affect optimal responding on the punishment trials, reaction time for either trial type, nor aversive subjective effects (e.g., "Bad MJ Effect"), relative to placebo ($p>0.05$). Smoked MJ of varied potency differentially altered reward-based associative learning in weekly MJ smokers, with MJ of relatively moderate strength increasing reward sensitivity, but MJ of relatively higher strength decreasing it. These findings contrast with the pattern of MJ's subjective rewarding effects, and suggests a partial dissociation between the hedonic and learning aspects of MJ reward.

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CANNABINOID AGONIST EFFECTS ON SIGN AND GOAL TRACKING

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The cannabinoid system has garnered attention recently as a target for treatment of several psychiatric disorders, including both addiction and post-traumatic stress disorder (PTSD). Cannabinoid agonists are known to increase dopaminergic activity, and increased dopaminergic responses to conditioned cues are the most consistently reported neurobiological correlates with increase attribution of motivational salience. Aberrant motivational salience attribution has been proposed as an etiological link between addiction and PTSD.

A behavioral model known as Pavlovian conditioned approach (PCA) can be used to measure motivational salience attribution by repeatedly pairing a neutral conditioned cue (e.g. a retractable lever) with the response-independent delivery of a reinforcer (e.g. a food pellet). Under these conditions, animals will begin to either sign-track, i.e. approach and contact the neutral cue, or goal-track, i.e. approach the location of impending reinforcer delivery. Goal-tracking indicates that the cue is being used merely as a predictor of reward. In contrast, sign-tracking indicates an attribution of motivational salience to the cue, and is correlated with vulnerability to both addiction- and PTSD-like behaviors in rats. We investigated the effects of a cannabinoid agonist on acquisition of sign and goal tracking behavior.

Sprague-Dawley rats were assigned to 4 conditions receiving vehicle or CP 55,940 in a dose of 10 μg/kg, 50 μg/kg, or 100 μg/kg, with 12 rats in each condition. Animals received injections followed by training daily for 7 days. Training sessions consisted of a retractable lever presentation followed immediately by food delivery into a magazine, repeated 25 times. Then 5 days of crossover training was conducted with drug rats switched to receiving vehicle and vehicle rats receiving 100 μg/kg of drug.

Rats receiving CP 55,940 showed fewer lever presses, greater latency, and lower probability of lever press. They also showed more magazine entries, a lower magazine entry latency, and higher probability of magazine entry. Their PCA index was significantly lower, indicating more goal tracking behavior in drug rats. These effects were dose-dependent. During the crossover phase, the drug rats showed decreased goal tracking while the vehicle rats showed deceased sign tracking. We conclude that the nonselective cannabinoid agonist CP 55,940 decreases acquisition of sign tracking and promotes acquisition of goal tracking behavior. This was the opposite of our original hypothesis and may be due to a disrupted timing of dopaminergic activity, such that cue can no longer generate a larger dopamine signal in comparison to the reward, thereby disrupting sign-tracking behavior. We are currently measuring cannabinoid receptor density and correlating it to spontaneous sign- and goal-tracking behavior in outbred rats.

ADOLESCENT CANNABINOID EXPOSURE PERSISTENTLY ALTERS NATURAL REWARD LEARNING, MOTIVATION, AND DOPAMINE CIRCUITS

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Adolescence is a critical period in cognitive and emotional development, during which synaptic pruning reshapes circuit connectivity in a long-term, perhaps permanent fashion. Endocannabinoids are involved in this process, and exposure to exogenous cannabinoid receptor (CBR) agonists like WIN55, 212,2 (WIN) during adolescence causes long-lasting deficits in memory and social interaction, and increases in reward seeking. This implies that adolescent cannabinoid exposure (ACE) alters the developmental trajectory of neural circuit maturation in a manner that causes enduring changes in cognition and reward.

Here, we exposed adolescent male rats to 14 daily injections of WIN (1.2mg/kg) from PD 30-43 (early adolescence), and examined effects of this ACE protocol on adult learning and motivation for a food reward, and responses to novelty. After 14 day washout (PD60, young adulthood), rats underwent Pavlovian conditioned approach (autoshaping, or sign/goal tracking), palatable food intake (novel and familiar foods, intake after acute hunger or satiety), and response to novelty tests (locomotion in a novel environment, novelty preference). We found altered attribution of incentive salience to food cues, increased intake of palatable food, increased preference for novelty, and altered homeostatic regulation of food intake. These effects were accompanied by alterations in effects of chemogenetic dopamine neuron manipulations, and altered endocannabinoid system function. These results show ACE alters mesocorticolimbic reward circuitry function, and increases reactivity to food rewards and food-predictive cues.

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LONG-LASTING IMPACT OF ADOLESCENT CANNABINOID EXPOSURE ON REWARD-RELATED BEHAVIORS: POTENTIAL INTERACTION WITH SCHIZOPHRENIA

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Adolescent cannabis use occurs commonly, affects neurodevelopment, and is a risk factor for schizophrenia, as well as other affective and addictive disorders. Schizophrenia, itself, is associated with high rates of alcohol and drug use as well as motivational and reward-learning deficits. Thus we were interested in whether Δ^9-tetrahydrocannabinol (THC) exposure during adolescence would influence reward related behaviors in adulthood, especially in the context of neurodevelopmental risk for schizophrenia. Using a neurodevelopmental model of schizophrenia, the neonatal ventral hippocampal lesioned (NVHL) rat, we assessed the effects of adolescent THC treatments (post-natal day 28-42; 6 mg/kg i.p.) on: (a) free-access 2-bottle choice alcohol (20% v/v) drinking; (b) context-based instrumental food reward acquisition, extinction, renewal and reinstatement; and (c) limited access sweet-fat food binge-like eating. In a subsequent study, we assessed the effects of adolescent THC treatment on the acquisition and extinction of Pavlovian autoshaping (sign-tracking) behavior. Neither NVHL-lesion status nor adolescent THC treatment altered free-access alcohol intake in adulthood. Adolescent THC treatment, however, significantly impaired the motivation to lever press for a food reward in both the NVHL and sham animals; both THC treated groups showed decreased responding throughout the entire acquisition period, consistent with decreased motivation to work for a reward in adolescent cannabis smokers. In contrast, only the THC-treated sham animals showed reduced food cup entries, while extinction or renewal of lever pressing did not differ between groups. Conversely, the NVHL animals displayed impaired reinstatement of lever-pressing if given food pellets in the extinction context. Lastly, THC-treated NVHL and sham animals displayed decreased binge-like sweet-fat food consumption in a limited-access paradigm. In the autoshaping study, adolescent THC treatment significantly increased sign-tracking compared to saline treatment (consistent with increased cue-reactivity in adolescent cannabis smokers), while resulting in decreased food cup entries. This study suggests the adolescent THC exposure may produce long-term impairments in reward and motivation. The discordant findings between instrumental and Pavlovian conditioning may provide important clues regarding the neurobiological and behavioral underpinnings of the potential "gateway drug" effects of adolescent cannabis use.

TOWARDS A TRANSLATIONALLY-RELEVANT PRECLINICAL MODEL OF CANNABIS-SEEKING BEHAVIOR

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Preclinical models of cannabis consumption that mimic the volitional nature and pulmonary route of administration common to human users represents a major challenge in the field. Although previous studies have shown that chronic exposure to synthetic CB1 receptor agonists or isolated cannabis constituents (i.e., Δ^9-tetrahydrocannabinol [THC] or cannabidiol [CBD]), can impact a number of diverse endpoints, there are several drawbacks with the traditional approach that limit translatability, including the route of administration (parenteral/intravenous vs. pulmonary), drug administered (natural vs. synthetic), and method of exposure (forced vs. volitional). With that said, very little is actually known about the effects of pulmonary *cannabis* self-administration, and whether these effects are similar to those demonstrated with synthetic CB1 receptor ligands. To this end, we have developed a novel, ecologically valid model of cannabis vapor self-administration that uses 'e-cigarette' technology to deliver discrete 'puffs' of vaporized cannabis extracts to rodents in a response-contingent manner.

In the current study, we trained adult male Sprague-Dawley rats (n=7-8/group) to self-administer either 1) a vaporized cannabis extract that is high in THC (29.2% THC/1.1% CBD), 2) an extract that is low in THC but high in CBD (1.96% THC/59.3% CBD), or 3) a vehicle vapor containing 80% propylene glycol/20% vegetable glycerol on a fixed ratio-1 (FR1) schedule of reinforcement. Each correct nose poke was rewarded with a 10-s vapor 'puff' paired with illumination of a cue light, and the vapor was maintained in the chamber for an additional 30 s using a programmable solenoid valve. The associated cue light remained illuminated during this time, indicating a timeout, and any responding on the inactive nosepoke or the active nosepoke during timeout was recorded but had no programmed consequences. Following acquisition of stable self-administration, rats underwent extinction training until criterion was met, at which point rats were subsequently tested for cue-induced reinstatement of cannabis-seeking behavior.

Rats in all groups showed stable rates of responding that produced physiologically relevant concentrations of THC and CBD, as indicated by detectable plasma cannabinoid levels and a significant reduction in locomotor activity in rats trained to self-administer high-THC vapor. The ratio of active to inactive nose pokes was significantly reduced across sessions in cannabis-exposed (but not vehicle-exposed) groups, thereby demonstrating response discrimination. Although rates of responding for cannabis extracts were not significantly different from vehicle, importantly, only rats that self-administered high THC or high CBD extracts showed a burst in responding on the previously active nose poke during extinction training. Moreover, rats trained to self-administer high-CBD extract showed impaired extinction learning. Finally, rats trained to self-administer high-THC vapor showed robust cue-induced reinstatement, which was not observed in vehicle-exposed rats. These preliminary data provide the proof-of-concept for a novel, translationally relevant model of pulmonary cannabis self-administration that produces physiologically relevant effects and shows hallmark characteristics of drug-seeking behavior.

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AM404 REDUCES HABITUAL ALCOHOL SEEKING AND DRINKING

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Alcohol use disorders affect 6.4% of the US population (SAMHSA, 2014), and carry with them an increased risk for serious health problems including liver damage and heart disease. We have established an alcohol self-administration model in mice to engender habitual alcohol drinking. We test for habit using contingency degradation that can be measured repeatedly within subjects, which adds to the sensitivity of our test for drugs to reduce habitual ethanol seeking. Additionally, this test has a measure of both seeking (the number of active nose port entries during the contingency degradation test) and taking (the number of entries into the magazine while the free dippers of ethanol are available). This provides a means of separately measuring ethanol consumption and ethanol seeking, and can demonstrate the capacity of a drug to reduce these habitual behaviors discretely.

Recent data from the lab demonstrate that AM404, an endocannabinoid reuptake inhibitor, reduces both habitual ethanol seeking and consumption. These data follow up on previous research by the Piomelli lab that demonstrated that AM404, as well as antagonists at the CB1 receptor, could reduce presumably goal-directed (FR1) ethanol self administration (Cippitelli et al., 2007). Likewise, we observed a reduction in habitual ethanol seeking and drinking with AM251, a CB1 receptor antagonist. Ethanol self-administration is known to increase the release of 2-arachidonoyl glycerol (2-AG) in the dorsal (Depoy et al., 2014) and ventral (Caille et al., 2007) striatum. We hypothesize that AM404 reduces habitual ethanol seeking and consumption by increasing the amount of anandamide in the striatum relative to the amount of 2-AG. Anandamide is a partial agonist at the CB1R, while 2-AG is a full agonist that causes desensitization of CB1R. We hypothesize that anandamide, in the context of ethanol self-administration, acts as an antagonist to counteract the 2-AG mediated drinking drive. This might explain why AM404 produces similar behavioral results as an antagonist of the CB1 receptor in this behavioral context. Research is underway using liquid chromatography mass spectrometry to directly test how habitual alcohol seeking affects endocannabinoid release, and how this is changed by AM404.

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CANNABINOID RECEPTOR 1 INVOLVEMENT IN COCAINE-TAKING AND COCAINE-SEEKING BEHAVIOR FOLLOWING STRESS-INDUCED ESCALATION OF COCAINE INTAKE

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Stress is an important contributing factor to addiction and is problematic as stress is unavoidable in daily life. Therefore, understanding the neurobiological mechanisms that underlie the contribution of stress to addiction is critical. Addiction can be characterized, in part, by a loss of control over drug intake that, in the rat, is modeled by escalating patterns of drug self-administration (SA). Notably, we have previously shown that in rats demonstrating stable cocaine SA under limited-access conditions (2-h/day), a stressor, electric footshock stress, administered daily at the time of training can escalate cocaine intake in a glucocorticoid-dependent manner. Stress-induced escalation of SA is likely the consequence of neuroplastic changes that persist long after periods of repeated stress end. These changes may involve neurobiological mediators that connect stress-responsive and reward systems in the brain, such as the endocannabinoid system (eCB) and may occur in regions implicated in both stress and reward, such as the ventral tegmental area (VTA). Importantly, cocaine-induced disinhibition of dopaminergic neurons in the VTA is eCB-dependent and restraint stress increases levels of eCBs in the VTA in cocaine-dependent mice. Therefore, we hypothesize that repeated stress at the time of SA induces a persistent increase in eCB signaling, particularly in the VTA, which results in escalation of cocaine use and increased susceptibility to later reinstatement. Male SD were trained to SA cocaine (0.5 mg/kg/inf) on a FR 4 schedule in 4 X 30 min SA sessions separated by 5-min drug-free periods. After rats displayed stable drug intake, some rats received footshock in the SA chamber during the 5 min drug-free period over 14 days. Footshock administration resulted in an increase in cocaine intake over 14 days and this effect persisted for at least 5 days after cessation of footshock. Systemic administration of the CB1R antagonist AM251 30 min prior to the SA session attenuated cocaine intake only in stress-escalated rats. Furthermore, intra-VTA administration of AM251 15 min prior to the SA session attenuates cocaine intake in stress-escalated rats. In separate groups of rats, once responding for cocaine was extinguished, rats were tested for reinstatement of drug-seeking behavior by administration of a priming injection of cocaine (2.5, 5, or 10 mg/kg, i.p.). Rats who received footshock during SA demonstrated augmented reinstatement to all doses of cocaine. Furthermore, as with SA, the CB1R antagonist AM251 given 30 min prior to injection of high-dose cocaine (10 mg/kg, ip) significantly attenuated cocaine-primed reinstatement only in rats with a history of stress-induced escalation of cocaine intake. These data suggest that stress-induced neuroplastic changes occur, likely in the eCB system, in regions of the brain that influence expression of escalated cocaine intake and augmented cocaine-primed reinstatement, such as the VTA, and that these changes may be glucocorticoid-dependent.

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PROBING MECHANISMS LINKING STRESS AND OPIATE DEPENDENCE: GENERATION OF A FAAH KNOCKOUT RAT

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The usage rate of heroin, and subsequent incidence of opiate overdoses, has vastly increased over the past decade in the United States, tripling to quadrupling in the past 5-10 years alone. Based on an underpinning of previous studies examining the role of opiate dependence altering brain stress systems, and that chronic stressors result in upregulation of fatty acid amide hydrolase (FAAH) activity, we have previously presented data showing chronic FAAH inhibition by PF-3845 can blunt the escalation of intake and increased motivation for heroin in rat self-administration models. Furthermore, FAAH inhibition reduced corticosterone release during heroin withdrawal, prevented heroin-induced deficits in reward threshold evaluated using intracranial self-stimulation (ICSS), and reversed upregulation of FAAH in the basolateral amygdala in heroin-dependent rats.

To facilitate more efficient examination of the role of FAAH in response to chronic stressors, opiate intake, and prolonged withdrawal, a Wistar rat deficient in FAAH activity was generated using a zinc-finger nuclease targeted deletion around an end region of exon 1. Three founders were generated, and a rat with a stable 65bp deletion was bred successfully. Wild-type, heterozygous, and FAAH knockout Wistar rats are currently being evaluated for basic behavioral phenotypic differences in pain and anxiety response, as well as being bred for biochemical validation of the deletion using Western blot, activity-based protein profiling, and mass spectrometry of fatty acid amide levels.

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IN VITRO EVIDENCE THAT GAT558 IS A POSITIVE ALLOSTERIC MODULATOR OF THE HUMAN CB_1 RECEPTOR

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The cannabinoid CB_1 receptor is a G protein-coupled receptor that is densely expressed in the central nervous system, and is thought to trigger a number of beneficial effects, including the amelioration of pain-related disease states and neurodegenerative disorders, when activated by endogenously released CB_1 receptor agonists (endocannabinoids) such as anandamide. CB_1 activation by exogenously administered agonists can also ameliorate such disorders. However, more unwanted side effects appear to be produced by exogenously administered CB_1 agonists than by endogenously released endocannabinoids.

One potential therapeutic strategy for optimizing the beneficial CB_1-mediated effects of endogenously released endocannabinoids would be to boost these effects by targeting an allosteric site on the CB_1 receptor with a positive allosteric modulator (PAM). Herein we present evidence that GAT558, a structural analogue of GAT211 (Laprairie et al., 2017), is a potent and efficacious CB_1 PAM. This was obtained by performing [^{3}H]CP55940 and [^{35}S]GTPγS binding assays with Chinese hamster ovary (CHO) cells stably transfected with human CB_1 receptors. More specifically, we found that at GAT558 behaved as a CB_1 ago-PAM (Schwartz & Holst, 2007). Thus, at a concentration of 1 μM, it significantly (1) enhanced the binding of [^{3}H]CP55940 to CB_1 CHO cell membranes ($P < 0.05$), (2) stimulated [^{35}S]GTPγS binding to these membranes ($P < 0.001$), and (3) increased the potency but not the maximal effect (E_{max}) of CP55940 for its stimulation of [^{35}S]GTPγS binding ($P < 0.05$). Importantly, in the [^{35}S]GTPγS assay, 1 μM GAT558 increased only the potency of the synthetic high-efficacy CB_1 agonist, CP55940, whereas, it increased both the potency and the E_{max} ($P < 0.01$) of the endogenous lower-efficacy CB_1 agonist, anandamide. Although GAT558 behaved, at 1 μM, as a CB_1 PAM of CP55940 and anandamide in the [^{35}S]GTPγS assay, this concentration of GAT558 abolished the inhibitory ("inverse agonistic") effect on [^{35}S]GTPγS binding to CB_1 receptors induced at 10 nM by the CB_1 inverse agonist/antagonist, SR141716A (rimonabant).

In conclusion, since GAT558 seems to be a positive allosteric modulator of the CB_1 receptor that can enhance the activation of CB_1 receptors by anandamide, it may also enhance the ability of endogenously-released anandamide to produce beneficial effects such as pain relief, a possibility that needs to be investigated by carrying out preclinical and clinical *in vivo* experiments with GAT558.

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Schwartz TW, Holst B (2007). Allosteric enhancers, allosteric agonists and ago-allosteric modulators: where do they bind and how do they act? Trends Pharmacol Sci 28: 366-373.

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IDENTIFICATION OF A NEW LEAD COMPOUND AS AN ALLOSTERIC MODULATOR OF THE CB_2 RECEPTOR

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There is good evidence that a number of beneficial effects can be induced both centrally and peripherally when CB_2 receptors are activated either by exogenously administered CB_2 receptor agonists or by endogenously released endocannabinoids. This evidence prompts a need to develop a new medicine that selectively increases the activation of CB_2 receptors, especially since these receptors do not share the ability of CB_1 receptors to induce unwanted psychotropic effects. One particularly effective way of meeting this need could well be to develop a positive allosteric modulator (PAM) that boosts beneficial effects of endogenously released endocannabinoids by selectively targeting an allosteric site on the CB_2 receptor.

Here, we present evidence that EC-21a, a novel structural analogue of a set of 2-oxopyridine-3-carboxamides that have been reported to be CB_2 receptor agonists, inverse agonists or antagonists (Lucchesi et al., 2014), is a CB_2 PAM, and also a CB_1 PAM. This evidence was obtained by carrying out [^{3}H]CP55940 and [^{35}S]GTPγS binding assays with membranes of Chinese hamster ovary (CHO) cells stably transfected with human CB_2 or human CB_1 receptors

We found that (1) [^{3}H]CP55940 binding to both CB_1 and CB_2 receptors was significantly enhanced by 1 nM to 1 μM EC-21a (Figure 1), (2) dissociation of [^{3}H]CP55940 from both CB_1 and CB_2 receptors was completely prevented by 100 nM EC-21a, and (3) the ability of CP55940 to stimulate [^{35}S]GTPγS binding to CB_1 and CB_2 receptors was significantly enhanced by 100 nM EC-21a. Some of these effects of EC-21a (effects 1 and 3) were greater in the CB_2 than in the CB_1 assays. At 100 nM, EC-21a also enhanced anandamide and arachidonoylglycerol-induced stimulation of [^{35}S]GTPγS binding to CB_2 receptors, albeit in some but not all experiments.

These results prompt a need for further research (1) that investigates whether EC-21a, by itself, can also produce signs of CB_1 or CB_2 receptor activation at sub-micromolar concentrations, and (2) that is directed at searching for a structural analogue of EC-21a that behaves as a selective and potent CB_2 PAM, or indeed, as a selective and potent CB_1 PAM.

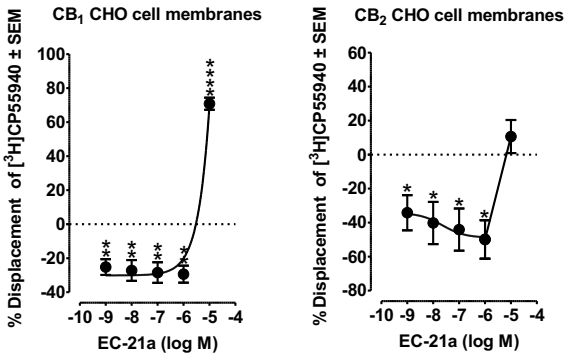

Figure 1: Effects of EC-21a on the binding of [^{3}H]CP55940 to human CB_1 and CB_2 receptors. Asterisks indicate values significantly different from zero (1-sample t test; *P < 0.05; **P < 0.01; ****P < 0.001; n=5 or 6).

Reference:

Lucchesi V et al. (2014). 1, 2-Dihydro-2-oxopyridine-3-carboxamides: the C-5 substituent is responsible for functionality switch at CB_2 cannabinoid receptor. Eur J Med Chem 74: 524-532.

DIARYLUREA-BASED ALLOSTERIC MODULATORS OF THE CANNABINOID CB1 RECEPTOR

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Allosteric modulators of the CB1 receptor have been shown to display pharmacological characteristics distinct from those of orthosteric agonists and antagonists and may offer a much needed alternative strategy to modulate CB1 signaling for therapeutic benefits. Several CB1 allosteric modulators have been reported thus far. We recently reported the first structure-activity relationship studies on one such modulator PSNCBAM-1. Here we will describe the design, synthesis and evaluation of a new series of diarylureas as CB1 receptor allosteric modulators. All target compounds were characterized by MS, NMR and HPLC.

These CB1 receptor allosteric modulators reduced the Emax of the orthosteric CB1 receptor agonist CP55940 in calcium mobilization assays, as expected with negative allosteric modulators (NAMs). Most compounds possessed low nanomolar IC_{50} values at CB1 receptor without any significant activities at the CB2 receptor. Their potencies in antagonizing CP55940-induced GTP-γ-[^{35}S] binding were consistent with calcium mobilization. One of the compounds attenuated prime induced reinstatement of cocaine seeking behavior with greater efficacy than PSNCBAM-1 in rats. The tested compound also demonstrated high metabolic stability in rat liver microsomes. These results will facilitate the development of potent and selective CB1 receptor modulators as potential medications for the treatment of drug addiction and related conditions.

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NOVEL ENDOCANNABINOID PROBES

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The endogenous *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG) act at the CB1 and CB2 receptors, two $G_{i/o}$-protein-coupled cannabinoid receptors (CBs) that are currently being targeted for a number of conditions including pain, inflammation, CNS disorders and cancer. The biological actions of AEA and 2-AG are terminated by a transport mechanism and enzymatic deactivation. In most tissues, AEA is metabolized hydrolytically by fatty acid amide hydrolase (FAAH), and 2-AG is metabolized by monoacylglycerol lipase (MAGL). However, recent investigations have demonstrated that oxidative enzymes of the arachidonate cascade, including lipoxygenases (LOX), cytochrome P450, and cyclooxygenase-2 (COX-2), can transform AEA and 2-AG into eicosanoid related bioactive products.

In an effort to understand the pleiotropic biological role of the endocannabinoid lipids we focus on the development of novel endocannabinoid probes that possess high CB receptor binding affinity as well as metabolic stability to the action of the COX-2, LOX, FAAH and MGL enzymes. Currently we are exploring the acyl chain and the head groups of the endogenous prototypes with special emphasis on the *bis*-allylic carbons and the omega position of the arachidonoyl chain. Inspired from the nature's elegant way to achieve molecular recognition, our design incorporates chiral centers and steric features at strategic positions within the templates. Synthetic approaches rely on highly stereoselective Wittig reactions, copper-mediated cross-coupling, peptide coupling and chemoenzymatic synthesis. A detailed SAR study along with a full biological evaluation of the novel analogs reported here is underway.

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SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF A DIPHENYL PURINE BASED PERIPHERALLY RESTRICTED CB1 RECEPTOR ANTAGONIST

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Type 1 cannabinoid receptor (CB1) antagonists might be useful for treating obesity, liver diseases, metabolic syndrome and dyslipidemias. Inhibition of CB1 receptors in the central nervous system, however, produces adverse effects, including depression, anxiety and suicidal ideation. Efforts are underway to produce peripherally selective CB1 antagonists to circumvent CNS-associated adverse effects. In this study, novel analogues of otenabant were explored in which the 4-aminopiperidine group was switched to a smaller piperazine group. To access a lipophilic binding site, the piperazine group was functionalized with aryl and heteroaryl groups, both with and without a carbon spacer. This resulted in mildly basic, potent antagonists of hCB1 possessing oral bioavailability, peripheral selectivity and significant hCB1 selectivity over hCB2. The 2-chlorobenzyl piperazine antagonist from this series of compounds was found to have sufficient oral bioavailability and peripheral restriction to advance to efficacy studies. This compound was tested in a mouse model of alcoholic liver steatosis. At an oral dose of 1.25 mg/kg b.i.d., this peripherally selective CB1 antagonist demonstrated efficacy in preliminary studies. Additional studies are planned with this and other similar compounds.

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MODULAR SYNTHESIS OF NOVEL CANNABINOID LIGANDS BASED ON THE COUMARIN MOTIF AS CB_1, CB_2, GPR55 AGONISTS AND ANTAGONISTS

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Coumarines are very common and wide spread natural motifs which find applications in medicinal chemistry. In previous investigations in our group was found out, that besides the general biological activities, modified coumarines also show biological activities against the cannabinoid receptor system. This led to the establishment of a modular microwave supported synthesis for modified coumarines (Braese et al., Eur. J. Org. Chem. 6 (2007) 943-952). The synthesis is starting from derivatized salicylaldehydes which are coupled with derivatized cinnamaldehydes in the present of an *N*-heterocyclic carben (NHC) as catalyst via a microwave supported condensation reaction. With this synthesis as tool further investigations led to the establishment of a small library of substituted 3-benzylcoumarines. In additional investigations the diversity of our library was extended by the 3-alkylcoumarin moiety. These structure moiety is easily accessible via Perkin-reaction by condensation with acetic anhydrides.

The biological activity validation against receptor type and specific receptor interactions is proven in different *SAR*-studies by our cooperation partners. The first assays were done by the group of C. Müller at the PharmaCenter Bonn, Germany. In a radioligand-binding-assay and the *β*-Arrestin-Recruitment-Assay our substances were tested on their activity against CB_1/CB_2 and GPR55/GPR18. Additional their effect as agonist respectively antagonist and their potency were tested (Braese et al., Biorg. Med. Chem. 17 (2009) 2842-2851; Braese et al., J. Med. Chem. 56 (2013) 4798-4810; Glaeser, PhD thesis, KIT, 2014). The results of this assays led to first trends about the different chemical structures and their corresponding biological effects.

Also we tested our coumarines for their anti-inflammatory effects on microglia cells in the Group of Dr. Fiebich at the University Medical Center Freiburg, Germany. Therefore they were evaluated on their effect against the arachidonic acid cascade, which leads in the expression of class-2-prostaglandins. In inflammatory diseases prostaglandins have an important role in genesis of pain and inflammation. A suppression of this signal cascade could lead to new possible therapeutics or therapies in the fight against neurodegenerative disorders. In this context we recently got promising results from a new study.

In ongoing investigations the results of all our biological assays will be examined about the relationships between chemical structure and biological activity. This will give us the opportunity for a better prediction of which chemical structure modification will result in which change of biological activity. Additional as well the extension and the increase in diversity of our library as further biological *SAR*-studies are still in progress.

STRUCTURE ACTIVITY STUDIES ON REGIOISOMERIC SUBSTITUTED INDOLES

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This presentation will compare the CB_1 and CB_2 receptor affinity data for a series of substituted indoles related to the cannabinoid drug of abuse 3-(1-naphthoyl)-1-pentylindole (JWH-018). The vast majority of indole based synthetic cannabinoid substances are 1-alkyl-3-acylindoles. We have prepared several series of regioisomers of the indole ring to evaluate the uniqueness of the 1,3-indole ring substitution pattern in cannabinoid receptor binding. Additionally, a number of potential designer-type molecular modifications of the 1,3-indole ring substitution pattern have been prepared and evaluated.

Our research has recently evaluated the 1-pentylindoles having the acyl group substituted at each of the six remaining available indole ring substitution positions. Synthetic and receptor binding comparisons will be made for the six benzoyl, the six (1-naphthoyl) and the six (2-naphthoyl)-1-pentylindole regioisomers.
A series of inverse isomers representing the specific reversal of the relative positions of the two common indole substituents found in the synthetic cannabinoids have been prepared and evaluated. These studies include compounds such as 1-(1-naphthoyl)-3-pentyl-indole and 3-(1-naphthoyl)-1-pentylindole as well as the 2-naphthoyl inverse isomer series.

Recently, we have evaluated the 3-(1-naphthoyl)-1-pentylindoles having additional groups substituted at the remaining five available indole ring substitution positions. These studies include groups such as methyl, methoxy and chloro substituted at positions 2-, 4-, 5-, 6-, and 7 of the indole ring.

Receptor binding profiles for these regioisomeric indole compounds at human cannabinoid CB_1 and CB_2 receptors will be compared to the standards JWH-018, Win55212 and CP55940. Numerous examples among these regioisomeric indoles display full agonist activity with affinities equal to or greater than the standard compounds. The results of these studies extends the structure activity relationship data for the indoles beyond the traditional 1,3-substitution pattern.

CHIRAL/ACHIRAL ANALYSIS OF NATURALLY OCCURRING CANNABINOIDS USING A NEW SUB-2 µM CHIRAL STATIONARY PHASE WITH ULTRA HIGH PERFORMANCE SFC-MS

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The interest in the medical use of *Cannabis Sativa L.* is steadily increasing because of its therapeutic efficacy towards a wide variety of ailments, and its unique chemistry, characterized by the presence of cannabinoids that are concentrated in leaves and in the female inflorescence. The fiber type of *Cannabis Sativa L*. is cultivated in Europe for textile production or for food (seeds, flour, and oil), and has a low concentration of psychoactive Delta 9-tetrahydrocannabinol (Δ9-THC) that is typically less than 0.2%. The main cannabinoid in the fiber type of *Cannabis Sativa L.* is cannabidiol (CBD) but there is also cannabidivarin (CBDV), cannabigerol (CBG), cannabinol (CBN) and the racemic cannabichromene (*rac*-CBC), each having various therapeutic actions. The analysis of the original composition of plant material is necessary for phenotype determination and quality control of medicinal cannabis used in therapeutic treatments.

The presence of natural racemic compounds (*rac*-CBC) in plant extract was investigated using Chiral Stationary Phases (CSPs) in *enantioselective* "*e*" Ultra High Performance Supercritical Fluid Chromatography (*e*UHPSFC). All of the analyses were performed using a new UHPSFC-compatible chiral column developed in the Sapienza University laboratory in collaboration with Regis Technologies. Kinetic evaluation using van Deemter plots showed excellent kinetic performance for the 150 x 4.6 mm column packed with 1.8 µm CSP, and resulted in efficiencies up to 265,000 theoretical plates per meter measured on the first enantiomer, and roughly 280,000 plates/m on the second enantiomer. The Chiral Stationary Phase (CSP) Whelk-O1 allowed resolution of the racemic compound cannabichromene (*rac*-CBC) in plant extract and the synthetic racemic Δ9-tetrahydrocannabinol. Good separation, in terms of chemio- and enantio- selectivity, was obtained with high resolution for all cannabinoids, and their acid forms, under isocratic conditions.

CHARACTERIZATION OF STRUCTURAL ANALOG OF CB_1 ALLOSTERIC MODULATOR ZCZ-011 WITH ENHANCED AGONIST ACTIVITY

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CB_1 allosteric modulators are a rapidly growing area of interest in cannabinoid pharmacology as they can offer an additional layer of texture to receptor signaling that could favor pathways of therapeutic relevance while avoiding those involved in untoward effects. Recently, positive allosteric modulators (PAMs) of the CB_1 receptor have been reported which exhibit positive binding cooperativity with the synthetic CB_1/CB_2 agonist CP55,940 and increase its potency and/or efficacy in functional assays. We evaluated the first reported CB_1 PAM, ZCZ-011, as well as structural analogs including GAT211, for their effects in agonist-stimulated [^{35}S]GTPγS and radioligand binding. Mouse cerebellar membranes were prepared as crude (P1) preparations and hCB_1 HEK293 cell membranes were prepared as enriched membrane preparations (P2).

In the [^{35}S]GTPγS functional assay, ZCZ-011 enhanced CP55,940 potency **[VEH: pEC_{50}=7.55±0.06 (7.43-7.68); 1 µM ZCZ: pEC_{50} = 8.74±0.16 (8.40 – 9.08)]** and efficacy **[VEH: E_{max} = 100.1±2.30 (95.5 – 104.7); 1 µM ZCZ: E_{max} = 114±2.29 (109.1 – 118.9)]** in mouse cerebellar membranes. Additionally, ZCZ-011 exhibited agonism in mouse cerebellar membranes **[pEC_{50} = 6.45±0.08 (6.29 – 6.61)]** and this effect was surmountable by the selective CB_1 antagonist SR141716, which is consistent with its negative binding cooperativity with SR141716 and suggests CB_1 mediation. ZCZ-011 also exhibited agonism in hCB_1 expressing HEK293 cell membranes **[pEC_{50} = 7.80±0.22 (7.32 – 8.27)]**. Because these cell preparations do not endogenously express endocannabinoids, these results suggest that ZCZ-011's effects are due to agonism and not enhancement of endocannabinoids.

Among the structural analogs tested, CAL010 **[pEC_{50} = 6.42±0.03 (6.35 – 6.49)]** emerged as the most efficacious compound, as it exhibited greater efficacy than ZCZ-011 in agonist-stimulated [^{35}S]GTPγS binding in mouse cerebellar membranes **[ZCZ-011: E_{max} = 79.4±3.70 (71.8 – 86.9); CAL010: E_{max} = 122.6±2.90 (116.5 – 128.7)]** and in HEK293 P2 membranes **[ZCZ-011: E_{max} = 46.86±3.65 (39.04 – 54.69); CAL010: E_{max} = 62.23±2.62 (56.61 – 67.85)].** CAL010 also enhanced CP55,940 potency to a similar degree to that of ZCZ-011 in mouse cerebellar membranes **[VEH: pEC_{50}=7.55±0.06 (7.43-7.68); 1 µM CAL010: pEC_{50} = 8.97±0.46 (7.98 – 9.97)]**. CAL010 also produced larger maximal increase in specific [^{3}H]CP55,940 binding than ZCZ-011 in radioligand binding **[ZCZ-011: E_{max} = 159.5±7.11 (144.5 – 174.5); CAL010: E_{max} = 235.6±7.10 (221.0 – 250.2)]** suggesting greater binding cooperativity.

These data demonstrate that structural modifications to the ZCZ-011 structure can further enhance agonist activity and binding. Additional analogs in this series are being screened and future studies will further characterize these in other signaling pathways. Behavioral studies assessing these compounds are also currently underway.

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THE CB_1 RECEPTOR ALLOSTERIC MODULATOR LDK 1258: *IN VIVO* PHARMACOLOGICAL EVALUATION IN MICE

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Manipulations of the CB_1 receptor elicit a wide variety of physiological and behavioral effects, including alterations in feeding behavior and energy storage, changes in motor behavior, and antinociceptive responses. Side effects associated with CB_1 receptor agonists (e.g., delta-9 tetrahydrocannabinol (THC)), such as psychoactive intoxication, short-term memory impairment, and dependence, diminished clinic clinical utility of these drugs. In addition, CB_1 receptor antagonists (e.g., rimonabant) reduce food intake and lipogenesis, but are not clinically available because of side effects such as depression and suicide ideation. Conversely, CB_1 receptor allosteric modulators offer a novel approach to maximize the therapeutic potential of cannabinoids and minimize unwanted side-effects. While agonists and antagonists such as THC and rimonabant bind to the primary CB_1 receptor binding site (defined as the orthorosteric site), allosteric modulators bind to a secondary (i.e., allosteric) site, resulting in a conformational change of the receptor that either increases (positive allosteric modulator; PAM) or decreases (negative allosteric modulator; NAM) the potency and efficacy of orthosteric ligands. A growing body of literature has characterized the cellular pharmacology of CB_1 receptor allosteric modulators in *in vitro* studies, but comparatively few studies have investigated the *in vivo* pharmacology of these compounds.

Here, we examined whether the novel CB_1 receptor allosteric modulator LDK 1258 alters feeding behavior through a CB_1 receptor mechanism, as well as elicits common cannabimimetic pharmacological effects. In initial studies, we compared the effects of LDK 1258 (3-30 mg/kg) and rimonabant (3-30 mg/kg) on food consumption in mice and whether the anorectic effects were mediated via the CB_1 receptor. In contrast to rimonabant, which reduced food intake in CB_1 (+/+) mice but not in CB_1 (-/-) mice, LDK 1258 produced anorectic effects regardless of genotype, indicating a CB_1 receptor dispensable effect. We next examined if this compound produced produces pharmacological effects in the tetrad assay, which consists of measurements of locomotor activity, catalepsy, antinociception, and hypothermia. LDK 1258 (30 mg/kg) produced significant decreases in locomotor activity and body temperature measures, but again these effects were CB_1 receptor dispensable. In the mouse drug discrimination paradigm, LDK 1258 (3-30 mg/kg) failed to substitute for either the high efficacy CB_1 receptor agonist CP55,940 (0.1 mg/kg) in in C57BL/6J mice or anandamide (6 mg/kg) in FAAH (-/-) mice. However, it dose-dependently decreased response rates. Ongoing studies are examining whether LDK 1258 elicits shifts in the dose-response curves of CP55,940 (0.01-0.1 mg/kg) and anandamide (1-6 mg/kg). The results of the present study demonstrate that LDK 1258 produces pharmacological effects in mice via a novel site of action, though ongoing experiments are examining whether this compound alters the potency or efficacy of orthosteric CB_1 receptor agonists. These finding underscore the importance of linking *in vitro* and *in vivo* pharmacological effects in the development of CB_1 receptor allosteric modulators.

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DISCOVERY OF LEAD INHIBITORS OF STEROL CARRIER PROTEIN-2

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Agents capable of augmenting endocannabinoid (eCB) signaling have potential in treating disorders of stress, anxiety, and pain. A great body of evidence supports the role of a putative anandamide (AEA) uptake transporter that facilitates sequestration of extracellular AEA and transport within cells. A number of AEA transport inhibitor probes have been reported, though the identity of the protein or proteins responsible for AEA transport remains a topic of much debate. We recently identified sterol carrier protein-2 (SCP-2) as a cytoplasmic binding and transport protein for eCBs (Mol. Neurobiol., 2014, 50, 149-158). Complete understanding of the physiologic role of SCP-2 in eCB signaling requires the development of selective SCP-2 inhibitor probes. Here, we describe the results of a preliminary study aimed identifying unique small-molecule scaffolds for lead SCP-2 inhibitors using virtual screening.

Based on an NMR structure of SCP-2 (1qnd, pdb.org), we virtually screened our in-house library of 11,000 compounds using AutoDock 4.2. From this *in silico* high-throughput screen, we selected four structurally diverse, "lead-like" compounds for evaluation *in vitro*. We also tested analogues of SCPI-1 and SCPI-5, two leads previously discovered from a high-throughput screen against *Aedes aegypti* (mosquito) SCP-2 (J. Lipid Res. 2005, 46, 650-657). Three of four compounds identified from computational screening inhibited binding of a reference probe molecule, NBD-stearate (NBDS) with EC_{50} values between 5-12 μM. Among analogues of known SCP-2 inhibitors, the SCPI-1 analogue, CWC-28-085 (*N*-(4-((4-(4-chlorophenyl)thiazol-2-yl)amino)phenyl)acetamide), produced the greatest inhibition of NBDS binding (~70%), whereas the aryl chloro isomer CWC-28-083 (*N*-(4-((4-(3-chlorophenyl)thiazol-2-yl)amino)phenyl)acetamide) only inhibited ~50% of NBDS binding. The low potency of SCPI-5 in this assay also suggests significant structural differences exist between human and mosquito forms of SCP-2. Taken together, these results provide a framework upon which to build small molecule SCP-2 inhibitor probes.

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IN VIVO CHARACTERIZATION OF AM7410, A POTENT ORALLY BIOAVAILABLE CB1R AGONIST

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CB1R agonists hold therapeutic promise for treating a broad range of diseases including pain, appetite loss, chemotherapy-induced nausea and vomiting, glaucoma and neurodegeneration. To date, cesamet (nabilone), dronabinol (synthetic Δ^9-THC), and sativex (mixture of cannabidiol and Δ^9-THC) have been the only approved cannabinoid based medications. The development of new generation CB1-agonist based pharmaceuticals has been hampered by the compounds' unpredictable oral bioavailabilities, poor pharmacokinetic profiles, and high lipophilicities.

Therefore, we aimed on improving these parameters and designing compounds that are also orally active. As part of our drug development program on controlled deactivation cannabinergic ligands, we recently identified AM7410, a structural analog of THC, as a potent CB1R agonist with improved physicochemical properties. The *in vivo* pharmacological effects of AM7410 were assessed in male CD-1 mice using two systemic routes of administration (p.o. and i.p.). In the CB1R mediated hypothermia assay, orally administered AM7410 (0.3 mg/kg) was approximately 3 times more potent when compared to intraperitoneal AM7410 (1 mg/kg). In the tail-flick ant nociception assay, the analgesic effect of orally administered AM7410 (1 mg/kg) was maximal (100% MPE). In both assays, AM7410 (p.o) displayed a fast onset of action and a relatively short duration of action when compared to other known classical cannabinoids. Future studies should assess the potential effects of orally administered AM7410 on processes such as tolerance and withdrawal.
The results presented here provide proof-of-concept for the development of orally active next generation CB1 agonists that may be useful for clinical research.

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INVESTIGATING POTENTIAL CARDIOTOXICITY OF JWH-073

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The synthetic cannabinoid (SCB) agonists JWH-073, JWH-018 and AM2201 are commonly found in preparations currently on the market as recreational drugs (i.e. Spice or K2). Several studies document chest pain and cardiac ischemia, and at least four case reports now exist of pediatric patients diagnosed with myocardial infarction after synthetic cannabinoid use. We aim to determine the cardiovascular effects of the SCB naphtoylindole JWH-073. We previously reported the results of observation of hematoxylin/eosin-stained organs from C57BL/6 mice treated once with 30 mg/kg JWH-073, 50 mg/mg Δ^9-tetrahydrocannabinol (THC) or vehicle (n = 4 each) (Breivogel et al, 2016 ICRS Symposium on the Cannabinoids). Analysis revealed cardiac cell death in 3 of the 4 mice treated with JWH-073, but none in the mice treated with THC or vehicle ($p < 0.05$ by Chi-squared analysis for JWH-073 vs THC or vehicle).

In follow-up to this pilot study, additional experiments have been performed. *In vivo* treatments of C57BL/6 mice with 30 mg/kg JWH-073, 50 mg/kg THC, or vehicle (n = 8 each) resulted in significantly lower serum cardiac troponin (a marker for cardiac cell death) in the JWH-073 (6.4 ± 0.3 ng/ml, $p < 0.05$ vs vehicle) and THC (6.0 ± 0.8 ng/ml, $p < 0.01$ vs vehicle) compared to vehicle-treated (8.4 ± 0.3 ng/ml) mice. Pre-incubation of isolated mesenteric arteries with 3 μM JWH-073 lowered the sensitivity to phenylephrine-mediated contraction (pD_2 6.12 ± 0.18 *vs*. 5.42 ± 0.08, n = 3, $p < 0.05$), and partially inhibited vasoconstriction induced by high concentrations of acetylcholine. In cultured H9C2 cardiac myocytes, no difference in cell viability was found after 4 days of treatment with 0.001-3.0 μM JWH-073 using the Sulfa Rhodamine B assay. Additional studies will assess hematoxylin/eosin-stained hearts from the aforementioned treated mice, the role of CB_1 and CB_2 in the vascular effects of JWH-073, and viability studies and reactive oxygen species determinations in H9C2 cardiac myocytes. We hypothesize that JWH-073 administration has the potential to induce peripheral vasodilatation that may contribute to the profound hypotension, and perhaps cardiac damage, reported in SCB intoxicated individuals. Further studies will clarify the potential direct effects of JWH-073 on cardiac cells.

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ANALYSIS OF CANNABIS FOR PESTICIDE RESIDUES BY GC/Q-TOF

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As of the November 2016 election, 29 states have approved the use of medical cannabis and eight states, representing 65 million people, have approved recreational use by adults. Canada allows medical use and is on a path to full legalization. Because the US Government still classifies cannabis as a Schedule 1 drug, all legislation controlling the growing, testing and use of cannabis products is done at the state level. There is no uniformity in the regulations and their enforcement. Pesticide use on cannabis plants is very controversial, but it is loosely regulated in most states. It is clear that pesticide residue testing is important for a product that may be eaten or inhaled. This paper describes the analysis of cannabis extracts for pesticide residues using an accurate mass high resolution GC/Q-TOF. Chromatograms are analyzed by applying an "all ions" approach using a personal compound database and library (PCDL) compounds in the PCDL in just a couple of minutes. Standards are only needed for those pesticides that are found and need to be quantified. This approach has also been used to identify pesticide residues in food and herbal extracts.

ANALYSIS OF PATIENTS EXPERIENCING TOXIC EFFECTS FROM SYNTHETIC CANNABINOIDS

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Synthetic cannabinoids (SCs), structurally heterogeneous and commonly used drugs of abuse, have become a paramount concern in recent years. Although most users prefer using cannabis whole plant for consumption, there are convenience, legal, and cost reasons driving the utilization of SCs. Clinicians need to be aware of pharmacologic and clinical similarities and differences between SC and cannabis use. Despite the widespread distribution and consumption of this substance, data regarding its effects on the human body are scare in the literature and often not in an easily presentable format for clinicians.

SCs act as full agonists of the cannabinoid type-1 receptor but display additional pharmacologic effects. There are numerous cases of patient harm and mortality in the United States, Australia, and Canada with many psychological, neurological, cardiovascular, pulmonary, and renal adverse events.
Providing physicians and healthcare professionals this information is crucial for diagnosis and patient treatment. Medical professionals and researchers can use this assessment to develop a categorized testing standard for patients exhibiting symptoms of SC ingestion. Consolidating all past emergency room occurrences of SC use plays a critical role in the recovery of patients, guiding medical personnel as they make decisions regarding testing and treatment possibilities. The aim of this study was to create a vital reference for medical professionals with the primary goal of assisting in diagnosing patients early, thus decreasing unnecessary diagnostic procedures and treatment initiation times.

Relevant publications were identified searching PubMed (inclusion from July 2009 to March 2017) using the following terms: synthetic cannabinoid, cannabinoids, emergency room, case studies). Our analysis indicated that 85% of patients were male and 15% female and ranged from 13 to 59 years in age with an average age of 23 years old (n=142). Thirty percent of the cases were children that were 17 or younger. Common symptoms included tachycardia (46 %), emesis (45% of patients), and hallucinations (27% of patients). In this review, the most commonly occurring symptoms present in patients presenting to the emergency room who are experiencing toxic effect(s) from synthetic cannabinoids.

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PRESYNAPTIC GPR55 RECEPTOR MODULATES [^{3}H]GABA RELEASE IN THE RAT SUBSTANTIA NIGRA

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GPR55 receptor has been proposed like a "atypical cannabiboid receptor" that can be activated by Δ^9-THC and lysophosphatidylinositol (LPI). It has been shown in HEK293 cells transiently expressing GPR55 and in dorsal root ganglion neurons that CPR55 activation mobilize Ca^{2+} from IP3R gated stores. On the other hand, mRNA expression of GPR55 has been reported in striatum. However, its presence in medium spiny neurons and if it can modulate neurotransmission at their terminals is unknown. The aim of this work was determinate if GPR55 receptor is expressed in striato-nigral synapses and if it can modulate GABA release and its signaling pathway.

By immunofluorescence we found co-expression of GPR55 and substance P in dorsolateral striatal neurons, suggesting its presence in the direct pathway of the basal ganglia. Their presynaptic location and effect on neurotransmitter release was evaluated. LPI increase K^+ induced [^{3}H]-GABA release in slices and synaptosomal fraction from the Substantia nigra pars reticulata in a concentration-dependent manner, this effect was prevented by the selective antagonist CID16020046 [200 nM] or cannabidiol [200 nM]. In order to evaluated the signaling pathway involved in GPR55 activation, the slices were pre-incubated with the SERCA blocker thapsigargine [2 μM] or with the PLC inhibitor U-73122 [10 μM], but neither of them couldn't prevent LPI effect on release. The PKA inhibitor H-89 [10 μM] blocked the effect of LPI on [^{3}H]-GABA release. The cAMP analogous 8Br-cAMP increases GABA release and had occlusive effects in presence of LPI 100 mM. Finally, 15 days after the dorso-lateral striatum lesion with kainic acid (1μg/μL) the expression of GPR55 decreases in synaptosomal fractions from the SNr. These results suggest that GPR55 is present in striato-nigral projections and its activation enhance cAMP production and GABA release via activation of PKA.

G PROTEIN-COUPLED RECEPTOR GPR55 PROMOTES COLORECTAL CANCER

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Background: Cannabinoid receptor 1 (CB1) has recently been shown to play a tumor-suppressing role in colon cancer (Wang 2008). This led us to investigate whether G protein-coupled receptor 55 (GPR55), signaling through different G proteins than CB1 and shown to be involved in various cancers, plays a role in colon carcinogenesis.

Methods: Colitis-associated colon cancer was chemically induced in GPR55 and CB1 knock out (KO) mice, GPR55/CB1 double knock out mice and wild type littermates (WTs). Tumor burden was evaluated macroscopically after 12 weeks and tumor biomarkers, receptor expression levels, cytokines and infiltrated leukocytes were analyzed from the collected tissue. Pharmacological inhibition of GPR55 with CID16020046 was performed in the same model. Additionally, a model of spontaneous colon cancer, i.e. without the induction of colitis, was carried out in GPR55 and CB1 KO mice. Patient data were obtained from a publicly available data base (http://r2.amc.nl) and from the OncoTrack EU project.

Results: GPR55 appeared to play a tumor-promoting role in colon cancer since both genetic and pharmacologic ablation of GPR55 led to reduced tumor numbers and areas as compared to control mice. Also, in colorectal cancer patients, low GPR55 expression correlated with an increased relapse-free survival. The mechanism by which GPR55 exerts its effects was found to involve the modulation of the tumor microenvironment. This was evident through the reduced expression of e.g. COX-2 and STAT3 and altered recruitment of CD8+ T cells (increased) and myeloid-derived suppressor cells (decreased) in tumors of GPR55 KO mice. The tumor-suppressing role of CB1 was confirmed since CB1 KO mice showed increased tumor numbers and areas. Additionally, we found that GPR55/CB1 double KO mice had a tumor burden equal to WTs.

qRT-PCR revealed that, in tumors of WT mice, GPR55 mRNA was up-regulated whereas CB1 mRNA was down-regulated compared to healthy control colon. Differential regulation of receptor expression was also found in patients where expression levels of both receptors correlated with disease severity (UICC staging), albeit in a differential manner.

Conclusion: (A-)typical cannabinoid receptors GPR55 and CB1 were found to play differential roles in colon carcinogenesis, where the former seems to act as oncogene and the latter as tumor suppressor. Since they share certain ligands, this is of importance when targeting the endocannabinoid system for future therapy of colon cancer.

ELUCIDATING GPR55 SPECIES STRUCTURAL DIFFERENCES IN TRANSMEMBRANE HELIX 2

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GPR55 is activated across human, mouse, and rat by Lysophosphatidylinositol (Lauckner et al., PNAS, 2008). However, GSK494581 and related benzoylpiperazine agonists have poor or no potency at mouse or rat GPR55, respectively, yet are potent at human GPR55(Brown et al., J. Pharmacol. Exp. Therap., 2011). Based on our currently published model of GPR55, sequence differences for residues facing into the transmembrane binding crevice regions reveal two distinct sites between human S2.64(84), M7.39(274) versus mouse P2.64(83), and L7.39(282)(Lingerfelt et al., Biochem., 2017). Human and rat differ in three sites, namely human S2.64(84), F6.55(246), M7.39(274) versus rat P2.64(83), L6.55(246), L7.39(274). Low sequence identity between these orthologs (~76%) is misleading,(Brown et al., J. Pharmacol. Exp. Therap., 2011) since the sequence identity is much higher for residues facing the binding crevice. Recently, we published a human F6.55L mutant that had a 92 fold loss in potency for the benzoylpiperazine agonist ML184, suggesting that in rat L6.55 plays a large role in the lack of potency for this class of agonists (Lingerfelt et al., Biochem., 2017). However, ML184 still had measurable potency in the F6.55L mutant, so the role of the helix kink induced by P2.64 comes into question. Significantly, transmembrane helix 2, TMH2, has two regions of flexibility in rodent, contained within the P2.58 i to i-4 region and the P2.64 i to i-4 region. Close proximity of the two regions, separated by only one residue, and the fact that the crucial residue K2.60 is contained in the second flexible region found only in rodent, suggests a conformational change is present here that impacts ML184 potency in rodent and not in human. We will discuss Conformational Memories (Whitnell, Hurst, Reggio, & Guarnieri, J. Comp. Chem., 2008) studies on mouse GPR55 TMH2 that create a unique conformation of the helix in rodent.

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MOLECULAR IMAGING OF MYOCARDIAL CANNABINOID TYPE 1 RECEPTOR IN MICE AND MEN

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An alarming number of recent studies indicate that acute/chronic use of marijuana or synthetic cannabinoids ("spice") may trigger severe cardiovascular side effects. Activation of myocardial cannabinoid type 1 receptor (CB1-R) by endocannabinoids has been proposed to contribute to cardiac dysfunction in various cardiomyopathies (including diabetic cardiomyopathy). Elevated plasma endocannabinoid levels also positively correlate with adverse cardiac events in obese subjects. Herein, we aimed to evaluate the feasibility of targeted imaging of myocardial CB1-R and to assess its potential upregulation in obese mice and humans using ^{11}[C]-OMAR and PET/CT.

Rimonabant significantly inhibited OMAR uptake in the mouse myocardium compared to vehicle, demonstrating the specific binding of OMAR to CB1-R in the heart muscle. Quantification of the myocardial OMAR retention with micoPET/CT in mice yielded significantly higher levels in obese than in lean mice. Absolute quantification of CB1-R gene expression using droplet digital PCR confirmed CB1-R upregulation in obese versus lean control mice. Obese mice also had elevated myocardial levels of anandamide and 2-arachidonoylglycerol compared to lean mice. In parallel, myocardial OMAR retention was significantly higher in 7 human subjects with advanced obesity compared to 5 normal weight human controls.

Thus, noninvasive imaging of cardiac CB1-R expression with ^{11}[C]-OMAR and PET/CT is feasible and signifies an upregulation of cardiac CB1-R expression in obesity. These observations warrant the further clinical testing of CB1-R targeted molecular imaging in cardio-metabolic disease.

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POTENTIAL EXPLOITATION OF THE ENDOCANNABINOID SYSTEM TO MODULATE BONE REMODELING ABOARD THE INTERNATIONALSPACE STATION

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Bone loss is a major thread to astronauts' health during Space missions, and thus the identification of novel biomarkers to be exploited in bone regeneration is a main focus of Space research.

Based on results obtained from our previous missions "ROALD" (Role Of Apoptosis in Lymphocyte Depression) and "ROALD2/RESLEM" (Role of the Endocannabinoid System in human Lymphocytes Exposed to Microgravity), that demonstrated the involvement of endocannabinoid signaling in growth and differentiation of human immune cells aboard the International Space Station (ISS), we proposed the "SERiSM" (Role of the Endocannabinoid System in Reprogramming Human Pluripotent Stem Cells under Microgravity) project. SERiSM has been selected by the Italian Space Agency (ASI) following the 2012 Life Science Research Announcement (ASI DC-MIC-2012-024), and has been approved by NASA to flight aboard the ISS in 2017. The aim of the project is to evaluate the role of endocannabinoid signaling during osteogenic differentiation in microgravity of an innovative stem cell model, i.e. human Blood-Derived Stem Cells (hBDSCs). These cells will be analysed by RT-PCR, morphological, cytochemical and immunochemical techniques.

Here, we will present the SERiSM rationale along with unpublished results showing mRNA and protein expression of endocannabinoid-binding receptors CB_1, CB_2, GPR55 and TRPV1, and endocannabinoid-metabolic enzymes NAPE-PLD, FAAH, DAGL and MAGL in hBDSCs, as well as in hBDSC-derived osteoblasts. In addition, we will show data obtained by exposing to simulated microgravity (0*g*) in a Rotary Cell Cultivation System both undifferentiated hBDSCs (t0) and hBDSCs stimulated for 5 days in order to differentiate into osteoblasts (t5). In particular, we will document a significant reduction in gene expression of CB_1 and CB_2 in hBDSCs at t0, but not at t5, compared to corresponding ground (1*g*) controls.

Overall, it can be anticipated that the SERiSM project will help to disclose the potential exploitation of the endocannabinoid system to modulate bone remodeling under authentic microgravity conditions.

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CHARACTERIZATION OF CB1R ISOFORMS IN FETAL AND MATERNAL TISSUES IN A BABOON *(PAPIO* SPP.) MODEL

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Introduction: Endogenous Cannabinoid system (ECS) plays an essential role in reproduction. Cannabinoid receptor 1 (CB1R) has been identified in maternal and fetal peripheral tissues such as adipose, hepatic, and pancreatic islets, both in murine and baboon models. Transcript variants encoding CB1R have been recognized as $CB1_aR$ and $CB1_bR$ in adult hepatocytes and β-cells and proposed as pharmacological targets for treatment of metabolic conditions. However, much information concerning the characterization of these two receptor isoforms in baboon (*Papio* spp.) is yet to be elucidated. Considering this, the purpose of this study was to characterize the expression of both $CB1_aR$ and $CB1_bR$ isoforms, isolated from fetal and maternal tissues in a baboon (*Papio* spp.) model.

Materials and methods: Maternal and fetal tissues [brain, placenta, maternal liver (ML), and fetal liver (FL)] were available from the tissue bank (*Papio* spp.). In order to assess the expression of the CB1R protein variants, Western blot (WB) was performed. A ChemiDoc-It^{TS3} Imager and Image J software were used to quantify proteins expression. Followed this, immunohistochemistry assays would permit visualize the co-expression of both $CB1_aR$ and $CB1_bR$ isoforms on placental tissue slides. Specific antibodies and blocking peptides were used. TaqMan mix (Roche, Applied Sciences, Indianapolis, IN, USA) and SYBR Green mix (Kapa Bio systems Inc. Woburn, MA, USA) were used for qRT-PCR and data were collected using LightCycler® 96 (Applied Biosystems/Roche, USA).

Results: CB1R (~58 kDa) was expressed in several tissues studied. $CB1_aR$ mRNA isoform showed expression in placental tissues, however, $CB1_aR$ protein isoform (~46 kDa) was only expressed in brain tissues. On the other hand, $CB1_bR$ protein isoform (~49 kDa) was expressed in FL compared to ML tissues as well as mRNA isoform. Protein isoform of $CB1_bR$ was not expressed in brain. Two more CB1R isoforms were found over-expressed (~30 kDa and ~33 kDa) in male and female fetal liver tissues.

Conclusion: Our study is the first one to demonstrate the expression of CB1R isoforms in fetal liver tissues compared to placental, brain, and maternal liver tissues.

CHARACTERIZATON OF ISOFORMS OF THE CALCIUM-INDEPENDENT *N*-ACYLTRANSFERASE PLAAT-1 IN HUMANS AND MICE

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N-Acylphosphatidylethanolamine (NAPE) serves as a precursor for anandamide and other bioactive *N*-acylethanolamines. *N*-Acyltransferases are responsible for transferring an acyl chain of glycerophospholipid, such as phosphatidylcholine, to the amino group of phosphatidylethanolamine, resulting in the formation of NAPE. We previously demonstrated that phospholipase A/acyltransferase-1 (PLAAT-1), which was originally isolated from mammals as a tumor suppressor, functions as a calcium-independent *N*-acyltransferase. We recently found the presence of an uncharacterized isoform of PLAAT-1 on NCBI database. This isoform contained an extra sequence at N-terminus, which was rich in arginine residues and formed a polybasic domain. In the present study, we compared the occurrence, intracellular localization and catalytic properties between this longer isoform and the original shorter isoform from humans and mice. Our PCR analysis revealed that human tissues express mRNA of the longer isoform with high expression levels in testis, skeletal muscle, heart, and brain. However, the shorter isoform was not detected anywhere. In contrast to humans, mouse tissues expressed both the isoforms with different tissue distribution. Unlike the cytoplasmic localization of the shorter isoform, the longer isoform was found in nucleus in addition to cytoplasm, implying that the polybasic domain possesses a nuclear localization signal. When their enzyme activities were examined by using purified proteins, neither isoform required calcium ion for full activity. The ratio of *N*-acyltransferase activity to phospholipase A_1/A_2 activity was also similar between the isoforms. Moreover, the overexpression of each isoform in mammalian cells remarkably increased cellular NAPE levels. In conclusion, the new longer isoform of PLAAT-1-was present in both cytoplasm and nucleus of humans and mice, and acted as a calcium-independent *N*-acyltransferase. These findings suggest a possible contribution of this PLAAT-1 isoform to the generation of NAPE in various membrane structures, including nuclear membrane.

ENDOCANNABINOID HYDROLASE ACTIVITIES ARE DIFFERENTIALLY EXPRESSED IN HUMAN BLOOD FRACTIONS

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There are multiple reports describing levels of endocannabinoids and congeners in human plasma, many of which show changes associated with physiological and pathological conditions, such as food intake and exercise. There are much fewer investigations describing the activities of the principle endocannabinoid hydrolysing activities, FAAH and MAGL, in human blood. We set out to apply established radiometric assays of FAAH and MAGL (using *N*-arachidonoyl-[^{3}H]-ethanolamine and 2-oleoyl-[^{3}H]-glycerol as substrates, respectively) to examine the distribution of these enzymes in human blood fractions, and whether repeated sampling from the same donors provided consistent levels of activity.
Following approval from the School of Life Sciences Ethics Committee, overnight fasting blood samples were taken in the morning from 18 otherwise healthy subjects (9 female and 9 male). The blood samples were collected at three visits and the period between each experimental visit was 15 days. Blood was collected into acid-citrate-dextrose as anticoagulant, or allowed to form a clot. ACD-treated blood was separated into plasma, washed platelets and erythrocyte fractions, while serum was isolated from the clotted fraction. Enzyme activities were normalised to the equivalent volume of blood from which they were derived and expressed as mol/min/mL blood in order to allow analysis of the relative contribution to blood endocannabinoid hydrolysis.

We found no differences between male and female donors in these analyses. MAGL activity was markedly higher in platelets (160 ± 13 nmol/min/mL blood) than erythrocytes (17 ± 1 nmol/min/mL), and much lower in plasma or serum, where it was variable or undetectable. Summing the activities from platelets, erythrocytes and plasma aggregated ~95 % of whole blood MAGL activity (188 ± 9 nmol/min/mL).

FAAH activity was highest in isolated erythrocytes (39 ± 3 pmol/min/mL), with much lower levels in platelets (1.0 ± 0.1 pmol/min/mL) and plasma (undetectable). Interestingly, FAAH activity was low but detectable in serum (0.5 ± 0.1 pmol/min/mL). Summing the activities from platelets, erythrocytes and serum aggregated ~610 % of the FAAH activity observed in whole blood (6.6 pmol/min/mL).

The activity of MAGL in platelets was relatively stable and reproducible within subjects over three different time points ($P>0.05$), while there was a significant difference in MAGL activity between subjects ($P<0.001$). On the contrary, there was no significant difference in the level of FAAH activity in erythrocytes either within or between subjects ($P>0.05$).

The levels of MAGL and FAAH activities rapidly degraded after being stored at -80 °C for up to 6 days ($P<0.0001$). More than 50 % of MAGL activity was lost in platelets and erythrocyte membranes after 3 days of storage, while FAAH activity was decreased ~ 30% in RBC after storage for 3 days at -80 °C.

Pharmacological analysis of the enzyme activities was consistent with inhibitor profiles; JJKK048 inhibited platelet 2-OG hydrolysis with a pIC_{50} value of 10.4 ± 0.1, while URB597 inhibited erythrocyte AEA hydrolysis with a pIC_{50} value of 8.3 ± 0.1.

Thus, although we have identified that storage at -80 °C results in a marked loss of activity, these studies identify that MAGL and FAAH activities are measurable in blood fractions and are differentially expressed in these fractions.

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INCREASES IN TEMPERATURE AND CAPSAICIN DRIVE THE PRODUCTION OF 2-AG AND RELATED LIPIDS WHILE DECREASING LEVELS OF AEA AND RELATED LIPIDS

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Cannabinoids and endocannabinoids (eCBs) have long been known to play a role in thermoregulation. Cross-talk between CB1 and TRPV1 have been indicated as part of the mechanism; however, there are many gaps in our understanding of how these signaling systems may work together to drive changes in both core temperature and the perception of heat and cold. Previously at ICRS we presented data that capsaicin-treated TRPV1 transfected HEK cells caused 2-AG production in these cells. We also presented data that acute THC decreases levels of 2-AG. Here, we further test the hypothesis that TRPV1 activation drives eCB production by examining how capsaicin dose and time course effect not only 2-AG production but its endogenous structural analogs, 2-oleoyl glycerol (2-OG), 2-linoleoyl glycerol (2-LG), and 2-palmitoyl glycerol (2-PG) as well as the eCB, Anandamide (AEA), and its endogenous structural analogs the NAEs. In addition to capsaicin-induced lipid production, we tested the hypothesis that changes in temperature that causes calcium-mobilization in the TRPV1-HEK cells would cause analogous changes in eCBs and related lipids.

TRPV1-transfected HEK cells were grown in 10% serum and DMEM at 37C and 5% CO2 until 80% confluency. Cells were then challenged with capsaicin (100nM, 1μM, or 10μM) for either 1 or 5 minutes. To stimulate with changes in temperature, TRPV1-HEK cells were grown in glass petri dishes were placed on a hot plate and heated to 45°C. Temperatures inside the dish were monitored to ensure consistent temperature increases from 37-45°C. Reactions were halted and lipids extracted with addition of 100% methanol. Cells and methanol were collected, the supernatants partially purified on C18 solid phase extraction columns and eluants analyzed using HPLC/MS/MS.

As previously shown with 10μM capsaicin, TRPV1 activation by capsaicin caused 2-AG production. Here we show that it also drives production of 2-LG and 2-OG, but not 2-PG. Capsaicin caused production of 2-AG, 2-OG, and 2-LG in a dose-dependent manner; however, all concentrations of capsaicin caused production of 2-AG, 2-OG, and 2-LG. Likewise, higher levels of production were measured at 5 minutes of stimulation compared to 1 minute of stimulation at each concentration. Importantly, an increase in temperature from 37 to 45°C also drives the release of 2-AG, 2-LG, and 2-OG; however, 2-PG is unaffected. In contrast, capsaicin and heat both ***decrease*** levels of AEA and related NAEs.

The relationship of the eCB system and thermoregulation is not well understood. Here, we show novel data that an increase in temperature increases the production of the eCB, 2-AG, while decreasing the levels of the eCB, AEA. This insight will open up new avenues for discovery in how the eCB system is involved in the regulation of temperature, but also how plant CBs like THC might be involved in thermoregulation.

DISTRIBUTION AND LOCALIZATION OF CB1R, NAPE-PLD, AND FAAH IN THE NUCLEUS ACCUMBENS CORE AND SHELL OF VERVET MONKEYS

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Dopamine (DA) release onto the nucleus accumbens (NAc) is central to the reward circuit, the dysregulation of which plays a role in addiction. The NAc is an ovoid structure divided into two regions delimited anatomically, the core and the shell. Both regions receive DA projections from the ventral tegmental area (VTA). VTA DA release onto the shell mediates feelings of reward associated with addiction, while the core is part of a motor circuit with the substantia nigra (SN) that encodes motor patterns for obtaining those rewards. There is belief from the rodent literature that the endocannabinoid (eCB) system present in the NAc might play a role in the modulation of DA release. Expression patterns of the cannabinoid receptor type 1 (CB1R), the synthesizing enzyme *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), and the degradation enzyme fatty acid amide hydrolase (FAAH) in the NAc have not been described in monkeys and humans. It is therefore the goal of the present study to characterize the expression and localization of the eCB system within the NAc of vervet monkeys (*Chlorocebus aethiops sabaeus*) using Western Blots and immunohistochemistry.

CB1R, NAPE-PLD, and FAAH are expressed across the NAc, both in the shell and core. CB1R is localized in Ctip2-positive cells, representing medium spiny neurons (MSNs), as well as in parvalbumin (PV)-positive cells, representing fast-spiking GABAergic interneurons (FSIs). We observed complementary expression, but not co-localization, between CB1R and TH-positive cells, corresponding to dopaminergic projections from the VTA and SN. Both enzymes, NAPE-PLD and FAAH, were also expressed in Ctip2- and PV-positive neurons, but not in TH-positive neurons. GFAP-positive astrocytes did not express CB1R, NAPE-PLD, or FAAH. These data indicate that the CB1R system is also present in the monkey NAc and we suggest that it may play an important role in the brain reward circuit through a modulatory action on dopamine release.

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TRPV1 AND FAAH DUAL BLOCKER MODULATES BONE MASS BY ENDOCANNABINOID/ENDOVANILLOID INTERACTION

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Bone is a metabolically active tissue that undergoes a continuous remodelling process during which, under normal conditions, bone resorption and formation remain in balance**.** Three key molecules tightly regulate the remodelling process: 1) receptor activator of nuclear factor kappa B ligand (RANKL), localized on osteoblasts, which enhances osteoclastogenesis via interaction with its receptor - RANK - localized on osteoclasts; 2) moreover, osteoblasts also produce osteoprotegrin (OPG), which protects the skeleton from excessive bone resorption by binding to RANKL and preventing it from interacting with RANK. Therefore, osteoblasts are important not only for the synthesis of bone, but also for the control of bone resorption. Disruption of bone metabolism leads to changes of bone structure, strength and mass, resulting in several bone diseases such as osteoporosis and osteoarthritis. Identifying the molecular pathways that regulate bone cell activity provides a key to understanding the causes of these diseases and to the development of new treatments.

Previous studies showed that cells and intervening nerves in the skeleton express cannabinoid receptors and the machinery for the synthesis and breakdown of endocannabinoids as well as the putative cannabinoid receptor GPR55, thus highlighting the importance of these molecules in bone mass regulation, loss and cell function. The transient receptor potential vanilloid type 1 (TRPV1), linked to many phytocannabinoid- and endocannabinoid-mediated effects, is known to play an important role in the regulation of pain and inflammation also due to its distribution also in bone cells. These findings suggest that the CB1, CB2, GPR55 and TRPV1 receptors, and the levels of endocannabinoids are important players in maintaining bone homeostasis. As a consequence, the endocannabinoid/endovanilloid system is considered an emerging target in bone disease therapies targeting bone forming cells.

On this background, we investigated the influence of the endocannabinoid system and TRPV1 receptors in osteoblast metabolism using a synthetic TRPV1 and FAAH dual blocker, OMDM-198. Human osteoblasts (HOb, Cell Applications, INC., USA) were treated with 10μM OMDM-198 to investigate its influence on osteoblast proliferation capacity (through the BrdU incorporation assay). We assessed changes in mRNA levels of endocannabinoid system proteins as well as osteoblast-specific transcription factor (RUNX2) and bone remodeling factors (OPG, RANKL) in response to OMDM-198 treatment. Moreover levels of endocannabinoids (anandamide, 2-AG) and related mediators (oleoylethanolamide and palmitoylethanolamide) in osteoblast culture media were evaluated by Liquid Chromatography–Mass Spectrometry (LC-MS) method.

Treatment with OMDM-198 significantly increased osteoblast proliferation capacity, concomitant with significant decrease in TRPV1 gene expression. Furthermore, in the culture media of stimulated cells, a strong elevation of anandamide and oleoylethanolamide levels was observed. This latter effect correlated with higher expression of NAPE-PLD, involved primarily in acylethanolamide synthesis, and a downward trend for the expression of anandamide-degrading enzymes: FAAH and COX2. Moreover, upon OMDM-198 treatment, the mRNA levels of CB1 were slightly elevated and those of GPR55 were significantly increased. Treated osteoblasts exhibited unchanged levels of OPG and RANKL. Gene expression of RUNX2 factor also remained at control levels.

The present results indicate the existence of a cross-talk between CB1, TRPV1 and possibly with GPR55 in bone forming cells. The higher levels of anandamide and oleoylethanolamide possibly correlate with the positive effect on osteoblast proliferation. Blockade of TRPV1 and FAAH maintain skeletal integrity. These findings suggest that the endocannabinoid system can protect bone from excessive resorption and could be a valuable therapeutic target for the prevention and treatment of bone diseases.

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INTERACTIONS BETWEEN ENDOCANNABINOID AND PROSTANOID SIGNALING PATHWAYS IN SPINAL ASTROCYTES

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Recognized as one of the most important endogenous cannabinoid ligand, 2-arachidonoylglycerol (2-AG) influences a plethora of physiological processes including neurotransmission. Effects of 2-AG are mediated mainly by type 1 cannabinoid receptor (CB1) in the central nervous system, and most of the mobilized 2-AG is broken down by monoglycerol lipase (MGL) to form arachidonic acid (AA). MGL, however, is not the only enzyme degrading 2-AG, since cyclooxygenase-2 (COX-2), among several other enzymes, also accepts 2-AG as substrate, resulting in the formation of prostanoids. Importantly, AA and also prostanoids are bioactive metabolites that can induce biological responses by activating GPR40 (FFA1) and prostanoid receptors, respectively.

Astrocytes not only express a complete molecular toolbox for cannabinoid and prostanoid signaling, but activation of CB1, GPR40 and prostanoid receptors all have been shown to induce calcium transients in these cells. Thus, here we investigated whether the metabolism of 2-AG in spinal astrocytes favors the formation of AA or prostanoids, and whether a consequential activation of the cells by the newly formed bioactive metabolites will occur.

Cultured spinal astrocytes were treated with 2-AG alone or following a preincubation with the CB1 antagonist AM251, COX-2 inhibitor nimesulide or MGL inhibitor JJKK-048, while the change in the intracellular calcium concentration was monitored. 2-AG induced calcium signals with short (<30s) and/or longer (>30s) latency in astrocytes. Pretreatment of astrocytes with AM251 caused an almost complete loss of the early component of 2-AG-evoked calcium signals, whereas neither nimesulide nor JJKK-048 had significant effect on the calcium transients. Simultaneous inhibition of MGL and COX-2, however, prevented the induction of delayed component of calcium signals.
Our results suggest that 2-AG induce calcium transients in spinal astrocytes not only through the activation of CB1, but AA and prostanoids, newly synthesized hydrolytic and oxidative metabolites of 2-AG, also increase the intracellular calcium concentration in these cells. Thus, CB1-dependent and CB1-independent mechanism also participate in mediating the effects of 2-AG in spinal astrocytes.

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CAN A LIGAND SWITCH CB1 SIGNALING FROM INHIBITORY (Gi) TO STIMULATORY (Gs) G PROTEIN?

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One of the most highly expressed receptors in the human central nervous system, the cannabinoid CB1 receptor (*CNR1*) belongs to class A, G-Protein Coupled Receptors (*Howlett et al., 2002, K Mackie, 2006*). CB1 is reported to signal predominantly via Gi/o proteins, but to some extent via Gs protein (*Glass et al., 1997*). The first CB1 allosteric modulator, ORG27569 (ORG), was reported by Ross and co-workers (*Price et al., 2005*). In our program to develop potent CB1 allosteric ligands, an analogue of ORG, PHR20 (*Reggio et al, 2015 WO2015195486 A1*) was synthesized by Seltzman and co-workers at RTI. Here we present the pharmacology of PHR20 using the orthosteric endogenous agonists of CB1 Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and synthetic agonists WIN 55,212-2 and CP-55,940 (*Howlett et al., 2002, Freund et al., 2003, K Mackie, 2006*).

Human Embryonic Kidney 293 (HEK293) cells were transfected with FLAG tagged human CB1 lacking the first 87 N-terminal residues. Cellular cAMP levels were measured using a homogenous time resolved fluorescence (HTRF) cAMP kit from Cisbio international. Ligand binding assays were performed on the membranes prepared from HEK293 cells stably expressing FLAG tagged hCB1 following earlier published protocols (*Feng et al., 2003, Kumar et al., 2014*).

The hCB1 binding assay using [^{3}H]CP-55,940, showed no displacement of CP-55,940 by PHR20. The cAMP assay showed that PHR20 produced no change in the forskolin stimulated cAMP accumulation, suggesting no Gi/o (or Gs) coupling. Pretreatment with PHR20 did not affect the forskolin stimulated cAMP accumulation produced by AEA, WIN 55,212-2 and CP-55,940 suggesting that PHR20 does not allosterically modulate the signaling of these ligands. However pretreatment with PHR20 resulted in an increase in forskolin stimulated cAMP accumulation for 2-AG, suggesting Gs signaling. This increase in cAMP accumulation produced by 2-AG in the presence of PHR20 could be decreased by cholera toxin (CTX). Pertussis toxin (PTX) treatment attenuated the cAMP accumulation by 2-AG however the increase in cAMP accumulation produced by 2-AG in the presence of PHR20 was retained. These results suggest that PHR20 is a selective, biased CB1 allosteric modulator that can switch G protein coupling from Gi/o to Gs for the endogenous ligand 2-AG at hCB1.

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STRUCTURAL RELATIONSHIP OF THE CLASS A ORPHAN GPCR, GPR6 WITH THE CANNABINOID CB1 AND CB2 RECEPTORS

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The orphan G-protein coupled receptor 6 (GPR6) is a constitutively active receptor coupled to stimulatory Gαs protein that raises intracellular formation of cyclic adenosine-3',5'-monophosphate (cAMP). Overexpression of GPR6 has been shown to boost neurite outgrowth in vitro. GPR6 is highly expressed in the striatopallidal in the basal ganglia. It has been shown that depletion of GPR6 causes both dopamine increase and striatal cAMP decrease. This suggests that GPR6 may have a role in the treatment of Parkinson disease as GPR6 depletion increases motor activity and decreases abnormal uncontrollable movements.

GPR6 usually is grouped with both GPR3 and GPR12 as they share at amino acids level more than 50% identity and 65% similarity. GPR3,6 and 12 belong to the GPCR Class A MECA cluster of receptors (melanocortin receptors (MCRs), endothelial differentiation G-protein coupled receptors (now called S1P and LPA receptors), cannabinoid receptors (CNRs), and adenosin binding receptors (ADORAs).

In this study, we have compared the sequences and identified similarities and differences between GPR3-6-12, the cannabinoid receptors CB1 and CB2, the lysophospholipid receptors S1P1 and LPA1, and the cannabinoid-related receptors GPR55 and GPR18. We have also evaluated similarities and differences between the sequences of human, mouse and rat GPR6. One important feature that we have identified for GPR6 is in its TMH6 sequence which contains five Gly residues (G6.33, G6.35, G6.42, G6.45 and G6.58). TMH6 has been shown to undergo a critical conformational change during G-protein dependent activation and Gly residues in key positions have been shown to endow additional flexibility in TMHs. We have previously shown that a Gly residue in CB1 TMH6, G6.49, confers additional flexibility in TMH6 that may be related to CB1 constitutive activity. We will present Conformational Memories results for GPR6 TMH6 that may shed light on the origin of the high G-protein dependent constitutive activity of GPR6. [Support: RO1 DA003934 and KO5 DA021358 PHR]

IN VITRO ESTERASE ASSAYS FOR HUMAN RECOMBINANT MONOACYLGLYCEROL HYDROLASES (MAGL, ABHD6, ABHD12)

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Although MAGL appears responsible for the bulk hydrolysis of 2-AG, ABHD6 has recently emerged as a minor, but potentially important additional monoacylglycerol hydrolase activity. This study aimed to establish *in vitro* assays for human recombinant enzymes in order to allow future high-throughput screening.

To this end, we examined esterase substrates: a chromogenic substrate (4-nitrophenolacetate, NPA) and two fluorigenic substrates (4-methylumbelliferyloleate, MUO and 4-methylumbellliferylheptanoate, MUH). The particulate and soluble fractions of the four enzyme transfects MGLX1, MGLX2, ABHD6 and ABHD12 transiently transfected into HEK293 cells were assessed for hydrolase activity at 37°C.

Background absorbance of freshly-prepared 2 mM NPA was relatively modest (A_{405} 0.04-0.06) and spontaneous hydrolysis was low over 120 min (in one experiment, 116 ± 2 % at 120' compared to 15'). However, background hydrolysis in mock-transfected cells was high (in one experiment at 60', particulate hydrolysis was 299 ± 11 % compared to tissue blank, while soluble hydrolysis was 199 ± 2 % as 10-fold original wet weight). Particulate hydrolytic activity showed the rank order: MGLX1 (549 ± 6) > MGLX2 (433 ± 4), while ABHD6 (302 ± 11) and ABHD12 (284 ± 14 % tissue blank) were ineffective. Soluble NPA hydrolysis showed the rank order: MGLX1 (523 ± 4) > ABHD6 (338 ± 6) > MGLX2 (288 ± 7), while ABHD12 was ineffective (239 ± 2 % tissue blank). Michaelis-Menten analysis using MGLX1 transfects suggested a K_M value of ~1 mM for both particulate and soluble fractions. Further dilution of particulate and soluble fractions resulted in an inability to distinguish MGLX1 transfect from mock transfect (in one experiment, 40-fold dilution of particulate fractions 122 ± 1 vs 99 ± 1 %; soluble fraction 125 ± 2 vs 103 ± 1 % tissue blank, respectively). Taken together, these data suggest that NPA is likely to be a poor substrate for detection of monoacylglycerol hydrolase activities; even with MGLX1 fractions, sample dilution means it is unlikely to be economical.

Using 12.5 µM MUO as substrate, background fluorescence was low (4-8 RFU) and not changed over time (in one experiment, 100 ± 6 % at 90' compared to 10'). Background hydrolysis at 60' in mock-transfected cells was approximately double tissue blanks and not altered substantially in hydrolase-transfected preparations. In one experiment at 60', the particulate rank order of activity was ABHD6 (273 ± 5) > MGLX2 (235 ± 6) > MGLX1 (210 ± 4) > mock (195 ± 4) > ABHD12 (169 ± 3 % tissue blank). The soluble fraction rank order was MGLX1 (246 ± 5) > ABHD6 (213 ± 14), mock (191 ± 8) > MGLX2 (167 ± 2), ABHD12 (162 ± 10). Taken together, these data suggest that MUO is a poor substrate for detection of monoacylglycerol hydrolase activities.

Using 25 µM MUH as substrate, background fluorescence was higher than with MUO (20-30 RFU) and increased over time (in one experiment, 132 ± 3 % at 60', 178 ± 5 % at 120' compared to 10'). In one experiment at 60', the particulate rank order of affinity was ABHD6 (719 ± 24) > ABHD12 (251 ± 5) > MGLX2 (178 ± 11), MGLX1 (166 ± 11) > mock (130 ± 4). Soluble MUH hydrolysis showed the rank order: ABHD6 (782 ± 29) > ABHD12 (256 ± 5) > MGLX2 (181 ± 13), MGLX1 (170 ± 7) > mock (124 ± 3 % tissue blank). Using ABHD6 particulate preparations, 25 µM MUH hydrolysis was inhibited in a concentration-dependent manner by WWL70 (pIC_{50} 7.4). At 1 µM, WWL70 evoked a large inhibition of MUH hydrolysis (21 ± 10 % control), while JZL184 evoked only a small inhibition (84 ± 5 % control).

In conclusion, NPA and MUO appear to be of limited value in the investigation of monoacylglycerol lipase activities in vitro. 4-MUH hydrolysis, by contrast, could be a promising assay for screening modulators of ABHD6.

DENSITY FUNCTIONAL THEORY STUDY OF ACYL MIGRATION IN VARIOUS-CHAIN MONOACYLGLYCEROLS

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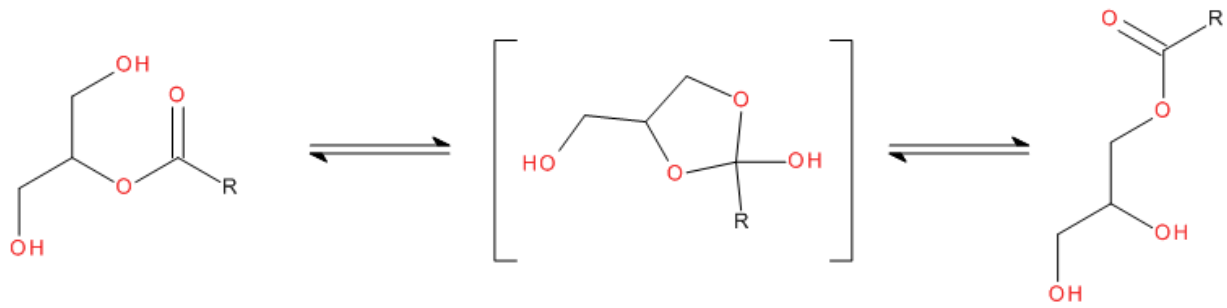
Monoacylglycerols (MAGs) are integral to endogenous cannabinoid physiology. The acyl group may exist bonded to any hydroxyl of the three-carbon glycerol- that is as 1(3)- and 2-MAGs. As the 1(3) isomer is generally energetically favorable over the 2, experimental data on the relative energies between isomers, as well as the ketal-containing intermediates, would be useful but costly. Computational thermochemical data may provide insight for later experimental profiling. Density functional theory (DFT) was employed at the B3LYP/6-31+G* level of theory to investigate the thermochemistry of a wide range MAGs with varying degrees of length (14 to 24 C atoms) and saturation (unsaturated to hexa-unsaturated).



Left: 2-MAG, Middle: ketal intermediate, Right: 1(3)-MAG*; Whereas 1(3)-MAG isomers are energetically preferable in comparison to 2-MAG, compounds such as 2-arachidonoylglcyerol (2-AG) bind to receptors more efficaciously than their 1(3)- counterparts.*

Results regarding free energy, enthalpic, and entropic differences between isomers, as well as higher levels of theory and relevant compounds are discussed, degrees of saturation are scrutinized to inspect for effects on changes in energy, and general implications of acyl migration in MAGs are reflected upon.

COMBINED CB2 RECEPTOR AGONIST AND PHOTODYNAMIC THERAPY SYNERGISTICALLY INHIBIT TUMOR GROWTH IN TRIPLE NEGATIVE BREAST CANCER

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Triple negative breast cancer (TNBC) is the deadliest form of breast cancer because compared with other types of breast cancer, it is more aggressive, diagnosed at later stage and more likely to develop recurrence. Many patients do not experience adequate tumor control after current clinical treatments involving surgical removal, chemotherapy and/or radiotherapy, leading to disease progression and significantly decreased quality of life. Here we report a new combinatory therapy strategy involving cannabinoid-based medicine and photodynamic therapy (PDT) for the treatment of TNBC. This combinatory therapy targets two proteins upregulated in TNBC: the cannabinoid CB2 receptor (CB_2R, a G-protein coupled receptor) and translocator protein (TSPO, a mitochondria membrane receptor). We found that the combined CB_2R agonist and TSPO-PDT treatment resulted in synergistic inhibition in TNBC cell and tumor growth. This combinatory therapy approach provides new opportunities to treat TNBC with high efficacy. In addition, this study provides new evidence on the therapeutic potential of CB_2R agonists for cancer.

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NEUROINFLAMMATORY EFFECTS AND BEHAVIORAL CORRELATES AFTER REPEATED EXPOSURE TO THE SYNTHETIC CANNABINOID JWH-018

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The synthetic cannabinoid (SC) 1-pentyl-3-(1-naphthoyl)-indole (JWH-018) has been detected in several samples of a smokable herbal mixture termed Spice/K2 drugs that are currently marketed as legal alternatives to *Cannabis*. Its use represents a growing public health worldwide. JWH-018 is a CB1/CB2 receptor agonist with higher affinity than Δ9-THC, the active ingredient of marijuana. JWH-018 shares with Δ9-THC CB1-dependent reinforcing and DA stimulant actions displaying a preferential effect on the NAc shell at the dose of 0.25 mg/kg i.p. (De Luca et al., Neuropharmacology 99 (2015) 705-714). Despite the increasing popularity of Spice drugs, the effects of their chronic use are unknown. Recently, an *in vitro* study showed that SCs induce cytotoxicity in forebrain neuronal cultures in a concentration-dependent manner (Tomiyama and Funada, Toxicol Appl Pharmacol. 274 (2014) 17-23). However, modulation of the endocannabinoid system has been associated with both neurotoxic and neuroprotective effects (Fowler et al Exp Neurol. 224 (2010) 37-47; Pope et al., Neurotoxicology 31 (2010) 562-571). In addition, we demonstrated that Δ9-THC reduces METH-induced brain damage via inhibition of nNOS expression and astrocyte activation (Castelli et al., PLos One 9 (2014)). In the present study, we evaluated the neuroinflammatory effects induced by a chronic treatment with JWH-018 in DAergic brain regions involved in emotional and cognitive processing. To this end, rats were administered once a day for 14 consecutive days with JWH-018 (0.25 mg/kg i.p.) or vehicle.

Levels of tyrosine hydroxylase (TH), dopamine transporter (DAT), glial fibrillary acidic protein (GFAP), ionized calcium binding adapter molecule 1 (IBA-1), and caspase were evaluated in the medial pre-frontal cortex (mPFC), nucleus accumbens (NAc), caudate-putamen and ventral tegmental area as signs of JWH-018-induced neurodegeneration and neuroinflammation. Besides, studies on anxiety-like (Elevated Plus Maze, EPM) and/or repetitive-like behaviors (Marble Burying, MB) and attentional processes (Prepulse Inhibition, PPI) were performed. Results showed that JWH-018 treatment: i) increases IBA-1 immunoreactivity in the NAc core and reactive astogliosis (GFAP) in the mPFC and NAc shell, ii) induces anxiety-like states as revealed by a decreased time spent in the open arms of the EPM, iii) causes repetitive-like behavior as revealed by the higher number of marbles buried in the MB test, and (iv) impairs the PPI of the acoustic startle reflex. Our findings demonstrated that the behavioral alterations induced by JWH-018 are associated with a neuroinflammatory phenotype thus contributing to understand the possible cause of detrimental effects of recurring use of Spice/K2 drugs.

ANANDAMIDE MODULATES EPIGENETIC REGULATION OF INFLAMMATORY GENES

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Objective – The endocannabinoid system regulates stress, food intake and emotional behavior. Endocannabinoids are also beneficial in the cardiovascular system to reduce blood pressure and infarct size. Whether they, however, also have anti-inflammatory functions is controversial. We therefor set out to identify the impact of endocannabinoids on inflammatory signaling in vascular cells.

Results – Anandamide (AEA) is the ethanolamide of arachidonic acid and is one of the best characterized endocannabinoids. We therefore focused on this compound. Pretreatment of murine aortic segments, murine lung endothelial cells and human aortic smooth muscle cells (hAoSMCs) with AEA decreases the IL1β- and TNFα-induced induction of inflammatory genes on mRNA and protein level. In line with this, supernatant of hAoSMCs stimulated with AEA attenuated monocyte migration. These effects were not mediated by PPAR or by the cannabinoid receptors CB1 or CB2 and therefore a potential of intracellular cannabinoid receptors. Importantly, NF-κB translocation into the nucleus was not affected by AEA, suggesting epigenetic mechanisms on the anti-inflammatory effects of AEA. Therefore, ChIP experiments were performed. AEA silenced inflammatory genes, by induction of repressive heterochromatin.

Conclusions – AEA reduces the inflammatory response of hAoSMC by epigenetic silencing of inflammatory genes.

EFFECTS OF Δ^9-THC ON CYTOKINE PRODUCTION FROM SPLENOCYTES DERIVED FROM IMMUNE COMPETENT AND IMMUNOSUPPRESSED MICE INFECTED SYSTEMICALLY WITH *CANDIDA ALBICANS*

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Previous studies have shown that Δ9-tetrahydrocannabinol (THC) suppresses the resistance to many microbial infections, but the effects of THC on fungal infections are unclear. Recent findings from our laboratory have shown that chronic THC treatment decreased the resistance to a secondary *Candida albicans (C. albicans)* infection in immune competent mice. However, the effect of THC on resistance to a fungal infection in immunosuppressed mice is unknown. The spleen plays a crucial role in host immune responses against systemic microbial infections, and the spleen is populated with numerous innate and adaptive immune cells. Therefore, we investigated the effects of chronic THC on cytokine production from splenocytes derived from immune competent and immunosuppressed mice infected with *C. albicans*. For our studies, we used two models of infection, an acute and a secondary systemic *C. albicans* infection. For both studies, c57BL/6 female mice were treated with vehicle (ethanol, cremophor, saline (1:1:18)) or THC in vehicle (16mg/kg/mouse, intraperitoneal (IP) injection) 4 days a week, for three weeks (experimental days 1-18). Mice that were to be immunosuppressed were injected with 5-fluorouracil (5-F, 0.1ml of 50mg/ml, intravenous (IV) injection) on day 16. For the acute infection model, the mice were infected with *C. albicans* ($5x10^5$ cells/mouse, IV) on day 19. For the secondary infection model, the mice were given a priming dose of *C. albicans* ($0.75x10^5$ cells/mouse, IV) on day 2 and a challenge dose ($5x10^5$ yeast cells/mouse, IV) on day 19. On day 22, spleens were harvested from mice (n=5) to establish a splenocyte culture. Splenocytes were treated with either Lipopolysaccharide (LPS, 1µg/ml), Concanavalin A (ConA, 5µg/ml) or heat killed (HK) *C. albicans* ($6.25x10^6$ yeast cells/ml). The splenocytes were then incubated at 37°C, and cell supernatants were collected at 48h to analyze the secreted cytokines (Interferon-gamma (IFN-γ), Interleukin 12(p40) (IL-12(p40), and Interleukin 6 (IL-6)). In addition, splenocytes were lyzed at 2h for RNA extraction to determine cytokine mRNA levels via RT-qPCR.

We found that, in both infection models, immunosuppression by 5-F decreased the cytokine secretion from the splenocytes. Interestingly, in both infection models, *C. albicans*-stimulated IFN-γ and IL-12(p40) levels were significantly lower in the THC group compared to the vehicle group in immune competent mice. In both infection models, compared to the vehicle treated group, THC did not significantly alter the ConA-stimulated IFN-γ secretion from the splenocytes derived from both immune competent and immunosuppressed mice. In the secondary infection model, compared to the vehicle group, THC significantly reduced the ConA stimulated secretion of IL-12(p40) from the splenocytes derived from immune competent mice. In the acute infection model, compared to the vehicle treated group, THC significantly reduced the LPS-stimulated IL-6 from the splenocytes derived from immune competent mice. These results strongly suggest that THC has an effect on splenic immune response against systemic *C.albicans* infection. Secretion of IL-17A from the splenocytes stimulated with ConA and HK *C. albicans* will also be analyzed, and the effect of THC on cytokine mRNA levels will be investigated using RT-qPCR.

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Δ^9-THC AND *CANDIA ALBICANS* INFECTION IN MICE

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Delta-9-tetrahydrocannabinol (Δ^9-THC) is known to suppress resistance to diverse microbial infections. However, little is known of the effects of Δ^9-THC on yeast infections. In our laboratory, we found that chronic Δ^9-THC treatment decreases resistance to a secondary systemic *Candida albicans* (*C. albicans*) infection in immune competent mice. We are now investigating the effects of Δ^9-THC on the resistance to systemic *C. albicans* infection in immune suppressed mice. For our studies, we use three infection models, a systemic acute yeast infection, a systemic secondary yeast infection, and a vulvovaginal (VVC) infection model. For these studies, c57BL/6 female mice were given an intraperitoneal (IP) injection of vehicle (VEH) control (ethanol, cremophor, saline (1:1:18)) or Δ^9-THC in vehicle (16mg/kg) on days 1-4, 8-11 and 15-18. On day 16, mice to be immunosuppressed were injected with 5-fluorouracil (5-F), a commonly prescribed anti-cancer drug which is a potent immune suppressor. For the acute yeast infection, the mice were challenged with *C. albicans* ($5x10^5$ *C. albicans* cells/mouse, IV) on day 19. For the secondary yeast infection, the mice were infected with *C. albicans* ($0.75x10^5$ cells/mouse, IV) on day 2 and were challenged with a higher dose of *C. albicans* ($5x10^5$ *C. albicans* cells/mouse, IV) on day 19. For the VVC infection model, mice were infected with *C. albicans* ($1x10^7$ cells/mouse, intravaginally) on day 19. Mice (n=7) were observed for 2 weeks for survival and morbidity. On day 22, tissues were harvested from some mice (n=5) to assess cytokine production and tissue fungal load. 5-F treatment, significantly decreased survival and serum IL-12p40 production and increased tissue fungal load in vehicle and THC treated mice in both systemic infection models. In both systemic yeast infection models, the survival rate and tissue fungal load in mice treated with THC+5-F are not significantly different from mice treated with VEH+5-F. However, compared to VEH+5-F treatment, THC+5-F treatment decreased serum IL-12p40 levels in the acute systemic yeast infection. In addition, splenocytes cultured from mice systemically infected with the yeast were treated with Concanavalin A (ConA) or heat killed *C. albicans* (HKCa). Splenocytes derived from 5-F treated mice secreted little to no cytokines regardless of *in vitro* treatment. However, splenocytes derived from THC treated immune competent mice secreted less IL-12p40 in response to ConA or HKCa challenge compared to splenocytes derived from VEH treated mice. In addition, splenocytes derived from THC treated mice secreted less IFN-γ in response to HKCa challenge, but not ConA, compared to splenocytes derived from VEH treated mice. These results suggest that THC may be able to further reduce the immune response in already immune suppressed mice. However, THC-caused cytokine suppression is not sufficient to significantly reduce survival in mice that have only been infected with *C. albicans*.

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EFFECTS OF THC ON THE SEVERITY OF *CANDIDA ALBICANS* VULVOVAGINAL INFECTION IN MICE

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Delta-9-tetrahydrocannabinol (THC), the psychoactive component in marijuana, has been widely reported to alter immune response to various pathogens. It is known that THC decreases resistance to bacterial, protozoan, and viral infections but less is known about the effects of THC on yeast infections. Vulvovaginal candidiasis (VVC) is a vaginal opportunistic fungal infection caused by *Candida* species. Over 75% of women experience VVC at least once in their life. Because approximately 10%–20% of women will suffer from chronic VVC requiring medical treatment, it is important to continue to broaden our understanding of VVC. For our experiment, we aim to determine the effects of chronic THC on *Candida albicans* (*C. albicans*) induced VVC in immunosuppressed mice. For this project, c57BL/6 female mice will be treated chronically with THC and immunosuppressed with 5-flourouracil, a chemotherapeutic agent. The mice will also receive Depo-Provera hormone injection to thin the mucosal lining of the vagina and facilitate uptake of the *C. albicans*. To infect the mice, the mice will be intravaginally challenged with 10µl of *C. albicans* ($1x10^7$ yeast cells/mouse) using a pipette. Two days post infection vagina lavage will be collected to evaluate infection intensity. Four days post *C. albicans* infection, vaginal lavage and tissues will be collected for fungal load and cytokine production evaluation. In our laboratory, we have found that THC suppresses cytokine production. Therefore, we expect that THC will cause suppression in the response to VVC. Now that over half of the USA has legalized the recreational and/or medicinal use of marijuana, it is imperative that we evaluate THC's effect on VVC. Our studies will further our knowledge in this area.

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THERAPEUTIC POTENTIAL OF CANNABINOIDS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE

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Early evidence suggests that marijuana or cannabinoid receptor stimulation may have a positive symptomatic effect on inflammatory bowel disease (IBD) patients due to analgesic and anti-inflammatory effects. The cannabinoid 2 receptor (CB_2R) is expressed primarily on immune cells, including $CD4^+$ T cells which are central to IBD pathogenesis, and is induced by active inflammation in both humans and mice.
We therefore investigated the role of the CB_2R in mice that develop chronic ileitis ($TNF^{\Delta ARE/+}$ mice) by evaluating the effect of stimulation with CB_2R-selective ligand GP-1a both *in vitro* and *in vivo*. We then compared cannabinoid receptor expression in the ilea and colons of healthy human controls to that of Crohn's disease patients. Finally, we assessed T cell phenotype in peripheral blood from adolescent IBD patients using marijuana to alleviate their symptoms.

Ileal expression of CB_2R and the endocannabinoid anandamide (AEA) were increased in $TNF^{\Delta ARE/+}$ mice compared to controls. CB_2R mRNA was preferentially induced on regulatory T cells (Tregs) compared to T effector cells, approximately 2.4-fold in WT and 11-fold in $TNF^{\Delta ARE/+}$ mice. Furthermore, GP-1a enhanced Treg suppressive function with a concomitant increase in IL-10 secretion. GP-1a attenuated murine ileitis as demonstrated by improved histologic scoring and decreased inflammatory cytokine expression. In IBD patients, active inflammation upregulates the CB_2R, while cannabis use increased production of the anti-inflammatory cytokine IL-10 two-fold, consistent with a potential therapeutic effect.

In summary, the endocannabinoid system is induced in murine and human active intestinal inflammation and CB_2R activation attenuates murine ileitis, establishing a critical anti-inflammatory role of the endocannabinoid system.

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CANNABINOID TREATMENT INDUCES REMISSION IN DRUG-RESISTANT PEDIATRIC INFLAMMATORY BOWEL DISEASE: A CASE REPORT

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Inflammatory bowel diseases (IBD) including Crohn's disease and ulcerative colitis are autoimmune-like diseases resulting from overactive inflammatory responses to intestinal microbes in genetically susceptible individuals. Patients with IBD are at high risk of developing infections and suffering worse outcomes, including higher rates of colectomy and death. Two clinical trials have shown that cannabinoid treatment improved weight gain, physical pain, diarrheal symptoms, depression, social functioning, and ability to work in patients with IBD who had failed conventional therapies. Despite these encouraging results, there has been no assessment of mucosal inflammation, therefore it is difficult to determine if treatment targets the underlying inflammatory process, or merely provides symptomatic relief. Furthermore, there is limited information about the use of cannabinoid therapies in pediatric patients with IBD.

This case report describes a 13-year-old boy with IBD who achieved clinical remission after receiving cannabinoid treatment. After years of disease, the patient had poor appetite, suffered from weight loss and stunted growth. Conventional therapies failed, including the TNF-α inhibitor infliximab (Remicade). The patient was also severely ill with repeated hospitalizations due to infection with *Clostridium difficile*. The family received approval for treatment with cannabis under the California Compassionate Care Act, and the patient began consuming edible chocolate bars that contained 3 mg of Δ9-tetrahydrocannabinol (THC) and 3 mg of cannabidiol (CBD), receiving three doses daily. The patient and family reported almost immediate symptomatic improvement, with increased appetite and body weight, reduced inflammatory scores and induced remission. Fecal levels of calprotectin, a reliable measure of active bowel disease fell from over 2,000 μg/g to 86 μg/g. Serum levels of inflammatory marker C-reactive protein were 13-18.5 mg/L from Jan 1, 2016 to March 3, 2016, and dropped to 0 mg/L on May 1, 2016, two months after initiating treatment in March 2016. Similarly, the serum erythrocyte sedimentation rate was reduced from 25 mm/Hr to 3 mm/Hr. The patient continues to consume cannabis for symptom management and is reported to still be in clinical remission for more than a year since beginning therapy. No adverse reactions were reported.

Oral cannabinoid therapy was effective and safe in a pediatric patient with IBD. Our case has added additional literature in accordance with previous reports supporting cannabis as effective and safe in patients with IBD. In addition, objective diagnostic measures of local, mucosal inflammation provide evidence that cannabinoid treatment may exert effects through cannabinoid receptors that line the intestinal tract and that are present on immune cells present within active inflammatory-mediated disease.

NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF KLS-13019 AND CANNABIDIOL IN *IN VITRO* AND *IN VIVO* MODELS OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

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Chemotherapy-induced peripheral neuropathy (CIPN) is a serious consequence of cancer therapy for which no effective treatments exist. Mitochondrial dysfunction, oxidative stress, and inflammation have all been implicated in its etiology, strongly suggesting that the development of CIPN likely involves an iterative process of neuronal dysfunction and inflammation in which each promotes the perseveration of the other. We have shown that the non-psychoactive cannabinoid cannabidiol (CBD) prevents the development of CIPN in a mouse model of paclitaxel-induced mechanical allodynia. CBD exerts a range of neuroprotective and anti-inflammation effects, including regulation of Ca^{2+} homeostasis, attenuation of reactive oxygen species (ROS) formation, and suppression of cytokines and chemokines involved in microglia, monocyte, and lymphocyte migration and activation. This wide range of effects clearly points to CBD as an exciting novel pharmacotherapy in the treatment of disorders associated with oxidative stress and inflammation, such as CIPN. Moreover, given the challenges with oral bioavailability of lipophilic compounds such as CBD, there is also much promise in pursuing the synthesis and characterization of novel small molecules with structural similarity to CBD but with improved pharmacokinetic profiles.

The present set of experiments were designed to determine the effects of CBD and the small molecule KLS13019 on ROS formation in an *in vitro* dorsal root ganglia (DRG) model of CIPN and on inflammation and mechanical sensitivity in an *in vivo* mouse model of CIPN. KLS13019 is a novel small molecule that possesses structural similarities to CBD, while exhibiting marked increases in potency, decreases in toxicity and improved PK properties from CBD. In preliminary studies we determined that CBD is protective against paclitaxel toxicity in DRG neurons. In follow-up studies we determined that KLS-13019 is also protective against paclitaxel toxicity in DRG neurons and that this protective effect was concentration-dependently inhibited by the mitochondrial Na^{+}/Ca^{2+} exchanger (mNCX) inhibitor CGP-378157. *In vivo*, we determined that administration of paclitaxel doses that produce mechanical sensitivity also lead to increased immune cell invasion into the spinal cord. Similar to the effects we observe with CBD, pretreatment with KLS13019 (2.5 mg.kg IP) significantly attenuated the development of paclitaxel-induced mechanical sensitivity. Interestingly, KLS13019 was more effective at attenuating microglial and T lymphocyte numbers in the spinal cord than CBD. However, additional cell culture studies demonstrate significant anti-inflammatory effects of CBD in microglia activated by paclitaxel exposure. Taken together these results demonstrate that CBD and KLS13019 can exert specific neuroprotective and anti-inflammatory effects highly relevant to a range of disorders, including CIPN.

TYPE-2 CANNABINOID RECEPTOR DEFICIENCY ALTERS ATHEROSCLEROTIC PLAQUE CALCIFICATION IN HYPERLIPIDEMIC LDLR-NULL MICE

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Background: Atherosclerosis, the most common type of heart disease, is a progressive inflammatory disease characterized by the formation of cholesterol-rich plaques within arterial walls. Calcification of advanced plaques leads to structural instability and is associated with increased vulnerability to rupture and risk of myocardial infarction. The mechanism of plaque calcification is unclear but is thought to occur through complex cellular and molecular mechanisms that resemble the osteogenic processes involved in normal bone development. The type-2 cannabinoid receptor (CB2) is a well-known modulator of bone remodeling and we previously showed that systemic CB2 gene deletion results in a significant increase in calcification of advanced plaques in Ldlr-null mice, a murine model of atherosclerosis, but the mechanisms involved have not yet been elucidated. Atherosclerotic calcification is known to involve vascular smooth muscle cell (VSMC) migration and transdifferentiation into osteoblast-like cells capable of depositing calcium. Therefore, we hypothesized that CB2 modulates plaque calcification by affecting the expression of osteogenic marker proteins involved in osteoblastic transdifferentiation (OBT) of VSMCs. We tested this hypothesis by evaluating the effects of systemic CB2 gene deletion on the expression of Runx2 and osteopontin (OPN) in atherosclerotic plaques from Ldlr-null mice. We further hypothesized that pharmacological targeting of CB2 would alter calcification of VSMCs and tested this by selectively activating and inhibiting CB2 in an *in vitro* cell culture model of VSMC OBT.

Results: Groups (n≥8) of 8-week old $Ldlr^{-/-}CB2^{+/+}$ (CB2-WT) and $Ldlr^{-/-}CB2^{-/-}$ (CB2-KO) were placed on a high fat diet (HFD) for up to 24 weeks. Immunohistochemical analysis of SMActin showed no difference in VSMC migration in aortic root plaques between CB2-WT and CB2-KO mice. Expression of OBT regulatory proteins in atherogenic aortas was determined by western blot. Runx2, a master transcriptional regulator of osteoblastogenesis, was increased ~2.5 fold in plaques from CB2-WT mice compared to CB2-KO mice ($p=0.03$). Expression of OPN, a potent inhibitor of vascular calcification, was found to be ~3.5 fold higher in plaques from CB2-WT mice compared to CB2-KO mice ($p=0.02$). Mouse VSMCs were cultured in an osteogenic media shown to induce OBT with and without a CB2-selective agonist (HU-308) and antagonist (SR144528). Alizarin red staining showed a 44% reduction in calcification of cells treated with HU-308 and a 238% increase in calcification of cells treated with SR144528 compared to the control.

Conclusion: These results support our hypothesis that CB2 modulates atherosclerotic plaque calcification, at least in part, by affecting the expression of regulatory proteins involved in OBT of VSMCs. Information from this study provides novel mechanistic insights into the function of CB2 signaling in atherosclerotic plaque calcification that may lead to the development of CB2-selective therapies aimed at reducing the burden of plaque calcification and therefore reduce rates of mortality in patients with heart disease.

TOTAL AND DIFFERENTIAL LEUKOCYTE COUNTS AMONG CANNABIS USERS THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY, 2005-2014

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Pre-clinical studies have reported immunomodulatory effects of cannabinoids. Evidence from population-based studies is scarce. The goal of the current study is to investigate whether cannabis use is associated with quantitative alterations in total and differential leukocyte counts using the National Health and Nutrition Examination Survey (NHANES), a series of nationally representative sample surveys of United States. NHANES employs a stratified multistage probability sample of the civilian non-institutionalized population. The current study included adult participants 20-59 years of age (n=14532) who underwent a detailed medical examination and were administered the drug use questionnaire in the mobile examination center. Computer-assisted self-interviews assessed cannabis use. The methods used to derive leukocyte parameters are based on the Beckman Coulter method.

Mean TLC was generally highest among recently active users followed by former and then never users. After adjusting for potential confounding variables, only recent users who used cannabis for ≥7 days in the 30 days prior to the interview had higher TLC when compared to never users ($\beta = 204$; 95% CI = 67, 341). Looking at the differential leukocyte components, modest differences were observed for monocyte and neutrophil counts, but not for lymphocytes, basophils, or eosinophils. In conclusion, a modest association between recent cannabis use and TLC was detected. Prospective studies with repeated measures may provide additional information and whether the observed quantitative alterations have a clinical significance.

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A NOVEL METHOD FOR POTENTIATING THE ANTIBIOTICS WITH CANNABINOID-BASED FORMULATIONS

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Antibiotics revolutionized medicine in the 20th century, and have (together with vaccination) effectively eradicated diseases such as tuberculosis in the developed world. Their abundances and effectiveness led to overuse, prompting bacteria to develop resistance to certain antibiotics and in certain cases to multi-drug resistance (MDR). There are currently considerable challenges with the treatment of infections caused by strains of clinically relevant bacteria that show multi-drug-resistance (MDR). Antimicrobial resistance has already been defined as a global threat with at least tens of thousands people die each year and many more are sick from antibiotic-resistant bacterial infections in western countries. New anti-bacterial agents are therefore urgently needed, but only one new class of antibacterial has been introduced in the last 30 years. Pharmaceutical combinations of antimicrobial agents with other agents capable of increasing the potency of the latter, while also decreasing the minimal therapeutic dosages of the antimicrobials, and thus minimizing the drug resistance development and biofilm formation, may represent a novel strategy to address this growing problem.

Previous investigations have demonstrated, to various degrees, antibacterial activity for selected cannabinoids including Δ9-tetrahydrocannabinol (THC). Moreover, we believe we can further enhance the antibacterial efficacy of THC by adding a cannabinoid-mimetic molecule, Palmitoylethanolamide (PEA), to the THC preparation and thus utilize the so-called "entourage effect".

Minimum inhibitory concentration (MIC) for THC and known families of antibiotics were determined in an array of standard and MDR bacteria strains Among the assessed antibiotics were Aminoglycosides (Gentamicin), Penicillins (Ampicillin, Carbenicillin), Glycopeptides (Vancomycin), and Quinolones (Ciprofloxacin). In addition, the impact of growing conditions on the antibacterial efficacy of THC with or without PEA, has also been accounted, when two growing media (rich and poor in nutrients) have been compared. Next, the combined effect of selected antibiotic and THC by itself or in combination with predefined concentration of PEA had been evaluated in 96-well plates.

Both THC and THC+PEA combnation synergized with the activities of assessed antibiotics in both standard and MDR strains of bacteria. For example, the cannabinoid combination were able to reduce the observed MIC value of Gentamicin by up to 8-fold in standard, non-resistant strain, and incredibly by up to 16-fold in MDR strain of the bacteria. It should be noticed, however, that the required minimal Gentamicin concentration for inhibition of MDR strain is much higher than the one required for the non-resistant strain. Similarly, the results have been observed for other antibiotics as well.

In summary, cannabinoid preparations have the potential to address the epidemic of antibiotics resistance infections.

CANNABIDIOL AND PALMITOYLETHANOLAMIDE SHARE SIMILAR INTRACELLUAR PATHWAYS IN PREVENTING INCREASED PERMEABILITY OF INFLAMED CACO-2 MEMBRANES

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Background: We have previously demonstrated that PEA and CBD decrease the transfer of ions across Caco-2 monolayers under inflammatory conditions. In the present study, we sought to identify if CBD and PEA also prevent the transfer of macromolecules across inflamed membranes, and to identify which receptors and intracellular cascades are involved.

Methods: Caco-2 cells were cultured for 14 days until fully confluent in the apical compartment of 12mm diameter 3.0μm pore polyester membrane inserts within polyester 24-well plates. Inflammatory conditions were simulated by adding to the media apical IFNγ (10ng/ml) for 18 hours, followed by TNFα (10 ng/ml) for 6 hours. PEA (10μM), CBD (10μM) or vehicle were added simultaneously with IFNγ. The effect of PEA or CBD on the transfer of fluorescent dextrans (FD4 and FD10) from the apical compartment to the basolateral compartment were determined by withdrawing 100μl aliquot samples from the basolateral compartment using a Fluoroskan Ascent FL2.5 fluorometer. Samples were taken at regular intervals for 36 hours. Target sites of action of PEA and CBD were explored with the antagonists AM251 (CB_1 100nM), AM630 (CB_2 100nM), GW6471 (PPARα 500nM), GW9662 (PPARγ 100nM), SB366791 (TRPV1 500nM), and CID16020046 (GPR55 500nM). Intracellular signalling mechanisms were explored using the following inhibitors: KT5720 (PKA 1μM), L-NAME (NOS 10μM), PD98059 (MEK/ERK 10μM), SQ22536 (adenylyl cyclase 1μM) and G06983 (PKC 1μM).

Results: Compared to control monolayers, IFNγ and TNFα caused an increase in the transfer of both FD4 and FD10 dextrans from the apical to basolateral compartment from 2hrs onwards until 36 hrs ($p<0.0001$). Both PEA and CBD prevented this increase in transfer from 2hrs ($p<0.001$) until the end of the experimental period at 36hrs. When applied together, CBD and PEA had no additive effect on the transfer of dextrans. The effect of CBD on dextran transfer was blocked by the CB_1 antagonist AM251 ($p<0.001$), and by the inhibitors KT5720, PD98059 and SQ22536 ($p<0.0001$). The effect of PEA was blocked by the PPARα antagonist GW6471 ($p<0.001$), and the inhibitors KT5720, PD98059 and SQ22536 ($p<0.0001$).

Conclusion: Both CBD and PEA prevent the transfer of macromolecules across colonic epithelial membranes during inflammatory conditions. These compounds act through the CB_1 and PPARα receptors respectively, but through similar intracellular second messengers, explaining a lack of additive effect between the two compounds. In future clinical practice, these agents may be of use in treating the increased colonic permeability and sepsis caused by inflammation of the gut.

EFFECTS OF CANNABINOIDS ON PAIN-STIMULATED AND PAIN-DEPRESSED BEHAVIOR IN MICE

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Most preclinical research with cannabinoids as candidate analgesics has been conducted with assays of "pain-stimulated" behaviors, which can be defined as behaviors that increase in rate, frequency or intensity after delivery of a noxious stimulus (e.g. withdrawal responses). However, preclinical-to-clinical translation of results with these procedures has been problematic. Novel procedures evaluate drug effects in assays of clinically relevant "pain-depressed" behaviors, which can be defined as behaviors that *decrease* in rate, frequency or intensity after delivery of a noxious stimulus (e.g. pain-related depression of locomotion or other natural behaviors). This study compared effects of THC and endocannabinoid catabolic enzyme inhibitors with effects of positive and negative controls in assays of pain-stimulated and pain-depressed behavior in mice.

Intraperitoneal administration of lactic acid (IP acid; 0.1-0.32% in a volume of 10 ml/kg) served as a visceral noxious stimulus to stimulate stretching and depress nesting in adult male and female ICR mice. Antinociception was tested for the following cannabinoids (doses in mg/kg subcutaneous): Δ9-tetrahydrocannabinol (0.32-10); the fatty acid amide hydrolase (FAAH) inhibitors URB597 (0.32-10) and PF3845 (3.2-32); and the monoacylglycerol lipase (MAGL) inhibitors JZL184 (1-32) and MJN110 (0.32-3.2). Comparators included two positive controls that are clinical analgesics [the NSAID ketoprofen (0.32-10); the mu opioid agonist oxycodone (0.1-3.2)] and two negative controls that alter motor behavior but do not produce reliable analgesia clinically [the $GABA_A$ receptor positive allosteric modulator and motor depressant diazepam (0.1-1.0); the dopamine//norepinephrine releaser and motor stimulant amphetamine (0.1-3.2)].

IP acid produced a biphasic stimulation of stretching (peak stretching at 0.18% IP acid) and a concentration-dependent depression of nesting (significant depression at 0.18 and 0.32% IP acid). All drugs decreased 0.18% IP acid-stimulated stretching. Ketoprofen and oxycodone also blocked depression of nesting produced by both 0.18 and 0.32% IP acid, and of the cannabinoids, only URB597 and JZL184 were effective. All other compounds failed to block IP acid-induced depression of nesting up to drug doses that disrupted control nesting in the absence of the noxious stimulus. Rimonabant (3 mg/kg) antagonized JZL184 antinociception, attenuated URB597 antinociception, and had no effect on ketoprofen antinociception. There were no significant sex differences in any effects. These results support further consideration of endocannabinoid catabolic enzyme inhibitors as candidate analgesics but suggest intriguing differences between drugs in the FAAH- and MAGL-inhibitor drug families. These results also support utility of pain-depressed behaviors for preclinical assessment of candidate analgesics.

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OF MICE AND RATS: BEST LAID SCHEMES (TO STUDY SEX DIFFERENCES) OFTEN GO AWRY

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Discovery of the mechanisms underlying cannabinoid action and their effects on physiology and behavior has been due, in part, to research conducted in animals, and specifically, in rodent models. For example, data from rodent models were used to evaluate the first CB_1 receptor antagonist rimonabant[4,6] and contributed to the structure-activity relationship information that facilitated recent discovery of the crystal structure of the CB_1 receptor.[3,5] Similarly, work with knockout mice missing crucial genes related to the endocannabinoid system has contributed to understanding of the physiological roles of this system.[1,2,9] Results from studies using rodent models have also suggested that CB_2 receptors may be present and functional in the brain.[8] Recently, in our work with rodent models, we discovered an intriguing species X sex interaction in the effects of Δ^9-THC in rats and mice, which suggests that findings of sex differences in cannabinoid pharmacology may vary by species.

Female and male Sprague-Dawley rats and C57/Bl6 mice were trained to discriminate Δ^9-THC in a standard two-choice operant procedure. While Δ^9-THC training dose differed across species (i.p., 1.7 and 5.6 mg/kg for rats and mice, respectively), it remained the same across sex within a species. Upon acquisition, a Δ^9-THC dose-effect curve was determined in each group. Results showed that Δ^9-THC was more potent at producing discriminative stimulus effects in female rats than in male rats (ED_{50}=0.30 mg/kg and ED_{50}=0.85 mg/kg, respectively), but response rates did not differ significantly between the sexes (main effect for sex, p=0.08). In contrast, beyond the obvious species difference in optimal training dose, Δ^9-THC showed nearly equal potency for discriminative stimulus effects across sex in mice (ED_{50}=1.89 mg/kg in females and male ED_{50}=1.93 mg/kg in males), but response rates for males were consistently higher than for females after vehicle administration and across all Δ^9-THC doses.

The greater sensitivity of female rats to the discriminative stimulus effects of Δ^9-THC observed here is consistent with our previous results with this species.[7] The lack of a similar difference in mice was not expected. We are continuing investigation of the generality and mechanistic underpinnings of this species X sex interaction, with results to be presented at the meeting.

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EFFECT OF THE FATTY ACID AMIDE HYDROLASE INHIBITOR URB937 ON RAT MODELS OF NAUSEA

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Elevation of 2-arachidonyl glycerol by monoacylglycerol lipase inhibition in the interoceptive insular cortex (IC)—a cortical site responsible for the experience of nausea—reduces acute nausea-induced conditioned gaping (a rat model of acute nausea; Sticht et al., Neuropharmacology. 102 (2016) 92-102), and contextually-elicited conditioned gaping (a rat model of anticipatory nausea; Limebeer et al., Behav Neurosci. 130 (2016) 261-6). In contrast, although systemic inhibition of fatty acid amide hydrolase (FAAH) via PF-3845, reduces both acute and anticipatory nausea in rats (Rock et al., Psychopharmacology (Berl). 232 (2015) 3841-3848), inhibition of FAAH in the interoceptive IC neither reduces acute or anticipatory nausea in rats, nor elevates AEA (Sticht et al., Neuropharmacology. 102 (2016) 92-102). Therefore, the site of action for FAAH inhibition's effects on nausea remains unclear. It is suggested that the anti-nausea action of FAAH inhibition may be peripherally, rather than centrally, mediated.

In the present study we evaluated whether URB937, a peripheral FAAH inhibitor (Clapper et al., Nat Neurosci. 10 (2010) 1265-70), could suppress acute and anticipatory nausea-like responding in rats, and examined its mechanism of action by measuring FAAH inhibition and levels of anandamide and related fatty-acid ethanolamides in central and peripheral regions.

URB937 (1 and 3 mg/kg, i.p.) reduced acute nausea in a dose-dependent manner (similarly to the globally active FAAH inhibitor, PF-3845; Rock et al., Psychopharmacology (Berl). 232 (2015) 3841-8) by a mechanism that involves peroxisome proliferator-activated receptors (PPARα). Furthermore, administration of the PPARα agonist GW7647 (3 mg/kg, i.p.) yielded similar results. URB937 (3 mg/kg, i.p.) also reduced anticipatory nausea, an effect that was blocked by rimonabant, a type-1 cannabinoid receptor (CB_1) inverse agonist. As expected, treatment with URB937 resulted in reduced FAAH activity in peripheral tissues but not in central regions, such as the prefrontal cortex, suggesting a peripherally mediated effect of FAAH inhibition on nausea. Interestingly, URB937 reduced FAAH activity in the area postrema (AP)—a brainstem region involved in the control of nausea, which is not protected by the blood-brain barrier— and increased the local concentrations of fatty-acid ethanolamides anandamide (AEA), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). Our results suggest that peripheral FAAH inhibition caused by URB937 reduces acute toxin-induced nausea by elevating peripheral levels of OEA and/or PEA, which can activate PPARα, and reduces anticipatory nausea by elevating peripheral AEA, which activates CB_1 receptors. They also point at the AP as a key anatomical region for the regulation of nausea by FAAH inhibitors.

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NAUSEA-INDUCED 5-HT RELEASE IN THE INTEROCEPTIVE INSULAR CORTEX IS REVERSED BY SYSTEMIC MAGL INHIBITION

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Considerable research suggests that forebrain serotonin (5-HT) release in the interoceptive insular cortex (IIC) is involved in illness-induced nausea as measured by the conditioned gaping model in rats (Grill & Norgren, Science (1978) 201 267-269; Parker et al., Eur J Pharmacol. 722 (2014) 122-133). We hypothesized that systemic lithium-chloride (LiCl) would enhance 5-HT release in the IIC as measured by HPLC-EC (HTEC-510, Eicom, San Diego, USA) analysis of dialysate collected by microdialysis. We further hypothesized that systemic MAGL inhibition (MJN110) would reduce the LiCl-induced increase in 5-HT levels by increasing 2-AG availability (Sticht et al., Neuropharmacology. 102 (2016) 92-102). Finally, we hypothesized that exposure to a LiCl-paired-saccharin solution would conditionally elevate 5-HT.

The first experiment evaluated the potential of LiCl-induced nausea to elevate 5-HT and of MJN110 to prevent the LiCl-induced elevation of 5-HT in the IIC. Following baseline microdialysis sampling, rats were injected with MJN110 (10 mg/kg, ip) or vehicle one hr prior to receiving an injection of LiCl (127 mg/kg, ip) or saline. The results revealed that among the vehicle pretreated rats, LiCl (but not saline) administration, resulted in an increase in 5-HT release in the IIC only during the first 20 min after LiCl injection, which is consistent with the time course of the illness-inducing effects of LiCl (Parker et al., Animal Learning & Behavior. 12 (1984) 307-315). Importantly, MJN110 pretreatment prevented this LiCl-induced elevation of 5-HT in the IIC, without having an effect on its own. These findings indicate that LiCl 'turns up' nausea-inducing 5-HT, and MJN110 'turns down' that nausea signal.

The second experiment evaluated the potential that conditioned nausea also elevates 5-HT in the IIC. Subsequently, rats were given three conditioning trials (every 48 hr) during which an intraoral infusion of saccharin was paired with an injection of LiCl or saline. On the test trial (3-5 days later) the rats received a 20 min infusion of saccharin during microdialysis sampling while conditioned gaping was also measured. The results indicated that infusion of LiCl-paired saccharin, but not saline-paired saccharin, elicited conditioned gaping responses and an elevation of 5-HT during the infusion. These results suggest that conditioned nausea, as indicated by gaping behavioral reactions, is mediated by a neurochemical release of 5-HT in the IIC. The potential of MJN-110 administered 60 min prior to testing to reduce this conditional release of 5-HT and the behavioral measure of gaping is currently being evaluated.

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FUNCTIONAL CHARACTERIZATION OF THE CANNABINOID RECEPTOR 1 IN ZEBRAFISH LARVAE USING BEHAVIORAL READOUTS

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The endocannabinoid system (ECS) is a central regulatory system that influences a wide range of biological processes. It consists of a group of molecules known as cannabinoids as well as the cannabinoid receptors that they bind to. Manipulation of cannabinoid receptor 1 (CNR1) has a strong effect on behavior, but published studies differ about the details.

The zebrafish has become a versatile animal model in biomedical research, and the objective of this study is to characterize the function of the CNR1 in relation to behavior in zebrafish larvae.

Behavioral responses were quantified in a visual motor response (VMR) test, which allows to analyze locomotion during basal conditions and upon a dark challenge. Zebrafish larvae were treated with the cannabinoid receptor agonists WIN55,212-2 (ranging from 2 to 8000 nM) and CP55,940 (ranging from 500 – 8000 nM). Results were validated for CNR1 specificity using a *cnr1*$^{-/-}$ mutant line, and by validation with CNR1 antagonist AM251 (500 nM) in wild type larvae.

Our data show that activation of CNR1 by the synthetic cannabinoids results in a strong dose-dependent CNR1-specific inhibition of locomotion, as measured by distance moved. Blocking CNR1 with the antagonist AM251 does not have an effect on locomotion, suggesting that endogenous cannabinoids do not alter the locomotor activity under these conditions. We also show that activation of CNR1 reduces the velocity and reaction time of the startle response after a dark challenge. Inhibition of locomotion rapidly decreased during the dark phase, which suggests a desensitization of CNR1. These CNR1-regulated inhibiting effects on locomotion can be antagonized by treatment with ethanol or nicotine.

Our data demonstrate that in zebrafish larvae exogenous cannabinoids CNR1 dependently decrease locomotor activity under basal conditions and alter the response to a challenge. These results show that the zebrafish larva coupled with the VMR test is suitable for testing the behavioral effects of CNR1 agonists. Interestingly, since pharmacological blockade of CNR1 does not have an effect in our assay, we conclude that endogenous cannabinoid activity is not involved in the modulation of locomotor activity of zebrafish larvae.

NOVEL CANNABINOIDS BASED ON THE COUMARIN MOTIF AND FAST EVALUATION THROUGH PHOTOMOTOR RESPONSE STUDY ON EMBRIONIC ZEBRAFISH

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Coumarins represent a class of privileged structures in medicinal chemistry with a broad spectrum of applications. Derivatives with 3-benzyl- and 3-alkyl-substituents have been proven to exhibit similar activities as classical cannabinoids. In various studies including radioligand binding and β-arrestin-recruitment-assays they showed activity towards cannabinoid receptors CB_1, CB_1, GPR55 and GPR18 as antagonists and agonists depending on structural motifs (Bräse et. al., Eur. J.Org. Chem., 2007 (6), 943-952; Bräse et.al. Biorg. Med. Chem. 2009, 17 (7), 2842-2851), thus revaluing the coumarin core as a lead structure for cannabinoid design.

The modular synthesis of these compounds was achieved through the preparation of substituted salicylic aldehydes, which are subsequently transformed via several synthetic routes. The synthesis of 3-benzyl coumarins is accomplished through an NHC-mediated condensation reaction with cinnamic aldehydes, whereas 3-alkyl coumarins are accessible via Perkin condensation with anhydrides. On the other hand, post-condensation modifications give access to 3-aryl and 3-styryl compounds. In addition, several synthetic approaches towards libraries of structurally derived compounds have been developed and are employed to create a large number of potentially cannabinoid active compounds.

In order to systematically characterize the effects of cannabinoids on the early developmental processes and to classify novel synthetic cannabinoid derivatives rapidly, we chose to use a photomotor response (PMR) assay with zebrafish (*Danio rerio*) as a model organism. Zebrafish embryos between 30 and 42 hours post fertilization (hpf) demonstrate a response to light. The PMR experiment consists of applying a bright light stimulus to the embryos for a very brief period and eliciting a series of behavioral responses (Kokel, et. al., Methods Cell Biol., 2011; 105:517-524.). We have developed a high-throughput PMR platform (Peravali, Proc. IEEE Eng. Med. Bio. Soc., August 2015) to screen both wild type and mutant zebrafish embryos and to evaluate a dose-response effect for our library of cannabinoids.

CONTROLLED DEACTIVATION CB_1 RECEPTOR LIGANDS AS A NOVEL STRATEGY TO LOWER INTRAOCULAR PRESSURE

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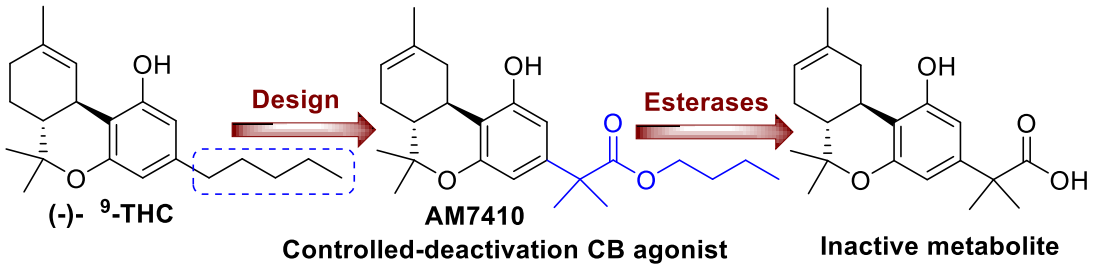
Nearly half a century has passed since the demonstration that marijuana and its chief psychoactive component Δ^9-THC lower intraocular pressure (IOP). Elevated IOP remains the chief hallmark and therapeutic target for glaucoma, a condition that places millions at risk of blindness. It is likely that Δ^9-THC exerts much of its IOP-lowering effects via activation of CB_1 cannabinoid receptors. However, the initial promise of CB_1 as a target for treating glaucoma has not thus far translated into a credible therapeutic strategy. We have recently shown that blocking monoacylglycerol lipase (MAGL), an enzyme that breaks the endocannabinoid 2-AG, substantially lowers IOP for 8 hours (Miller et al., 2016). Another strategy is to develop cannabinoid CB_1 receptor agonists that are optimized for topical application to the eye. Recently we have reported on a controlled deactivation approach where the "soft" drug concept of enzymatic deactivation was combined with a "depot effect" that is commonly observed with Δ^9-THC and other lipophilic cannabinoids (Sharma et al., 2013). This approach allowed us to develop novel cannabinoids with predictable duration of action and improved druggability and is particularly attractive for the design of CB_1 activators for ophthalmic use with limited or no psychoactive effects.

We have tested a novel class of compounds using a combination of electrophysiology in autaptic hippocampal neurons, a well-characterized model of endogenous cannabinoid signaling, and measurements of IOP in a mouse model. We now report that AM7410 is a reasonably potent and efficacious agonist at CB_1 in neurons and that it substantially (30%) lowers IOP as long as 8 hours after a single topical treatment. This effect is absent in CB_1 knockout mice. Our results indicate that direct targeting of CB_1 receptors with controlled deactivation ligands is a viable approach to lower IOP in a murine model and merits further study in other model systems.



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HARNESSING ENDOCANNABINOIDS FOR RETINAL NEUROPROTECTION

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The endocannabinoid system (ECS) components, including receptors, endocannabinoid ligands, and their biosynthetic and degradative enzymes, are expressed in ocular tissues. Modulation of the ECS by pharmacological inhibition, or by genetic deletion of enzymes responsible for endocannabinoid degradation, may be neuroprotective in experimental models of retinal ganglion cell (RGC) injury. Therefore, we examined the effects of pharmacological and genetic block of the enzyme monoacylglycerol lipase (MAGL), which degrades 2-arachidonoyl glycerol (2-AG), and fatty acid amide hydrolase (FAAH), which degrades 2-arachidonoyl ethanolamide (AEA), respectively, on RGCs survival in two different models of optic nerve injury.

The first model are Nee mice, which exhibit elevated intraocular pressure (IOP) and consequent loss of RGCs. Nee mice were treated daily with intraperitoneal MAGL and FAAH inhibitors (JZL184 and URB597, respectively) for three weeks. The IOP in Nee mice was measured using rebound tonometry (Tonolab).

The second model is axotomy involving a cut to the optic nerve in C57Blk (WT) and MAGL and FAAH knockout mice. In the axotomy model we also performed a pharmacological inhibition in WT animals using JZL184 or URB597 for 7 days. RGC survival, in both models, was evaluated by immunohistochemical staining with anti-Brn3a antibody.

We now report that genetic deletion of MAGL or FAAH hydrolytic enzymes, or their pharmacological inhibition, did not improve survival of RGCs following optic nerve injury via axotomy. However, chronic treatment of Nee mice with JZL184 was neuroprotective for RGCs, an effect independent of IOP modulation, since no change in IOP was observed upon drug administration.

In summary, **c**hronic administration of MAGL blocker JZL184 was neuroprotective in the Nee model of ocular hypertension, but *not* in the axotomy model. This suggests that the observed neuroprotection depends on the type of optic nerve injury and that in the Nee mice it occurs independently of lowered IOP.

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INTRAOCULAR PRESSURE LOWERING EFFICACY OF A Δ^9-TETRAHYDROCANNABINOL PRODRUG, NB1111, IN A NORMOTENSIVE RABBIT MODEL

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Δ^9-Tetrahydrocannabinol (THC), an active ingredient of the plant *Cannabis sativa*, could potentially act as an anti-glaucoma agent. However due to its lipophilic nature, THC does not permeate well across ocular tissues. NB1111 is a relatively hydrophilic prodrug of THC with improved ocular bioavailability. The aim of this study was to evaluate the effect of single and multiple day application of NB1111 loaded solid-lipid nanoparticles (SLNs) on the intraocular pressure (IOP) of normotensive rabbits. IOP lowering efficacy of a single application of NB1111-SLNs was also compared to that of marketed ophthalmic formulations of pilocarpine and timolol maleate. NB1111 was incorporated into SLNs, prepared by ultrasonication at concentrations of 0.98%. Fifty microliters, of SLN or nanoemulsion, was instilled topically in the cul-de sac of the left eye of normotensive rabbits (n=6), twice a day, for five consecutive days. For the single day IOP-Time profiling, after the first application on Day 1, IOP was measured every 30 min till IOP returned to 90 % of the baseline. All animal studies were conducted following UM IACUC approved protocols.

The data obtained on normotensive rabbits showed a significant IOP lowering effect by NB1111 when formulated in SLNs. The maximum percent drop in the IOP from the baseline IOP (ΔIOP_{max}) was 27.1%. The time for peak IOP reduction (T_{max}), was 90 minutes and the duration of action for NB1111-SLN, or the time required for the ΔIOP to reach 90% of baseline IOP, was 480 minutes. The NB1111-nanoemulsion formulation did not have a significant effect on the IOP of normotensive rabbits. The ΔIOP_{max} was 11.7% and the T_{max} was 60 minutes. The ocular concentrations obained with the NB1111-nanoemulsion were also significantly lower than those obtained with the NB1111-SLN formulation. The effect of THC-SLNs was also studied and it did not have any effect on the IOP of normotensive rabbits. From the single dose studies, it was observed that the marketed formulation, Pilocarpine HCl (2 %w/v) showed a ΔIOP_{max} of 15.9% at 30 minutes and its IOP lowering effect lasted for 120 minutes (Fig. 7). Dosing with Timolol Maleate (0.25 % w/v) resulted in a more intense ΔIOP_{max} of 23.1% at 60 minutes with a duration of action of 180 minutes. The results show that NB1111-SLN produced a significant increase in the intensity and duration of action, compared to NB1111-nanoemulsion, THC-SLN, timolol and pilocarpine formulations; demonstrating the positive effects of the vehicle, as well as the prodrug derivatization of THC on the IOP lowering efficacy of THC.

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PHYTOCANNABINOIDS, TETRAHYDROCANNABINOL (Δ^9-THC) AND CANNABIDIOL (CBD), REDUCE COLD-INDUCED OCULAR PAIN IN A MOUSE MODEL OF CORNEAL HYPERALGESIA

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Background: Damage to corneal tissues resulting from chemical burn, surgeries, trauma, neurological diseases, infections etc., produces strong ocular pain and can result in sensory nerve dysfunction. Existing pharmacotherapies for ocular pain frequently fail to provide adequate pain relief and can produce side-effects. Activation of the endocannabinoid system (ECS), which includes cannabinoid 1 (CB_1R) and cannabinoid 2 (CB_2R) receptors, is analgesic and anti-inflammatory. The cornea expresses cannabinoid receptors, notably CB_1R, and activation of these receptors may be useful in reducing ocular pain and inflammation.

Purpose: To establish a temperature threshold required to induce ocular pain response in a mouse model of corneal injury and to investigate the antinociceptive properties of the phytocannabinoids Δ^9THC and CBD using this model.

Methods: The central cornea was injured using a chemical cauterization model in wild type (WT) mice. Injured corneas were stimulated with cold saline of different temperatures (4/10/15 degree Celsius) to establish the temperature threshold for ocular pain responses. Cauterized eyes were treated with 5 µl of topical Δ^9THC or CBD (1-5% w/v) at: 30, 60 and 120 min (M) post-injury. The ocular pain score was quantified from 1 minute video recordings of the behavioral pain responses (blink response, squints and eye wipes) measured at 6 hours post- injury following topical saline stimulation.

Results: Corneal cauterization resulted in an increased pain response to 4°C, 10°C and 15°C topical saline at 6 hours post-injury compared to their respective sham control eyes ($p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively). Topical application of 1% Δ^9THC and 5% CBD significantly reduced ocular pain responses in WT mice measured using 10°C saline stimulation ($p < 0.001$ and $p < 0.01$, respectively).

Conclusion: Phytocannabinoids, Δ^9THC and CBD, reduce ocular pain resulted from cold stimulation of injured cornea. Phytocannabinoid therapeutics that include THC and CBD may offer a novel target in the treatment of acute and chronic ocular pain.

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CHRONIC VERSUS ACUTE Δ^9-TETRAHYDROCANNABINOL TREATMENT OF INFLAMMATORY PAIN IN MALE VERSUS FEMALE RATS

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Δ^9-Tetrahydrocannabinol (THC), when given acutely, is more potent at causing antinociception in female compared to male rats with inflammatory pain. However, chronic THC has been shown to cause greater antinociceptive tolerance in female rats compared to males, suggesting that cannabinoids may produce greater analgesia in males when used daily. The purpose of this study was to compare chronic and acute THC effects on antinociception and edema in male and female rats using a model of persistent inflammatory pain. After baseline measurements on day 1, inflammation was induced by intraplantar injection of complete Freund's adjuvant (CFA). One h post-CFA, THC (0.0, 1.0, 2.0 or 4.0 mg/kg, i.p.) was administered. The same dose of THC was administered at 1700 on day 1, and at 0800 and 1700 on days 2 and 3. At 0800 on day 4, rats that had previously received THC were injected with either the same dose of THC or vehicle, while rats that had previously received vehicle were injected with either vehicle or an acute dose of THC (1.0, 2.0 or 4.0 mg/kg). Mechanical allodynia, heat hyperalgesia, biased weight-bearing and locomotor activity were assessed at 30, 60, 120 and 240 min post-THC injection, and edema was assessed at 240 min. Rats were tested again on day 8, on each measure, but did not receive any injections on days 5-8.

In males, both acute and chronic THC dose-dependently reduced mechanical allodynia on day 4, but these effects were gone by day 8. Similar effects were found in females on day 4, but these effects lasted to day 8. Chronic THC caused no anti-allodynic tolerance in either sex. In males, acute THC also caused dose-dependent anti-hyperalgesia, while chronic THC caused anti-hyperalgesia that was not dependent on dose, and all effects were gone by day 8. In females, acute and chronic THC caused dose-dependent anti-hyperalgesia, and these effects were smaller but still apparent on day 8. Both males and females developed partial tolerance to THC's anti-hyperalgesic effect. Neither acute nor chronic THC increased weight-bearing on the inflamed paw in either sex. Acute THC dose-dependently decreased locomotion in both sexes, but rats that received chronic THC developed complete tolerance to this effect. Chronic but not acute THC also decreased paw edema in both sexes. The present results suggest that both acute and chronic THC administration may provide some relief from inflammatory pain in both sexes, but these effects may not persist after termination of THC treatment. Additionally, tolerance appears to develop more readily to THC's sedative effect than to its anti-allodynic or anti-hyperalgesic effects.

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CANNABINOID ANTINOCICEPTION AGAINST PACLITAXEL-INDUCED NEUROPATHIC PAIN

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Chemotherapy-induced peripheral neuropathy (CIPN), a chronic pain condition caused by degeneration of nerve tissue, is a common side-effect of the chemotherapeutics used to treat many types of cancer. CIPN occurs in as many as 68 percent of patients treated with these drugs and causes a multitude of symptoms ranging from mild numbness or tingling in the extremities to severe and inescapable pain. While there are ways to manage the pain once it has developed, the condition is often permanent. There is no approved prophylactic against development of CIPN; however, preclinical studies have shown that synthetic cannabinoid agonists including WIN 55,212-2 and AM1710 are not only effective in treating the pain associated with CIPN, but in preventing it as well. Given that the naturally occurring phytocannabinoid Δ^9-tetrahydrocannabinol (THC), in the form of cannabis, is already widely used to treat cancer pain, the purpose of the present study is to determine the efficacy of chronic THC as a prophylactic against development of CIPN. Rats were treated on days 1, 3, 5, and 7 with saline or 4.0 mg/kg paclitaxel to induce neuropathy. THC dissolved in a 1:1:18 ethanol:cremophor:saline vehicle was administered at doses of 0.0, 1.0, 2.0, or 4.0 mg/kg via subcutaneous injection once daily on days 1-9. Rats were tested for mechanical and cold allodynia on days 1, 7, 14, and 21. Preliminary data suggest that THC administered chronically during the course of paclitaxel treatment decreases the development of mechanical allodynia in both male and female rats. Investigation into whether this effect is dose-dependent or differs across sex is still underway. This information will be useful for developing clinical treatment options to prevent the development of CIPN and improve the quality of life for cancer survivors.

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TARGETING THE ENDOCANNABINOID AND GLUCOCORTICOID SYSTEM TO ATTENUATE INFLAMMATORY ARTHRITIS

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Inflammatory arthritis is a debilitating autoimmune disease characterized by chronic joint inflammation and pain that compromises mobility and decreases quality of life. Current treatments induce adverse side effects and are often ineffective at reducing joint pain. For example, synthetic glucocorticoids attenuate inflammation and associated pain, but can induce harmful metabolic side effects. An alternative approach is to combine drugs to use lower doses of each drug, thereby decreasing the adverse side effects**.** Although this strategy is successful in acute inflammatory pain models, paucity of research focuses on the effects of combination treatments in models of chronic inflammatory pain. The present study tested the hypothesis that a low dose of the MAGL inhibitor JZL184 potentiates the anti-inflammatory and anti-allodynic effects of the synthetic glucocorticoid dexamethasone, in the chronic, collagen-induced arthritis (CIA) model. CIA was induced in male DBA1/J mice with an immunization of an emulsion of collagen and complete Freund's adjuvant (CFA). A secondary "booster" exposure to the collagen was administered three weeks later to induce anti-collagen autoimmunity. CIA significantly increased clinical signs of arthritis (e.g., paw redness and digit swelling), paw edema, proinflammatory cytokines (e.g., TNFα, IL-1β, IL-6, per multiplex ELISA), and mechanical allodynia (e.g., decreased paw withdrawal threshold). Chronic administration of the combination of JZL184 (4 mg/kg, ip) and dexamethasone (0.015 mg/kg, ip) significantly reduced CIA-induced paw swelling, but did not affect CIA-induced mechanical allodynia. These results suggest that the endocannabinoid and glucocorticoid systems may work together to decrease pain and inflammation associated with inflammatory arthritis.

DIFFERENTIAL EFFECTS OF CANNABINOID/MORPHINE COMBINATIONS IN TWO RODENT PAIN ASSAYS

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Opioid addiction and overdose are a major medical and societal problem. This project examines the potential utility of cannabinoid-opioid combination therapy for pain relief, where the dose of opioid could be reduced while maintaining a desired therapeutic effect. Two cannabinoids, WIN55,212-2 (WIN; CB1 and CB2 agonist) and GP1a (CB2-selective agonist) were tested alone and in combination with morphine, in two standard assays assessing pain, the rat carrageenan and mouse formalin models. In the carrageenan test, the latency (secs) to paw withdrawal after exposure to a beam of radiant heat was used as the antinociceptive index and was scored by the Hargreaves Plantar Test Apparatus. Rats were injected i.p. with either WIN or GP1a (both in 10% DMSO). Fifteen minutes later (t=0), the animals were given an intraplantar injection into the left hind paw of 0.1 ml of a 2% carrageenan solution (FMC, Philadelphia, PA) in saline. At t= +180, all animals were tested by exposure to the heat beam 3 times at 5-minute intervals to establish the baseline level of pain. At t = +195 minutes the animals received a second injection of cannabinoid or vehicle. At t = +210 minutes, morphine (3.0 mg/kg) or saline s.c. was given into the dorsal surface of the body. Latency of paw withdrawal to radiant heat was measured at 240 minutes and expressed as a percentage change from baseline. The percent of maximal possible antinociception (%MPA) for each animal at each time was calculated as %MPA = [(test latency at t = +240 – baseline latency at t = +180)/(22-baseline latency at t = +180)] x 100. A cutoff limit of 22 seconds was set to avoid damage to the paw. Morphine gave a dose-dependent analgesic response in this assay. GP1a alone did not show significant analgesia in doses ranging from 0.5 to 10.0 mg/kg. However, the combination of a suboptimal dose of morphine (3.0 mg/kg) and an ineffective dose of GP1a (5 mg/kg) resulted in an increased level of analgesia. WIN alone also did not cause significant analgesia and did not alter the level of morphine analgesia. Contrasting results were obtained using the formalin pain assay. In this assay, mice were injected s.c. with either morphine or a cannabinoid, alone or in combination. Animals were given an intraplantar injection of a 5% formalin solution diluted in 0.9% saline. The number of seconds of licking the injected paw was scored for each animal from 20 to 35 minutes after formalin injection. GP1a alone gave approximately a 50% reduction in licking over a range of doses from 1.0 to 50 mg/kg without a clear dose-response effect. When combined with morphine in this assay, the results were sub-additive. In contrast, when WIN was combined with morphine in the formalin assay, robust synergistic analgesic activity was observed. CB1 and CB2 antagonists demonstrated that WIN was acting via the CB1 receptor.

These results show that cannabinoids used in combination with opioids have the potential to reduce the dose of opioids needed for analgesia. Synergistic effects can be mediated by cannabinoids acting at the CB1 or the CB2 receptor and are dependent on the pain assay utilized.

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SEX DIFFERENCES IN CANNABINOID SENSITIVITY AND THE DEVELOPMENT OF TOLERANCE IN A MOUSE MODEL OF INFLAMMATORY PAIN

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Tolerance to the antinociceptive effects of cannabinoid agonists may limit their therapeutic utility. While previous research in our lab has demonstrated that mice expressing a desensitization-resistant form of the CB_1 receptor (S426A/S430A) develop tolerance in a sex- and agonist-specific manner in an acute model of thermal pain, these effects have yet to be examined in a more clinically relevant model of inflammatory pain. Therefore, the objective of the present study was to determine whether the S426A/S430A mutation altered tolerance to either the full cannabinoid agonist CP55,940 or the partial cannabinoid agonist Δ^9-THC and whether these effects differed in a sex-specific manner. Tolerance to CP55,940 and Δ^9-THC was assessed using the formalin model. In this model, intraplantar injection of 2.5% formalin into the hindpaw results in a biphasic pain response comprised of a rapid acute phase (0-15min) followed by a prolonged inflammatory phase (15-60min) during which pain behaviors are quantified. Dose response curves for each drug (0-0.2 mg/kg CP55,940; 0-6 mg/kg Δ^9-THC) were generated for male and female S426A/S430A mutant and wild-type mice. Male wild-type mice were more sensitive to the antinociceptive effects of both CP55,940 and Δ^9-THC compared to female wild-type mice. Cannabinoid tolerance was assessed following daily administration of either 6 mg/kg of Δ^9-THC (14 days) or 0.1 mg/kg of CP55,940 (28 days). Following 14 days of treatment with 6 mg/kg of Δ^9-THC, male wild-type mice developed tolerance to 6 mg/kg Δ^9-THC while male mutants and female mice of either genotype did not. Following chronic dosing with CP55,940, wild-type males were tolerant by day 21 whereas mutants were not tolerant by day 28. Males were more sensitive to the effects of 0.1 mg/kg CP55,940 as this dose failed to produce significant antinociception in females. Taken together, these results suggest that female mice are less sensitive to the antinociceptive effects of both Δ^9-THC and CP55,940 and that the S426A/S430A mutation delays tolerance development to both Δ^9-THC and CP55,940 in the inflammatory pain model. Additionally, male wild-type mice became tolerant to Δ^9-THC more quickly than female wild-type mice, raising the possibility that the mechanisms of tolerance may be partially sex-specific.

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THE EFFECTS OF PHARMACOLOGICAL BLOCKADE OF PPARs ON FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR, FEAR-CONDITIONED ANALGESIA AND CONDITIONED FEAR IN THE PRESENCE OF NOCICEPTIVE TONE IN RATS

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Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors and targets for endocannabinoids and *N*-acylethanolamines. There is evidence for their involvement in pain and cognition, however, their role in pain-fear interactions is unknown. The amygdala, particularly the basolateral amygdala (BLA), plays a key role in pain, conditioned fear and fear-conditioned analgesia (FCA). This study aimed to investigate the effects of systemic and intra-BLA administration of PPARα, PPARβ/δ and PPARγ antagonists on formalin-evoked nociceptive behaviour, FCA, and conditioned fear in the presence of nociceptive tone in rats.

Male Sprague-Dawley (SD) rats received footshock or no footshock in a conditioning arena. 23.5 hours later, rats received intraplantar injection of formalin into the right hind paw and intraperitoneal administration of vehicle, PPARα (GW6471; 2mg/kg), PPARβ/δ (GSK0660; 1mg/kg) or PPARγ (GW9662; 2mg/kg) antagonists and, 30 minutes after, were re-exposed to the conditioning arena for 15 minutes. Nociceptive and fear-related behaviours were assessed and tissue levels of neurotransmitters/endocannabinoids measured post-mortem in the BLA and central amygdala (CA) by LC-MS/MS. In a second experiment, male SD rats underwent the same FCA protocol; 15 minutes after the formalin administration, antagonists of PPARα, PPARβ/δ, PPARγ (all at 10 μg/0.5 μl), or vehicle, were microinjected bilaterally into the BLA and their effects on pain- and fear-related behaviours were assessed for 30 minutes.

Systemic administration of PPARα, PPARβ/δ and PPARγ antagonists significantly potentiated context-induced freezing without altering nociceptive behaviour. GW9962 attenuated FC-induced reductions in 2-AG and AEA in the contralateral (to formalin injection) BLA. FC was associated with significantly higher GABA levels in the contralateral CA, and lower glutamate levels in the ipsilateral CA, effects not observed in rats receiving GW6471 or GSK0660. These two antagonists also significantly increased GABA levels in the contralateral BLA and PEA levels in the ipsilateral CA of FC rats. PPARα and PPARβ/δ antagonists significantly increased PEA levels in the CA of FC rats. Intra-BLA administration of GW6471 or GW9962 significantly potentiated context-induced freezing.

PPARs, and particularly PPARα and PPARγ expressed in the BLA, may play a role in the short-term, within-trial extinction of conditioned fear in the presence of nociceptive tone but do not appear to mediate FCA. Further work is required to determine the extent to which the neurochemical alterations observed are causally implicated in PPAR-mediated regulation of fear-related behaviour.

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THE DEVELOPMENT AND MAINTENANCE OF INFLAMMATORY AND NEUROPATHIC PAIN IS PRESERVED IN GPR55 KNOCKOUT MICE

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The G-protein coupled receptor GPR55 has been postulated to serve as a novel cannabinoid receptor. In a previous report, GPR55 knockout mice failed to develop mechanical hyperalgesia, suggesting a potential pro-nociceptive role for GPR55 in the control of nociceptive responding (Staton et al. (2008) Pain 139: 225-236). However, GPR55 knockout mice remain incompletely characterized in models of pathological pain and their responsiveness to different modalities of cutaneous stimulation (i.e. heat, cold, non-noxious mechanical stimulation) remains largely unexplored. The present study was conducted to fully characterize GPR55 knockout and GPR55 wild-type mice in multiple models of inflammatory and neuropathic pain. Inflammatory sensitization was produced in mechanistically distinct models of inflammatory nociception induced by intraplantar administration of capsaicin, complete Freund's adjuvant or formalin. Strikingly, no differences were observed between GPR55 knockout and wild type mice in the development of inflammatory pain in any of the models tested. Neuropathic pain was induced in a mouse model of traumatic nerve injury by performing a partial ligation of the sciatic nerve, which induces hypersensitivity to mechanical, cold and heat stimulation. Toxic neuropathy was induced by treatment with the chemotherapeutic agent paclitaxel, which induces hypersensitivity to mechanical and cold stimulation only. No differences were observed between GPR55 knockout and wildtype mice in the development or maintenance of hypersensitivity to mechanical, cold or heat hypersensitivity in either model of neuropathic pain. In conclusion, genetic deletion of GPR55 did not alter the development of pathological pain in adult mice in any chronic pain model evaluated.

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CANNABINOID AGONISTS (CP55,940, ACEA AND AM1241) FOLLOWING CHRONIC ADMINISTRATION CAUSE CHANGES IN THE ESTRUS CYCLE IN AN OPTIMIZED CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN MODEL

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Cannabis-like compounds have demonstrated antinociceptive properties in various chronic pain models. Synthetic cannabinoid such as CB1 (ACEA), CB2 (AM1241) and CB1/CB2 (CP55,940) agonists can modulate chronic pain perception. Indeed, they have previously been shown to alleviate chemotherapy-induced neuropathic pain. However, the impact of chronic administration of these compounds on the estrus cycle needs to be investigated. The goal of this study is to evaluate the role of these different (ACEA, AM1241 and CP55,940) cannabinoid agonists on the estrus cycle following chronic administration in a chemotherapy-induced neuropathic pain model in C57BL/6J mice. This study is evaluating the effects on the estrus cycle following chronic systemic administration of these different cannabinoid agonists (ACEA, CP55,940 and AM1241) in an optimized chemotherapy-induced neuropathic pain (cisplatin 5 mg/kg intraperitoneal and 4 % sodium bicarbonate subcutaneously weekly) mouse model. We tested the estrus cycle by daily vaginal lavage prior to daily injection with the different synthetic cannabinoids. Further staining of the slides with crystal violet and identification of the estrus cycle under the microscope following cell type identification enable the identification of the stage of the estrus cycle (proestrus, estrus, metestrus and diestrus).

Our results suggest a compound-specific effect, which may be influenced by hormonal changes and could be mediated by receptor selectivity. The nonselective CB1/2 agonist CP55,940 shifts the cycle towards metestrus - the infertile stage of the cycle. The CB2 selective agonist AM1241 shifts the cycle towards the fertile, estrous stage of the cycle. The CB1 selective agonist ACEA is also changing the estrus cycle. Further studies investigating the mechanism behind these compound- or receptor-specific influences on cycle progression is needed to fully appreciate the impact of these behavioral and pharmacokinetic differences on hormonal responses and potential influence on pain perception. A better understanding of the cannabinoid-specific mechanisms responsible for changes in the estrus cycle and possible hormonal role in pain perception are mandatory to advance the development of long lasting, highly efficacious, and personalized pain therapies.

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THE ROLE OF 2-AG OXIDATION IN MECHANICAL HYPERALGESIA IN A HUMANIZED MODEL OF SICKLE CELL DISEASE

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Sickle Cell Disease (SCD) is a painful inherited blood disorder that affects millions of people worldwide. Intense ongoing and episodic pain is a frequent complaint of people living with SCD. Transgenic HbSS-BERK mice express human sickle hemoglobin and mirror symptoms of human SCD, including chronic pain. Previously it was established that COX-2 oxygenates 2-AG forming a pronociceptive lipid mediator, prostaglandin E_2 glycerol ester (PGE_2-G). Here we determined the contribution of PGE_2-G to hyperalgesia in HbSS mice. Mechanical hyperalgesia was defined as an increase in withdrawal frequency in response to a von Frey monofilament with a force of 3.9 mN applied to the plantar surface of the hind paws. The level of 2-AG in dorsal root ganglia (DRG) was determined by HPLS-MS. Quantitative RT-PCR was conducted to determine expression of monoacylglycerol lipase (MGL), the primary enzyme that hydrolyzes 2-AG. The amount of cycloxygenase-2 (COX-2) protein in DRGs was measured by Western blot. The direct effect of PGE_2-G on dissociated DRG neurons was defined by measuring ($[Ca^{2+}]_i$) *in vitro*.

A decreased level of 2-AG in DRGs was associated with mechanical hyperalgesia in HbSS mice. The decrease in 2-AG was independent of MGL, as the expression of MGL mRNA in HbSS and control mice (HbAA) was the same. In contrast, COX-2 protein was elevated in DRGs of HbSS mice. COX-2 oxidizes 2-AG to PGE_2-G which contributes to hyperalgesia. Intraplantar (i.pl.) injection of PGE_2-G (1 μg/10 μl) produced mechanical hyperalgesia in HbAA- mice. PGE_2-G (10 μM) evoked brief Ca^{2+} transients in 42% of the DRG neurons tested, confirming the direct effect of PGE_2-G on small diameter nociceptive DRG neurons. *R*-flurbiprofen (30 μg/10 μl, i.pl.), a preferential inhibitor of the glycerol binding site of COX-2, blocked mechanical hyperalgesia in the injected paw of HbSS mice. Therefore, it is likely that COX-2 is responsible for the reduction in 2-AG levels by converting it to an algogenic lipid. Targeted inhibition of the glycerol binding site in COX-2 may be a promising strategy for the management of pain in SCD.

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CONTRIBUTION OF *FAAH* GENOTYPE TO LOW BACK PAIN SENSITIVITY AND PAIN BURDEN

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A major research emphasis has been focused on defining the molecular changes that occur from acute to chronic pain in order to identify potential therapeutic targets for chronic pain. As the endocannabinoid system is dynamically involved in pain signaling, a plausible mechanism that may contribute to chronic pain vulnerability involves genotypic variation of endocannabinoid genes, particularly *FAAH*. The enzyme fatty acid amide hydrolase (FAAH), which metabolizes the endocannabinoid anandamide, is a well known mediator of pain processing.

First, this study sought to describe and compare peripheral and central pain sensitivity among chronic and acute low back pain subjects. Individuals were classified as acute LBP if their pain resolved within the next 6 weeks or chronic LBP if they developed persistent low back (≥ 6 months). Using a previously established quantitative sensory testing protocol, we comprehensively assessed somatosensory parameters among 40 acute and 40 chronic LBP participants as well as 20 healthy volunteers. Samples of whole blood were drawn to extract genomic DNA. *FAAH* genotypes of three SNPS (rs324420, rs932816, rs4141964) were examined for their contribution to pain sensitivity and burden.

Chronic LBP subjects displayed higher mechanical and cold pain sensitivity compared to acute LBP participants at the initial visit. A significant association was observed between *FAAH* SNP genotype and self-report pain measures, mechanical and cold pain sensitivity among LBP subjects

Chronic LBP participants showed selective pain sensitivity enhancement compared to acute LBP participants, which may be important in distinguishing vulnerability to chronic pain. Further research to characterize pain-associated somatosensory changes in the context of altered endocannabinoid mRNA expression levels may provide insight on the molecular underpinnings of maladaptive chronic pain.

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MATERNAL AND FETAL HEPATIC ENDOGENOUS CANNABINOIDS RESPONSE TO MATERNAL HIGH FAT DIET

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Introduction: Liver plays a central role in fat metabolism and insulin resistance. Maternal BMI influences fetal liver and brain development, being the strongest predictor of fetal fat accumulation and offspring adiposity (*PMID: 24740157).* Specifically maternal obesity has been linked to autism spectrum disorders, non-alcoholic fatty liver diseases (NAFLD), diabetes and other life-threatening diseases. Endogenous cannabinoids (eCBs) play a vital role in obesity. However, information regarding eCBs physiology in obesity, associated with pregnancy, is sparse. The different patterns of maternal obesity: pre-pregnancy obesity vs. pregnancy-related weight gain, over-eating vs. high - fat - high calorie diet make studies of mechanisms of fetal programming challenging. Animal models represent an opportunity to dissect specific mechanisms of dietary patterns and provide important data for development of interventional strategies in humans. The aim of this study was to describe the influence of a maternal high fat diet on the baboon feto-maternal hepatic axis.

Materials and Methods: Baboons *(Papio spp)* were fed a diet of 45% fat (HFD, n=11), while controls (CTR, n=9) ate 12% fat from at least 9 months prior to conception. Fetal and maternal serums samples were collected at term Cesarean Section. qRT-PCR (Quantitative Reverse Transcription Real Time - PCR), immunohistochemistry (IHC) and western blot analysis were performed to quantify expression of CB1 and CB2 receptors and the central enzyme regulating eCBs tone - Fatty Acid Amid Hydrolase (FAAH) in maternal and fetal liver. Data were presented as mean ± SEM (Standard Error of Mean) and statistical analysis was performed using Mann-Whitney test.

Results: Fetal and maternal hepatic gene expression for *CB1R, CB2R* and *FAAH* did not differ between two groups. CB1R protein expression (full length receptor, ~ 55 kDa), was increased in HFD dams, carrying male fetuses by 40% and decreased in their fetuses by 75%. Full length hepatic CB1R was not detectable in the HFD female fetuses and their mothers. Maternal hepatic CB2R and FAAH protein expressions did not differ between groups. Fetal hepatic expression of CB2R and FAAH were decreased in both – HFD male and female fetuses compared to CTR.

Conclusions: High fat diet consumption in pregnancy decreases the abundance of the main enzyme degrading eCBs and decreased ability of fetal liver to counteract changes, associated with hepatic fat deposition.

REVERSAL OF FATTY LIVER BY PERIPHERAL CB1 RECEPTOR BLOCKADE IS SIRT1/PPARα DEPENDENT

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Non-alcoholic fatty liver disease (NAFLD) is the most chronic liver disease worldwide, and becoming an important public health problem due to the rising incidence of obesity. Emerging evidence suggests that the endocannabinoid (eCB) system plays a crucial role in the development of NAFLD. High-fat diet (HFD)-induced fatty liver depends on the activation of hepatic cannabinoid-1 receptor (CB_1R), whereas CB_1R blockade ameliorates it. However, the underlying molecular mechanism by which CB_1R blockade contributes to the reversal of fatty liver remains unclear.

Here, we utilized a HFD-induced hepatic steatosis model in mice with a specific genetic ablation of Sirtuin-1 in hepatocytes ($LSIRT1^{-/-}$) as well as a global deletion of PPARα ($PPAR\alpha^{-/-}$). Both SIRT1 and PPARα are critical signaling molecules associated with modulating fat deposition in the liver. Treating these mice and their littermate controls with the peripheral CB_1R antagonist, AM6545 (10 mg/kg, ip), resulted in an equal reduction in body weight and food intake, and improved glycemic and hormonal control. On the other hand, reversal of the HFD-induced liver dysfunction and hepatic steatosis by AM6545 was only documented in wild-type littermate control mice. Moreover, decreased mRNA and protein expression levels of SIRT1 and PPARα and its target genes were documented following either exposing HepG2 cells to a fatty environment, which was associated with increased eCB tone, or treating the cells with the CB_1R specific agonist, arachidonyl-2-chloroethylamide. Both of these effects were ameliorated by blocking CB_1R with AM6545.

Collectively, our findings suggest that SIRT1 and PPARα are important signaling molecules downstream of hepatic CB_1R, and play a significant role in the antisteatotic effect of peripheral CB_1R blockade. In fact, decreased activation of hepatic CB_1R, promotes the SIRT1/PPARα signaling pathway, which may increase fatty acid β-oxidation and reduce hepatic *de novo* lipogenesis. These results may further support the rationale for the clinical testing of peripherally restricted CB_1R antagonists for the treatment of NAFLD.

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A ROLE FOR FATTY ACID AMIDE HYDROLASE IN THE LEPTIN-MEDIATED EFFECTS ON FEEDING AND ENERGY BALANCE IN FASTED MICE

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Endocannabinoids (eCBs) and leptin are both regarded as important regulators of feeding and metabolic processes. Whereas enhanced eCB signalling is generally associated with hyperphagia and increased body weight, enhanced leptin signaling is rather associated with hypophagia and decreased body weight. It has been demonstrated that leptin inhibits eCB production in the hypothalamus in order to mediate its hypophagic effects. In support of these findings, genetic ablation of leptin (ob/ob mice) is associated with heightened eCB signaling in the hypothalamus. Moreover, CB1 receptor antagonism induces a hypophagic response in ob/ob mice. Despite a clear interaction between leptin signaling and the eCB system, a mechanism linking the two in the context of whole body energy metabolism remains elusive. Unpublished work from our group has shown that systemic leptin administration not only decreases hypothalamic anandamide levels, but furthermore increases fatty acid amide hydrolase (FAAH) activity, the primary enzyme responsible for anandamide degradation. Leptin-deficient mice, by contrast, were shown to have decreased FAAH activity together with increased hypothalamic anandamide and 2-arachidonoylglycerol. Finally, FAAH inhibition blocked the effects of systemic leptin on body weight and feeding in fasted mice. Collectively, these unpublished findings from our group suggest that leptin acts through FAAH in order to suppress eCB signaling.

To extend these findings, we are examining the effects of leptin on energy expenditure and substrate utilization in order to determine whether FAAH inhibition prevents leptin-mediated effects on whole body energy homeostasis or whether its effects are selective for feeding. Moreover, using the FAAH C385A knock-in mice, which model a common human mutation in the FAAH gene leading to decreased FAAH expression, we are examining how genetic variance in FAAH modulates feeding, body weight and metabolic responses to leptin. Our findings will shed light on the mechanistic basis of leptin-mediated reductions of eCB production. Furthermore, our results from the FAAH C385A mice will have high translational value, potentially identifying FAAH C385A as a novel variant SNP underlying individual variations in leptin sensitivity.

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THE PRELIMBIC CORTICAL ENDOCANNABINOID SYSTEM MEDIATES STRESS-ENHANCED COCAINE-SEEKING RELAPSE VULNERABILITY IN BOTH SEXES

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There are different populations of cocaine addicts within whom relapse may be precipitated through distinct neurobiological mechanisms, implying that a one-size-fits-all treatment is unlikely to exist. Probing for more individualized therapies is therefore more likely to identify effective relapse preventatives. An important population difference emerges under the context of stress, as there is variability in the ability of stress to directly trigger relapse. However, under conditions where stress alone cannot induce the reinstatement of cocaine seeking, we have found that it instead acts by augmenting reactivity to other triggers. Following cocaine self-administration and extinction, neither stress-response levels of corticosterone (CORT; 2 mg/kg) nor low-dose cocaine (2.5 mg/kg, i.p.) alone induce the reinstatement of cocaine seeking, but do so in combination. CORT (50 ng/side) can act directly within the prelimbic cortex (PrL) to potentiate reinstatement, an effect likely associated with CORT (100nM)-provoked depolarization-induced suppression of inhibition of the PrL pyramidal neurons which drive drug-seeking behavior. Endocannabinoids (eCBs) are implicated in this phenomenon, as intra-PrL cannabinoid receptor-1 (CB1) activation (AM251; 300 ng/side) and 2-arachidonoylglycerol synthesis (DO34; 0.1 & 1 µg/side) are necessary for potentiated reinstatement. Based on these findings, we hypothesize that stress-level CORT acts through the PrL eCB system to render pyramidal neurons more excitable by ordinarily subthreshold stimuli, resulting in greater vulnerability to reinstatement.

However, these data were solely collected from males, and a key population difference in cocaine addiction also exists between the sexes. As female cocaine addicts display enhanced relapse susceptibility compared to males, particularly during periods of stress, we investigated whether there are sex differences in CORT-potentiated reinstatement. Despite equivalent levels of cocaine intake during self-administration (0.5 mg/kg/inf i.v., 2 hr/day *x* 14 days), females exhibit CORT-potentiated reinstatement to a lower subthreshold dose of cocaine (1.25 mg/kg, i.p.) than males. Additionally, intra-PrL CORT application is sufficient, while intra-PrL CB1R activation is necessary, for potentiated reinstatement in females. Although the parallel results suggest no sex difference in PrL CORT-induced eCB-mediated enhanced relapse vulnerability, that does not explain the augmented vulnerability observed in females. Since females exhibit relapse variability across the ovarian hormone cycle, testing is underway to correspond estrous phase to CORT-potentiated reinstatement responding. Our preliminary results indicate that greater CORT-potentiated reinstatement is observed when circulating estrogen (E2) levels are rising while progesterone is low. Furthermore, voltage-clamp recordings reveal that E2 augments excitatory but does not affect inhibitory neurotransmission in female PrL layer V pyramidal neurons. Therefore, we hypothesize that E2 may complementarily interact with CORT-induced CB1R-mediated disinhibition of pyramidal neuron output from the PrL, effectively amplifying relapse vulnerability in females and facilitating cocaine-seeking behavior.

Regardless of sex, the findings from these experiments implicate the PrL eCB system as a treatment target for the prevention of stress-promoted relapse. Furthermore, the importance of these findings extends beyond addiction, and should guide our understanding of how hormones and eCBs may regulate and contribute to a range of neuropsychiatric disorders.

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RECRUITMENT OF 2-AG SIGNALING IN THE BASOLATERAL AMYGDALA MAY RELATE TO THE ONTOGENY OF STRESS HABITUATION

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In response to repeated exposure to a homotypic stressor that does not present a threat to an animal's survival, a progressive reduction in stress-induced glucocorticoid secretion occurs. This adaptation, termed habituation, is crucial to limit negative health consequences resulting from prolonged or unnecessary glucocorticoid release. The endocannabinoid system is an important regulator of the stress response system and plays a key role in habituation. We, and others, have previously established that in adult rats, repeated exposure to stress results in an elevation in 2-AG signaling within the amygdala, and that systemic or intra-basolateral amygdala (BLA) blockade of the CB1 receptor results in dishabituation to repeated homotypic stressors. This suggests that the progressive recruitment of 2-AG signaling in the BLA may be a significant mechanism underlying stress habituation. While these changes are reliably seen in adult rats, unpublished data from our group has demonstrated that adolescent rats fail to exhibit habituation of the corticosterone (CORT) response to repeated stress, as well as do not exhibit an increase in amygdala 2-AG levels following 10 day of repeated restraint.

To investigate whether the inability to elevate 2-AG signaling in the BLA contributes to the lack of stress habituation in adolescent rats, we are examining whether the impact of acutely elevating amygdala 2-AG in adolescents (through local administration of the MAGL inhibitor KML29) is sufficient to reduce HPA axis responses to repeated stress. To this extent, adolescent male rats (PND 35) were subject to daily 30-minute restraint stress for 10 days and plasma CORT levels were measured immediately following stress on days 1 and 10. On the last day of restraint, rats were administered KML29 (200 ng/0.2 µl per side) or vehicle directly into the BLA 20 minutes prior to stress onset. Our preliminary data indicate that, consistent with previous reports, vehicle-treated adolescent rats failed to display a reduction in CORT levels across 10 days of restraint stress. Interestingly, those treated with KML29 demonstrated a trend towards a decrease in CORT secretion, suggesting that elevations in 2-AG signalling within the BLA may promote stress habituation. Ongoing studies are examining this phenomenon in more depth, as well as the impact of 2-AG depletion (through intra-BLA administration of the DAGL inhibitor DO34) on stress habituation in adults to determine the necessity of 2-AG signalling to contribute to stress habituation. Collectively, these results will help to inform us the mechanisms sub-serving the ontogeny of stress habituation from adolescence into adulthood, as well as determine the importance of 2-AG signalling in the BLA for this process.

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INDEPENDENT EFFECTS OF REPEATED STRESS AND AM251 TREATMENT IN ADOLESCENCE ON ANXIETY, SOCIALITY, AND NEUROENDOCRINE STRESS RESPONSES, AND ON RELEVANT PROTEIN EXPRESSION IN THE PREFRONTAL CORTEX AND HIPPOCAMPUS, IN FEMALE RATS

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Recent studies of CB1 receptor blockade with the antagonist AM251 in adolescent male rats found limited effects on socioemotional behaviour and hypothalamic-pituitary-adrenal (HPA) axis responses to stressors (Lee et al., 2015; Simone et al., 2017), but females may be more sensitive to such blockade (Rubino et al., 2015). Further, effects may be greater when combined with stressors, given the role of the ECS in regulating the HPA axis. To test this hypothesis, female rats were treated daily on postnatal days (P)30-44 with either no injection, vehicle, or AM251 (1 mg / kg), and half of each group was either returned to the homecage (NoStress) or underwent 1h confinement (Repeated Stress; RStress). All testing was conducted > 24h after the last stress and drug treatment. On P45, measures were obtained in subgroups on anxiety in the elevated plus maze (EPM), plasma corticosterone (CORT) release to confinement stress, and baseline expression of cannabinoid-associated proteins in the hippocampus and prefrontal cortex. Another subgroup was tested on P46 for activity in a novel environment and interactions with a novel conspecific, then tested as adults (P70) in the EPM, and again at P73 to determine CORT release to restraint stress.

No interaction of Stress and Drug treatment was found for any measure. In adolescence, AM251 increased social interactions ($p = 0.035$), and tended to decrease anxiety in the EPM, but the effect did not meet statistical significance (time on open arm, $p = 0.066$; entries to open arm, $p = 0.087$). RStress increased activity in the EPM (entries to closed arms; $p = 0.04$) and in an open field ($p = 0.009$). RStress tended to increase baseline CORT ($p = 0.07$), and AM251 rats had increased CORT after confinement stress than did no injection ($p = 0.06$) and vehicle injection ($p = 0.04$) rats. Compared with NoStress rats undergoing a first confinement, RStress had lower CORT concentrations 60 min after confinement stress ($p = 0.001$). RStress rats had reduced expression of spinophilin in the dorsal hippocampus ($p = 0.05$), increased expression of GAD67 in the ventral hippocampus ($p = 0.039$), and increased expression of FOS in the prefrontal cortex ($p = 0.006$). AM251 treatment increased GAD67 expression in the prefrontal cortex compared with no injection ($p = 0.002$) and vehicle ($p = 0.009$). No effects of Stress or Drug were observed on anxiety or CORT responses to restraint stress in adulthood. Although both repeated treatments had discernible effects when tested soon after treatment ended, the results suggest that there is substantial recovery from treatment with time.

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SEX DIFFERENCES IN ALCOHOL WITHDRAWAL-INDUCED NEGATIVE AFFECT AND CORTICOAMYGDALAR ENDOCANNABINOIDS

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Alcohol withdrawal is associated with stress-related anxiety. The endocannabinoid (ECB) system is critical to the homeostatic function of the HPA-axis response to stress, and changes in the ECB system, particularly in the corticoamygdalar circuit, contribute to alcohol withdrawal-induced anxiety. Male alcoholics experience more pronounced withdrawal symptoms than female alcoholics, and previously demonstrated sexual dimorphisms in the ECB system, which are influenced by ovarian hormones, may contribute to this sex difference in withdrawal.

In the present experiment**,** intact male and female rats, and ovariectomized (OVX) female rats with or without estradiol (E2) replacement, were exposed to 6 weeks of chronic intermittent alcohol vapor (14 hr on/10 hr off, 7 days/week). During acute withdrawal (6-8 hrs after the vapor turned off), air-puff-induced ultrasonic vocalizations (USVs) and elevated plus maze (EPM) behavior were measured. On a subsequent day, during acute withdrawal, the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) were harvested for rtqPCR analysis of CNR1, NAPE-PLD, FAAH, MAGL, and DAGL-α mRNA expression or quantification of ECBs [anandamide (AEA) and 2-arachidonoylglycerol (2-AG)].

Alcohol exposed male (but not female) rats emitted significantly more 22-kHz USVs and displayed increased anxiety-like behavior in the EPM during acute withdrawal. In the BLA, male (but not female) rats had reduced NAPE-PLD, MAGL, and DAGL-α mRNA expression, but displayed a significant decrease only in AEA content. In the mPFC, there was also a reduction in NAPE-PLD expression in males, but significant decreases in AEA content were unexpectedly observed only in alcohol-exposed females, with males instead showing a decrease in 2-AG content during withdrawal. Similar to alcohol-exposed males, OVX females exhibited anxiety-like behavior and reductions in NAPE-PLD expression and AEA content in the BLA, as well as widespread reductions in all ECB-related genes measured in the mPFC. Moreover, in alcohol-exposed OVX females, AEA content was decreased and 2-AG content was increased in the mPFC. Importantly, none of these withdrawal-induced alterations in the ECB system were prevented by E2 replacement except for the reduction in AEA content in the BLA, and this was not sufficient to prevent the expression of withdrawal-induced anxiety. Overall, these data indicate that alcohol withdrawal produces sexually dimorphic effects on anxiety-like behavior and ECB expression in the BLA and mPFC, which may in part be due to a protective effect of ovarian hormones (but not E2 alone). Furthermore, these data point to a potential mechanism by which sex-specific affective symptoms could emerge during alcohol abstinence and withdrawal.

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TARGETING THE THERAPEUTIC PROPERTIES OF CANNABIDIOL: FOCUS ON DEPRESSION AND PAIN

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Cannabidiol (CBD), a non-psychotropic constituent of cannabis, is thought to have antidepressant and analgesic effects (Metchoulam et al., 2007, Chem Biodivers. Aug;4(8):1678-92), acting via the serotonin 5-HT1 subtype receptor (Rock et al., 2012, Br J Pharmacol. Apr;165(8):2620-34). Here, we investigated the acute (0.05-1.0 mg/kg, i.v.) and chronic (5.0 mg/kg/day,i.p. for 7 days) effect of CBD on serotonin (5-HT) neurons of Dorsal Raphe Nucleus (DRN) in male Sprague-Dawley rats, alone or with the 5-HT_{1A} receptor antagonist WAY100635 (0.3 mg/kg, i.v.) or CB_1 receptor antagonist AM251 (1.0 mg/kg, i.v.). Forced Swim Test (FST) was performed to evaluate the antidepressant-like effect of CBD. The analgesic effect of CBD was also tested after 14 days post Spared Nerve Injury (SNI).

Cumulative injection of CBD (0.1-1.0 mg/kg) decreased 5-HT DRN activity ($P<0.001$, $n=9$), effect prevented by WAY100635, but not by AM251 ($P<0.001$, $n=4$). Chronic CBD increased the firing rate of DRN 5-HT neurons ($P<0.001$). Acute and chronic treatment with CBD did not affect immobility time in the FST in naïve rats. Chronic treatment with CBD prevented mechanical allodynia ($P<0.05$) and thermal hyperalgesia ($P<0.05$) after 14 days post-SNI in CD1 mice. These data confirm that CBD administration decreases 5-HT activity via 5-HT_{1A} receptor, regimen which did not affect depressive behavior, but was effective in treating neuropathic pain condition.

EFFECTS OF STRESS ON PLACE CONDITIONING PRODUCED BY Δ^9-TETRAHYDROCANNABINOL IN SPRAGUE DAWLEY RATS

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The compound Δ^9-tetrahydrocanabinol (THC), a partial agonist of the cannabinoid 1 (CB1) receptor within the endocannabinoid (eCB) system, is one of the most commonly used illicit drugs in the world. Human users generally report feelings of euphoria and relaxation, however, dysphoric effects are common as well. Animal research on the rewarding/aversive effects of THC has produced inconsistent results with most studies finding that THC is aversive to rats. It still remains unclear what factors modulate rewarding/avoidance behaviours elicited by THC.

Recent research indicates that exposure to prior stressors results in a deficit in the functioning of the eCB system. It is known that foot shock stress enhances anxiety-like behaviours in rodents when they are tested 24h later, and causes a reduction in brain anandamide (AEA) concentrations. Raising AEA concentrations by blocking its catabolic enzyme fatty acid amide hydrolase (FAAH) prevents the enhancement of anxiety-like behaviours, only in previously stressed animals via a CB1 mechanism. Therefore, CB1 eCB signaling is needed to terminate the stress response. Since THC acts as a partial agonist of the CB1 receptor, prior stress may also alter the rewarding effects of THC.

The effect of prior stress exposure on the rewarding or aversive effects of THC (1, 0.1, 0.5mg/kg) were determined using an unbiased place conditioning procedure. Sprague Dawley rats were either exposed to footshock stress (0.8 mA footshock every min for 6 min) or no stress 24h prior to each conditioning trial. To take advantage of the prior fear conditioning training, the potential of the same doses of THC used to condition place preference/aversion were used to determine if it also modifies the expression of conditioned fear.

A dose of 1 mg/kg THC produced a conditioned place aversion that was not modified by stress. At this same dose foot shock tended to reverse locomotor suppression by THC. There were no effects on conditioned place preference or locomotor activity by 0.5 or 0.1 mg/kg THC. The expression of conditioned fear was found to be reduced by 1 mg/kg THC in previously stressed rats. However, 0.1 mg/kg THC prolonged the expression of freezing. A dose of 0.5 mg/kg of THC had no effect on the expression of conditioned fear. In conclusion, footshock stress 24h prior to conditioning did not alter a conditioned place aversion/preference produced by THC.

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CB_1 RECEPTOR ANTAGONISM IN THE BED NUCLEUS OF THE STRIA TERMINALIS INTERFERES WITH AN ACUTE NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL-INDUCED PLACE AVERSION

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The bed nucleus of the stria terminalis (BNST) is a region of the extended amygdala that is implicated in addiction, anxiety and stress related behaviours. This region has been identified in mediating the aversive state of naloxone-precipiated morphine withdrawal and cannabinoid type 1 (CB_1) receptors have been found to modulate neurotransmission within this region. Previous findings suggest that the CB_1 antagonist/inverse agonist, AM251, administered systemically or by infusion into the central nucleus of the amygdala (CeA) prevented the aversive affective properties of morphine withdrawal as measured by conditioned place aversion (CPA) learning. As well, when administered systemically or by infusion into the basolateral nucleus of the amygdala (BLA) or the interoceptive insular cortex, the monoaclyglycerol lipase (MAGL) inhibitor, MJN110 (which elevates 2-arachidonlyglycerol), also prevented a naloxone-precipitated morphine withdrawal induced CPA. Given the connectivity of these regions and the BNST, the present study sought to determine whether cannabinoid modulation of the BNST would also prevent the affective properties of naloxone precipitated morphine withdrawal-induced CPA. Acute morphine withdrawal induced CPA occurs when naloxone is administered 24 hr following a single exposure to a high dose of morphine. Prior to conditioning trials, rats received intra-BNST infusions of AM251 or MJN110. AM251, but not MJN110, prevented the establishment of the MWD-induced place aversion. The current findings emphasize an important role for the BNST in opioid withdrawal and suggest that the ameliorative effects of systemically administered CB_1 antagonists are mediated, in part, by their actions within this region.

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REGULATION OF THE ESTABLISHMENT OF AN ACUTE MORPHINE WITHDRAWAL CONDITIONED PLACE AVERSION, BUT NOT MORPHINE PLACE PREFERENCE, BY OLEOYL GLYCINE

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Considerable preclinical evidence suggests that modulation of the endocannabinoid system may have therapeutic potential in the regulation of addiction. Oleoyl Glycine is a newly discovered endogenous cannabinoid-like compound that may have therapeutic potential in the treatment of addiction. Here we evaluated the potential of Oleoyl Glycine (administered 20 min prior to conditioning) to modify both a conditioned place aversion produced by morphine withdrawal and a conditioned place preference produced by morphine in rats. Acute morphine withdrawal induced place aversion was produced by administration of naloxone (1 mg/kg sc) 24 hr following a single exposure to a high dose (20 mg/kg sc) of morphine and placement in a distinctive box for two conditioning trials. Morphine place preference was produced by administration of morphine (10 mg/kg sc) prior to placement in a distinctive box on each of four conditioning trials. Oleoyl Glycine was found to successfully block an acute naloxone precipitated morphine withdrawal CPA at a dose (5 mg/kg, ip) that had no motivational properties on its own, while exhibiting no effect on the rewarding properties of morphine (at doses of 5 or 30 mg/kg, ip). Administration of CB1 antagonist/inverse agonist, AM251 (1 mg/kg ip), but not PPAR alpha inhibitor, MK886 (1 mg/kg ip), blocked the effect of Oleoyl Glycine on the morphine withdrawal induced CPA, suggesting a CB1 dependent mechanism.

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Δ^9-THC WITHDRAWAL ACTIVATES THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AND ALTERS SOCIAL BEHAVIOR

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Cannabis withdrawal is characterized in humans by changes in sleep, increased craving, anxiety, depression, and irritability. Preclinical cannabinoid withdrawal models predominantly evaluate somatic signs of withdrawal. Previous work from our lab and others also demonstrates that THC withdrawal significantly alters cognition and behaviors related to emotionality and motivation. It is unknown whether the irritation and aggression reported following cannabis withdrawal may also be modeled in rodents. In the present study, tolerance was induced in male C57BL/6 mice after 6 days of THC (10 mg/kg, b.i.d., s.c.) or vehicle treatment, then withdrawal was precipitated using rimonabant (3 mg/kg, i.p.). We hypothesized that THC withdrawal would increase plasma corticosterone levels. Plasma was collected 30 min after rimonabant treatment, and corticosterone was quantified by ELISA. Corticosterone was elevated by chronic THC treatment when compared to vehicle treated mice regardless of rimonabant treatment and in THC mice that did not receive rimonabant. We also hypothesized that THC withdrawal would decrease time spent near a novel target mouse in the social interaction test. Vehicle treated mice occupied the novel target mouse chamber more than an empty chamber, but mice subjected to THC withdrawal did not occupy the target mouse chamber any more than any other. A resident intruder paradigm was also used to assess aggressive and prosocial behavior in male mice. These data suggest that disruption of CB_1 function, following chronic THC administration, activates a classic neuroendocrine stress response and decreases social interaction in mice.

THE 'SACRED MAYA INCENSE,' *PROTIUM COPAL* (BURSERACEAE), ELICITS ANXIOLYTIC EFFECTS IN ANIMAL MODELS

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The Maya of Central America have used 'copal', the resin of *Protium copal*, as a sacred incense during ceremonies since pre-Columbian. When burned, copal is described as mentally uplifting and calming. However, despite the extensive use of this resin across Central America, the behavioral effects of copal are largely speculative, with few scientific data and no behavioral studies reported to date. The objective of this study was to determine whether copal incense elicits anxiolytic-like activity in rats and, if so, through what mechanism(s). The effects of copal incense in rats were assessed using the elevated plus maze (EPM), social interaction (SI) conditioned emotion response (CER) and Novel object recognition (NOR) paradigms. Rats were exposed to burning copal resin (100 mg and 250 mg) or smoke-flavoured, nicotine-free, e-cigarette liquid for 5 minutes within a smoking chamber apparatus then immediately tested in behavioral models. Whereas no significant effects were observed in the EPM and NOR models, exposure to copal reduced anxiety-like behavior in the SI and CER tests. The window of anxiolytic effect after inhalation of copal resin appeared short and within 10 minutes but was more profound in the social interaction context. Phytochemical analysis of the resin by HPLC-MS/MS identified an abundance of the triterpenes lupeol, alpha-amyrin and beta-amyrin, which are known to act at benzodiazepine receptors and to inhibit monoacylglycerol lipase (MAGL). Subsequent *in vitro* assessment revealed potent inhibition of MAGL by both the crude resin (IC_{50} = 810 ng/mL) and each of the identified triterpenes (IC_{50} < 1.8 μM). To investigate the potential mechanisms of copal's bioactivity, rats were administered (*i.p.*) the CB1 antagonist AM251 or the $GABA_A$ antagonist flumazenil prior to exposure to incense. Both antagonists completely reversed the copal-induced increase in social interaction. Our results demonstrate the anxiolytic-like activity of inhaled copal incense in animal models, particularly with regard to social behavior, effects that likely contribute to the ceremonial value of copal in Mayan traditions.

CONSEQUENCES OF DAGL-α DISRUPTION ON SPATIAL LEARNING AND MEMORY PROCESSES IN C57BL6/J MICE

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A growing body of evidence implicates the importance of the endogenous cannabinoid 2-arachidonoyl glycerol (2-AG) in the regulation of learning and memory. The biosynthesis of 2-AG occurs through diacylglycerol lipase (DAGL), in which DAGL-α is the principal regulator of 2-AG on neurons, and DAGL-β regulates this eCB on microglia and macrophages. Because DAGL-α knockout mice show decrements in synaptic plasticity (Gao et al., J. Neuorsci. 30 (2010) 2017-2024, Tanimura et al., Neuron 65 (2010) 320-327) and compromised hippocampal neurogenesis (Jenniches et al. Biol Psychiatry. 79 (2016) 858-868), the present study used complementary pharmacologic and genetic approaches to examine whether interruption of DAGL-α will disrupt acquisition, expression, and extinction of spatial memory tasks of the Morris water maze (MWM) in C57BL/6J mice. *Experiment 1: genetic deletion of DAGL–α*. To assess MWM acquisition DAGL-α knockout and wild type mice received 10 Fixed Platform MWM training days (i.e. a submerged platform remained in the same location across days), and then were assessed for expression of spatial memory on day 11 in a single MWM Fixed Platform Probe Trial (i.e. the submerged platform was removed). *Experiment 2: pharmacological inhibition of DAGL–α*. C57BL6/J Mice received a 2h pretreatment of the DAGL inhibitor DO34 (Ogasawara et al. PNAS. 113 (2016) 26-33) (0.3, 3, and 30 mg/kg) or vehicle (VEH) on each of 10 Fixed Platform MWM training days. In order to assess whether DO34 affects the expression of spatial memory, separate groups of mice were given 10 days of drug-free Fixed Platform training. On day 11, each subject was administered 30 mg/kg DO34 or VEH, and 2h later underwent a single 2 min MWM Fixed Platform Probe Trial. The extinction of spatial memory was then assessed, in which mice continued to receive their respective injections of 30 mg/kg DO34 or VEH (2 h pretreatment) and tested in 2 min probe trials at 1, 2, 3, 4 and 6 weeks post-acquisition.

DAGL-α knockout mice failed to acquire the Fixed Platform task and showed no evidence of spatial bias during the probe trial. In contrast, DO34-treated mice displayed small yet significant dose-dependent delays in MWM acquisition rate, without any performance deficits in either the probe trial or extinction task. The severely impaired MWM performance of DAGL-$\alpha^{-/-}$ mice compared to C57BL6/J mice receiving DO34 may reflect developmental consequences of constitutive DAGL-α gene deletion absent from drug-treated mice. The impaired acquisition rate, but not expression or extinction of DO34-treated mice may suggest that DAGL-α contributes to the homeostatic regulation of specific aspects of spatial learning and memory. Therefore, the performance deficits resulting from 2-AG biosynthesis interruption point to the eCB's regulatory, and perhaps developmental importance in spatial learning and memory.

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IS *NEUREGULIN 1* A STRONG CANDIDATE FOR GENE-CANNABIS INTERACTIONS IN SCHIZOPHRENIA

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Background: *Neuregulin 1* (*NRG1*) is a risk factor for schizophrenia. In line with the Two-Hit hypothesis, the cumulative environmental risk factor cannabis induces more pronounced neuro-behavioural consequences in transmembrane domain *Nrg1* mutant mice. However, NRG1 biology is complex and seven different isoform types are known. We recently validated a novel mouse model for Nrg1 type III isoform overexpression for schizophrenia. Here, we compare the behavioural effects of cannabis exposure (i.e. delta-9-tetrahydrocannabinol: THC) in various *Nrg1* mouse models.

Methods: Adult *Nrg1 type III* transgenic as well as knockout mice and appropriate controls were exposed to different doses of acute or chronic THC. All mice were assessed for behaviours relevant to schizophrenia and cannabis including locomotion, anxiety, social domains, and sensorimotor gating.

Results: *Nrg1 type III* knockout and control mice were susceptible to THC effects in a dose-dependent manner. THC reduced open field locomotion, increased anxiety-related behaviours, and also had a moderate effect on sensorimotor gating. Different to what has been found in other *Nrg1* mouse models, *Nrg1 type III* knockout mice appeared not to be more sensitive to the behavioural effects of THC. These data will be compared with the widely published impact THC has on *Nrg1* transmembrane domain mutant mice, which are more sensitive to the neuro-behavioural effects of cannabinoid exposure.

Conclusion: Our findings show that it is highly likely that the reported *Nrg1*-cannabis interaction in the context of schizophrenia is dependent on the *Nrg1* isoform type. This also suggests that mutant *NRG1*-cannabis interactions found in humans should be investigated in more detail thereby considering the particular *NRG1* isoform types affected.

ALTERATIONS IN CB_1 RECEPTOR AND IL-6 EXPRESSION IN THE PHENCYCLIDINE MOUSE MODEL OF SCHIZOPHRENIA

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Schizophrenia is a chronic psychotic disease that affects 1% of the world population. Clinical studies indicate that inflammatory processes are involved in the pathophysiology of schizophrenia, specifically, abnormal level of the cytokine IL-6 was found in patients. These changes were found in different studies in the CSF and blood. It has also been documented that increased use of cannabis is a risk factor associated with the onset of psychosis. Accordingly, a number of studies have found significant changes in the endocannabinoid system in patients with schizophrenia. Since these changes were found in other studies it remains unclear whether all these changes occur in the same areas of the brain. The aim of the current study was to test the mRNA expression level of CB_1 receptor and IL-6 in a mouse model of schizophrenia. Schizophrenia-like symptoms were induced by sub-chronic injections of phencyclidine to mice. Phencyclidine is a glutamate NMDA receptor antagonist that induces in human psychosis and other impairments and in mice schizophrenia-like behaviour. The mRNA expression level of the selected genes was examined by RT-PCR. The study has focused on the prefrontal cortex, a brain area that is involved in the pathophysiology of schizophrenia. In this model, the expression level of IL-6 and CB_1 was significantly altered at age 26 days. This study contributes to our understanding of the involvement of endocannabinoid system in the pathophysiology of schizophrenia.

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CANNABIDIOL IMPROVES BEHAVIOURAL AND GLUTAMATERGIC DEFICITS IN A PRENATAL INFECTION MODEL OF SCHIZOPHRENIA

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Disruption to the glutamatergic system, particularly hypofunction of the *N*-methyl-D-aspartate receptor (NMDAR), may underlie the symptoms of schizophrenia (Merritt et al., Front. Psychiatry 4 (2013) 1-8). Antipsychotic drugs (APDs), which primarily target the dopaminergic system, have minimal benefit for the cognitive deficits and one-third of patients with negative symptoms (e.g. social withdrawal) are APD-resistant (Lindemayer et al., Schizophr. Res. 147 (2013) 241-252). APDs can also cause adverse side effects (e.g. metabolic and motor disturbances); therefore, it is essential to find new therapeutic agents for schizophrenia (Weston-Green et al., CNS Drugs 27 (2013) 1069-1080). Cannabidiol (CBD) possesses antipsychotic properties, but its effect on the cognitive/negative symptoms and underlying glutamatergic dysregulation in schizophrenia is unknown (Osborne et al., Neurosci. Biobeh. Rev. 72 (2017) 310-324). Therefore, the aim of this study was to examine whether CBD can improve negative/cognitive behavioural phenotypes and NMDAR dysfunction in a prenatal infection (poly I:C) model of schizophrenia. Pregnant dams were administered either poly I:C (POLY; 4 mg/kg) or saline (CONT) on gestation day 15. At the beginning of the post-pubertal period (postnatal day 56), female offspring were administered either vehicle (CONT+VEH, POLY+VEH) or CBD (10 mg/kg; CONT+CBD, POLY+CBD) twice daily for 3 weeks (n=12/group). The novel object recognition (recognition memory), T-maze (working memory) and social interaction (social behaviour) tests were used to assess cognition and negative schizophrenia-like phenotypes. Binding density of the NMDAR was assessed in the prefrontal cortex (PFC), cingulate cortex and striatum.

CBD treatment improved recognition memory deficits in poly I:C offspring ($p<0.05$ vs. POLY+VEH), but did not affect recognition memory of control offspring ($p>0.05$ vs. CONT+VEH). All groups showed comparable working memory performance in the T-maze test. CBD treatment ameliorated social interaction deficits in poly I:C offspring ($p<0.01$ vs. POLY+VEH), but impaired social interaction in control offspring ($p<0.05$ vs. CONT+VEH). CBD restored poly I:C-induced reductions in NMDAR binding density in the PFC ($p<0.001$ vs. POLY+VEH), with a tendency to increase NMDAR binding density in the cingulate cortex ($p=0.097$). Overall, CBD treatment increased NMDAR binding density in the striatum compared to VEH ($p<0.01$). Interestingly, CBD administration in control offspring reduced NMDAR binding density in the PFC only ($p<0.05$ vs. CONT+VEH), a region highly involved in social behaviour. These preliminary findings suggest that CBD has the potential to treat aspects of the cognitive/negative symptoms of schizophrenia and its therapeutic benefits may be linked to NMDAR up-regulation in specific brain regions. These results also suggest that CBD may have adverse effects on social behaviour coupled with PFC NMDAR deficits in healthy offspring; however, further research is needed to confirm this.

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DAILY USE OF CANNABIDIOL (CBD) EXTRACTS MAY LEAD TO POSITIVE Δ^9-TETRAHYDROCANNABINOL (THC) DRUG SCREENS

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Background: Standard drug tests for cannabis use measure THC-COOH, a non-psychoactive metabolite of delta-9-tetrahydrocannbinol (THC). This study was conducted to determine whether individuals who use CBD oil on a daily basis would test positive on urine cannabis drug tests. The rationale for this is two-fold. First, though touted as purified CBD/hemp extracts, it is likely that retail CBD extracts contain small concentrations of THC and other cannabinoids. The cross-reactivity of CBD, its metabolites, or other cannabinoids with urine enzyme immunoassay (EIA) drug tests is currently unknown. Second, recent work suggests that CBD may be converted to delta-9 and delta-8 THC within the gut following oral administration (Merrick et al., 2016; Wannabe et al, 2007). It is unknown whether daily oral administration of purified botanical CBD extracts can lead to detectable THC metabolites in urine.

Methods: In this ongoing study, participants were adult residents of Colorado who report daily oral ingestion of CBD extracts for at least the prior 30 days and no use of THC-based products over the previous 8 weeks. Participants completed a brief survey assessing demographics and use of products labeled as containing CBD and/or THC, and provided a urine specimen for testing. Each specimen was subject to qualitative drug testing using two commercial EIA "dipstick" tests with a cut-off of 20ng/mL THCCOOH and two with a cut-off of 50ng/mL THCCOOH. Aliquots were also sent to Dominion Diagnostics for quantitative THC-COOH testing.

Results: Preliminary findings indicate that daily use of CBD extracts consistently result in positive qualitative urine drug screens at the 20ng/mL cut-off. Mixed results were observed at the 50ng/ml detection cut-off.

Conclusions: Daily use of CBD extracts likely results in positive urine cannabis screens when a cut-off of 20ng/mL is used, and may result in positive screens at a cut-off of 50ng/mL. Future work is needed to determine whether the drug test results observed here are caused by cannabinoids other than CBD being present in the extract, and/or metabolism of CBD to THC or THCCOOH. Additional studies are also needed to determine the impact of frequency of hemp oil use, age, sex, or other individual characteristics on drug test results.

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CANNABIS-RELATED PROBLEMS IN ADULT, NON-TREATMENT-SEEKING RECREATIONAL CANNABIS USERS

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Cannabis is the third most commonly used psychoactive substance globally (after alcohol and tobacco), yet there is relatively little systematically collected data about its adverse consequences in non-treatment-seeking adults. A convenience sample of 385 non-treatment-seeking adult recreational cannabis users who had made a "serious" (self-defined) quit attempt without formal treatment during their life were recruited from the community. All participants completed the 176-item Marijuana Quit Questionnaire (MJQQ) to assess their lifetime history of cannabis use and presence of 4 types of cannabis-related problems. The association between number of cannabis-related problems and respondent characteristics was evaluated using Pearson correlation coefficient.

Respondents had a mean (SD, range) age at time of interview of 29.2 years (9.3, 18-65), age at first regular (weekly) cannabis use of 16.6 years (3.6, 9-36), and duration of regular cannabis use of 11.3 years (9.0, <1-11.3). Cumulative lifetime frequency of cannabis use was <100 for 6.8%, 100-999 for 22.0%, 1,000-4,999 for 28.3%, 5,000-10,000 for 14.3%, and >10,000 for 28.6%. Respondents reported a mean of 1.7 (1.1, 0-4) lifetime cannabis-related problems: 19.5% reported no problems and 3.1% 4 problems. 62.3% reported a psychological/emotional problem; among the 13% who specified their problem, 56% reported poor memory/concentration, 14% guilt/shame, 12% depression, 10% paranoia, and 2% anxiety. 54.8% reported interpersonal problems; among the 19.5% specifying their problem, 28% reported with family/relatives, 24% friends, and 22.7% spouse/partner. 37.1% reported physical health problems; among the 17% specifying their problem, 39.8% reported cough, 22.7% headache, and 18.2% trouble breathing. 11.7% reported a physical injury to self or others. Respondents reported a mean of 6.7 (2.4, 0-10) DSM-5 diagnostic criteria for cannabis use disorder (CUD)-- 98.6% met putative criteria for CUD (89.1% moderate-severe). There were significant correlations between number of cannabis-related problems and lifetime frequency of cannabis use (r-0.10, p=0.05) and age at first regular use (-0.14, 0.006), but not duration of regular use (0.008, 0.87). There was no significant correlation between age of respondents and number of cannabis-related problems (-0.04, 0.49) or lifetime frequency of use (0.06, 0.21), suggesting the internal validity of study findings. These findings suggest that many adult, non-treatment-seeking recreational cannabis users experience a variety of cannabis-related problems, and that adolescent cannabis use (more than duration of use) may be a factor in generating such problems.

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SYMPTOMS OF OBSESSIVE-COMPULSIVE DISORDER PREDICT CANNABIS MISUSE

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Cannabis use has been linked to several psychological disorders, including social anxiety disorder, and to anxiety more generally. Further, more people report using cannabis to reduce negative affect and to deal with their problems (i.e., to cope) than any other drug. Therefore, it is of paramount importance that researchers, cannabis users, and society, more generally, understand the consequences of cannabis consumption. To date, there is a paucity of research investigating the relationship between cannabis use and obsessive-compulsive disorder (OCD). The present study was conducted to explore the putative link between OCD and cannabis by examining associations between severity of OCD symptoms, cannabis use, and cannabis misuse. Further, we sought to determine whether these associations exist above and beyond the potentially confounding influence of symptoms of anxiety, depression, and stress. Finally, we tested the mediating role of cannabis coping motives (i.e., using cannabis to cope with negative affect and other problems) to determine whether OCD is related to cannabis because people use the drug to cope.

A large sample of young adult cannabis users (n = 430) completed an online survey containing measures of OCD symptoms (the Obsessive-Compulsive Inventory-Revised [OCI-R]), cannabis use (the Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory), cannabis misuse (the Marijuana Problems Scale and the Cannabis Use Disorder Identification Test-Revised), cannabis coping motives (the Marijuana Motives Measure), and symptoms of anxiety, depression, and stress (the Depression, Anxiety, and Stress Scales). Almost 60% of the participants reported using cannabis at least once a week (2.6 times per week on average), and approximately 13% of the sample met or surpassed the clinical cut score on the OCI-R.

Severity of OCD symptoms was unrelated to frequency and quantity of cannabis use, but it was significantly, positively related to increased cannabis misuse (both marijuana problems and cannabis use disorder symptoms). These effects persisted after controlling for anxiety, depression, and stress. In addition, the specific feature of obsessing was found to consistently predict cannabis misuse. Finally, an indirect effect of severity of OCD on cannabis misuse via coping motives was discovered. Together, these findings indicate that there is an association between OCD and cannabis misuse that is independent of anxiety, depression, and stress and that is partially driven by the use of cannabis to cope with negative affect and other problems. Based on these findings, we recommend that individuals with OCD symptoms avoid using cannabis, especially as a coping mechanism, because they may be more vulnerable to the development of problematic use and cannabis use disorder. In other words, cannabis may not be a viable treatment option for managing the symptoms of OCD, and using cannabis to cope may function as more of a "Band-Aid," rather than addressing the underlying mechanisms maintaining the symptoms of the disorder.

THE ROLES OF SEX, COPING MOTIVES, AND NEGATIVE AFFECT IN THE RELATIONSHIP BETWEEN STRESS AND CANNABIS USE

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Cannabis is widely reported to produce feelings of relaxation and tension reduction, and many individuals report using cannabis to cope with negative affect. Consistent with these reports, a link between stress and cannabis has been established in previous research. However, the roles of several important extraneous variables in this link have not been examined. To our knowledge, the present study is the first to examine whether sex moderates the relationship between stress and cannabis and whether coping motives (i.e., using cannabis to cope with negative affect and problems) mediate the stress-cannabis link. Additionally, we sought to examine whether negative affect mediates the relationship between stress and cannabis.

A sample of 573 young adult cannabis users (i.e., individuals who had used cannabis at least once within the last 3 months) completed an online survey containing established measures of early life stress, chronic stress, frequency of cannabis use, cannabis problems, motives for using cannabis, depressive symptoms, and symptoms of anxiety. The results revealed that early life stress, but not chronic stress, was related to increased frequency of cannabis use. Further, both early life stress and chronic stress were related to increased cannabis problems. Moderation analyses revealed that the relationship between chronic stress and cannabis problems was present in both sexes but was significantly stronger in males. In contrast, the relationship between early life stress and frequency of cannabis use was significant in females only. We then conducted mediation analyses, controlling for sex, to determine whether a latent negative affect variable (comprising symptoms of depression and anxiety) functions as a mediator of the stress-cannabis link. Results revealed that negative affect mediated the relationship between chronic stress and cannabis problems and between early life stress and cannabis problems, but not between early life stress and frequency of cannabis use. Finally, we tested the role of coping motives as a mediator of the relationship between stress and cannabis, controlling for sex. Results revealed that coping motives mediated the relationships between chronic stress and cannabis problems, early life stress and cannabis problems, and early life stress and frequency of cannabis use.

Findings from the present study reveal the important roles of sex, negative affect, and coping motives in the link between cannabis and stress. Specifically, findings from the present study suggest that chronic stress is related to increased cannabis problems, and that this link is especially strong in males. Further, early life stress may have long-term consequences that lead to increased cannabis use in females. We also identified negative affect as an important component of the stress-cannabis link, with evidence suggesting that both chronic and early life stress lead to increased levels of negative affect, which in turn leads to increased levels of cannabis problems. Moreover, we found evidence that both chronic stress and early life stress predict increased use of cannabis for purposes of coping, and that this, in turn, leads to increased cannabis problems and frequency of cannabis use. The findings help to elucidate the conditions under which stress may lead to cannabis use as well as the mechanisms through which stress exerts its effects.

MARIJUANA STRAIN CHEMOTYPES ELICIT DISTINCT SUBJECTIVE FEELINGS

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Inhaling vaporized marijuana flower extract elicits a feeling of euphoria that sometimes includes nuanced states of mind such as creativity, relaxation, and liveliness. We investigated marijuana's potential to invoke states of mind in users by measuring subjective feelings before, and at two time points after, subjects inhaled vaporized flower extracts for each of three marijuana strains with diverse chemotypes. Data from our double-blinded psychopharmacological studies suggests that each chemotype elicited a high with distinct feelings, and that specific feelings measured from one chemotype differed from feelings measured from another chemotype. Sets of feelings that scored most strongly helped identify prominent states of mind that were raised by each chemotype. Our results encourage the formulation of purified cannabinoids and terpenes for eliciting user-desired states of mind.

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CANNABIS USE FREQUENCY AND MOOD ON CREATIVITY

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This study examines the relationship between cannabis use (infrequent, moderate, and heavy use) and one's mood (neutral, positive, and negative) on creativity. Folk ideas of creativity and the relationships between cannabis use and mood may not reflect the real relationship between these factors (e.g. regarding cannabis use, it is *perceived* to be linked with higher rates of creativity; regarding mood, negative states (i.e. tortured artist) are thought to fuel creativity). Although both cannabis use (Chermahini & Hommel, 2010) and mood (Baas, De Dreu, & Nijstad, 2008; Davis, 2009) have been found to influence creativity independently, the current study is unique in its aims to identify whether cannabis use and mood *interact* to influence one's creativity.

The relationship between cannabis and creativity is not linear and is informed by dopamine levels. Cannabis is linked to increased dopamine levels (Sami et al., 2015), which may therefore influence creativity (Kowal et al., 2015). In fact, the relationship takes an inverse U-shaped pattern, where too little and too much dopamine is associated with low levels of creativity, while a moderate amount of dopamine is associated with the highest levels of creativity, as tested through the creativity measure of the Alternative Use Task (AUT) (Chermahini & Hommel, 2010). The AUT is a measure of a subset of creativity, known as divergent thinking. In this task, one's ability to "think outside the box" is measured by the number of alternate uses of common objects produced by participants. In the area of mood, it has been found that any mood state, outside of neutral, is associated with increased creativity (Baas, De Dreu, & Nijstad, 2008; Davis, 2009). Thus, we predict that infrequent users will perform better in positive and negative moods and worse in neutral. Moderate users will perform best overall, particularly in the neutral mood. Heavy cannabis users will perform better in neutral, compared to positive and negative moods.

Participants (n=219) engaged in an AUT over three different mood blocks (neutral, positive, and negative), where mood was induced via sound stimuli. The first block (neutral) presented white noise, while the second and third blocks presented positive and negative music (Koelsch, Fritz, Cramon, Müller, & Friederici, 2006), counterbalanced. Divergent thinking was measured by the number of alternative uses for common objects produced by the participants. The AUT was followed by a cannabis use survey and the Creative Achievement Questionnaire (Carson, Peterson, & Higgins, 2005).

Although no significant interaction or main effects of cannabis use frequency and mood was found, post hoc analysis of the survey data suggest self-report creativity and one's education level are linked to higher rates of creativity. Post-hoc analyses also suggest that being under the influence of cannabis while engaging in divergent thinking tasks does not lead to higher rates of creativity. Limitations to this study include a failed manipulation check of mood inducement Future research directions and implication of of this study will be discussed.

ASSOCIATIONS BETWEEN MICROBIOTA, MITOCHONDRIAL FUNCTION, AND COGNITION IN CHRONIC MARIJUANA USERS

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Marijuana (MJ) use is associated with cognitive deficits. Both mitochondrial (mt) dysfunction and gut dysbiosis were also reported to affect cognition. We examined whether cognition is related to peripheral blood mononuclear cells' (PBMCs) mt function and fecal microbiota in people who chronically used MJ. Nineteen chronic MJ user and 20 non-users were evaluated using the Cognition Battery in NIH Toolbox, their mt function for ATP production, and basal and maximal respirations were measured in PBMCs using the Seahorse XFe96 Analyzer, and the abundances of *Prevotella* and *Bacteroides* (associated with plant-based and animal product-based diet, respectively) were calculated from stool microbiota analysis. *Prevotella*:*Bacteroides* ratio was ~13-fold higher in nonusers than users (p=0.06), and both *Prevotella*:*Bacteroides* ratio (p=0.09) and mt function (p=0.0027-0.0057) inversely correlated with lifetime MJ use. *Prevotella* abundance positively, and *Bacteroides* abundance inversely, correlated with mt function in all participants (p=0.0004-0.06). *Prevotella* abundance correlated positively with scores of fluid cognition, flanker inhibitory control and attention, list sorting, and dimension change card sort in MJ users, but not in non-users (interaction-p=0.018-0.05). Similarly, mt function correlated positively with scores of fluid cognition and flanker inhibitory control and attention in MJ users, but not in non-users (interaction-p=0.0018-0.08). These preliminary findings suggest that MJ use is associated with alterations of gut microbiota and mt function, which further associated with cognition; and MJ-associated low vegetable and fruit intake may contribute to these changes. Longitudinal studies are needed to further delineate the relationships among diet, microbiota, mt, and cognition in the context of MJ use.

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IMPLICIT CANNABIS COGNITIONS AND EARLY USE AMONG CANADIAN ADOLESCENTS

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Cannabis is the most commonly used drug in the world (Rehm & Fischer, 2015). In 2015, the Government of Canada committed to legalizing and regulating cannabis in the near future (Government of Canada, 2016). Canadian adolescents report using cannabis more than any other illicit substance (George & Vaccarino, 2016), with 22.4% of youth aged 15 to 19 reporting past-year usage (Statistics Canada, 2015). An emerging body of research suggests that early cannabis use can lead to safety risks (Young & Jesseman, 2014), poorer mental health outcomes (Lev-Ra et al., 2014), and an increase in the use of other drugs (Silins et al., 2014). In light of current use and potential harms, it is imperative to understand the factors that influence cannabis use among adolescents.

Much research has established that changes in youths' cognitions about substances accompany and precede changes in substance use (Fulton, Krank, & Stewart, 2012). Recent findings highlight the role played by implicit cognitions in early substance use initiation (Payne, Lee, Giletta, & Prinstein, 2016). Past research has shown that youth who show positive implicit associations to alcohol and cannabis are more likely to initiate use in the near future (Fulton et al., 2012). Further, research shows that social influence from parental or peer use changes implicit cognitions and mediates the future initiation of substance use (Payne et al., 2016).

The present study examined social cognitive factors that predict early initiation of cannabis use. The primary goal of this research study was to examine implicit cognitions that youth hold regarding cannabis. This study measured social influences and predictors of cannabis use in youth and is unique in looking at the implicit affective reactions of youth to brief presentations of substance-related pictures.

We recruited 535 eighth grade students from two school districts in British Columbia. Implicit cognitions were measured using the affect misattribution procedure (AMP) (Payne & Lundberg, 2014). This procedure measured implicit reactions to visual stimuli through affective misattributions. The reliability and validity of this procedure is well-established with other stimuli, including alcohol-related images (Cameron, Brown-Iannuzzi, & Payne, 2012). Though widely used, the AMP has never been used to explore implicit attitudes about cannabis. Attitudes toward substance use, social norms, and intention to initiate using cannabis were also measured. Preliminary analyses suggest that adolescents with increased affective activation to visual cannabis cues positively correlate with current use of cannabis and will predict changes in patterns of use.

To our knowledge, this study is the first to use the AMP to predict the pattern and trajectory of cannabis use in adolescents. Complete data analysis is forthcoming. Comprehensive results, implications, and directions for future research will be discussed.

SUBSTITUTING CANNABIS FOR ALCOHOL: CONTEXTUAL FACTORS AND REASONS FOR PREFERENCE

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Recent examinations of substance use patterns in medical cannabis users have identified a "substitution effect", whereby patients report substituting cannabis for alcohol and prescription medication. However, little research has examined substituting cannabis for alcohol among college students who use cannabis in a nonmedical setting. The characterization of cannabis substitution for alcohol among this group is important given high levels of problematic alcohol use among college students.

This study examined substituting cannabis for alcohol among undergraduate students who reported using both cannabis and alcohol within the past 6 months (N=569). Overall 26% (n=145) reported that they preferred cannabis to alcohol, and 52% (n=297) reported that they preferred alcohol to cannabis. Approximately 22% (n=127) indicated no preference between alcohol and cannabis. Using cannabis to purposely reduced drinking was reported by 7% (n=40) of respondents. Purposeful substitution of cannabis for alcohol was associated with higher levels of problematic alcohol use as measured by the AUDIT ($r = .17$, $p < .001$), suggesting that active substitution may reflect attempts to reduce problematic behavior. With regard to concurrent use of cannabis and alcohol, 49% (n=278) reported drinking less when using cannabis and 2% (n=10) reported that cannabis use was associated with craving alcohol.

Among those who reported an overall preference for cannabis reasons for choosing cannabis over alcohol included: "because it is safer" (50%; n=71), "because I prefer the feeling" (72%; n=104), and "to avoid hangovers" (55%; n=78). Among those who reported a preference for alcohol, the top reasons, whereas reasons for choosing alcohol over cannabis included: "because the effects are more predictable" (47%; n=138), "because I can use it in public" (46%; n=134), and "because it is legal" (43%; n=126). Regarding contextual factors that may impact preference, 58% of all participants indicated a preference for cannabis on a night in alone; only 7% would choose cannabis on a night out with friends, and 28% indicated a preference for cannabis on a night in with friends.

The findings add to our understanding of factors that influence cannabis substitution for alcohol in a nonmedical context, and suggest that cannabis use may reflect efforts to reduce alcohol-related harms among college students. Longitudinal research is warranted to better understand the public health consequences of cannabis substitution in this context.

CANNABIS USE IN INDIA: CHARACTERISTICS, ACCESS, AND REASONS FOR USE

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Cannabis has been used in India for thousands of years for spiritual and therapeutic purposes (Russo, 2005). Although it is part of the traditional Indian medical (i.e., Ayurvedic) pharmacopeia most forms of cannabis are now illegal except for an edible preparation of the leaves (i.e., bhang) that can be purchased from government-licensed shops (Chandra & Chandra, 2015). Despite widespread and ancient use there is a paucity of research examining the parameters of contemporary cannabis use in India. We report findings from a cross-sectional study of medicinal and non-medicinal cannabis use in India.

We examined motives for cannabis use, patterns of use, modes of access, and substitution of other drugs among cannabis users in India ($N = 100$). Participants aged 22-69 ($M = 32.2$, $SD = 7.5$; 18% female), were recruited through Amazon's Mechanical Turk (AMT)—an internet crowdsourcing marketplace. All participants had used cannabis in their lifetime and 70% ($n = 70$) reported currently using cannabis (e.g., ganja/bhang/charas).

Participants had several different motivations for using cannabis. A majority (59%) indicated they consumed cannabis to celebrate holidays or sacred events (i.e., Holi) followed by the use for cultural tradition (28%). Purchasing cannabis from a friend was the most often cited source (49%), however some purchased cannabis from a store (23%), while others collected it from the wild (6%). Findings in accordance with the only legally available form of cannabis indicated that most participants consumed cannabis in a liquid (e.g., bhang lassi) or food (63%) while others mixed it with tobacco (32%). Consistent with the Indian Hemp Drugs Commission (1894) the majority reported occasional use, 3 or less times per month (66%) with less than a third (29%) using multiple times per week. In regards to substitution, respondents indicated preference for cannabis over alcohol, and reported using less alcohol when using cannabis. These findings are especially relevant given the high rates of alcohol-related problems and death in India (Prasad, 2009). Finally, the majority of respondents reported feeling comfortable discussing cannabis for therapeutic purposes with their physician despite the illegality of cannabis use and indicated they rarely or never felt discriminated against by their physician for using cannabis (63%).

To our knowledge this is the first study to examine cannabis use patterns and substitution for alcohol, nicotine, and pharmaceuticals in India. Our findings indicate that in many cases the division between social and therapeutic use is blurred. Cannabis is linked to traditional medical, cultural, and spiritual practices which may explain in part why individuals in India who use cannabis report relatively low levels of discrimination. Detailed comparisons to a North American sample are forthcoming.

IDENTIFYING HIGH CBD CANNABIS STRAINS FOR EPILEPSY TREATMENT

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Epilepsy is the most common and serious brain disorder worldwide which can afflict people of any age, ethnic, social, or geographic category. There are many treatments available (BNF, 2011) but all have notable side effects (Ortinski and Meador, 2004; Schachter, 2007) and 30% of cases remain pharmaco-resistant, resulting in poorly controlled seizures (Hitiris et al., 2007). The *Cannabis species Cannabis sativa* and *Cannabis indica* have been documented as useful agents to treat epilepsy for centuries (Devinsky et al., 2014). However, interest in this area increased in 2013, after reports of children who experienced benefits from treatment with cannabidiol (CBD)-rich extracts of *Cannabis* (Maa and Figi 2014). Recently in Israel, the use of cannabis as an anticonvulsant was introduced as a treatment option for pharmaco-resistant children suffering from epilepsy. These pharmaco-resistant epileptic children are treated with cannabis extracts enriched with CBD, however, while some extracts show anticonvulsant properties other do not. Cannabis oil extracts contain more than 140 different cannabinoids and other biologically active compounds that have different pharmacological properties; however, most of these compounds have largely been ignored by researchers and medical professionals when choosing a specific strain to treat epilepsy.

The aims of this research is to first identify the cannabinoids in the different strain of cannabis used for the treatment of epilepsy in Israel; the second aim is to evaluate the potential use of these differing *Cannabis* strains as anticonvulsants. In order to accomplish the first aim we are utilizing mass-spectrometry, coupled to liquid chromatography (LC-MS), to comprehensively profile the cannabinoid composition for a variety of *Cannabis* strains that are clinically used in Israel. After profiling the cannabinoids we are evaluating their anticonvulsant effects using the pentylenetetrazol (PTZ) test–a validated model to identify drug treatments that are effective in the treatment of epilepsy in humans. We have found that not all high CBD extracts have the same anticonvulsant ability. We are also finding that the terpenoid content in the cannabis extracts are important for the anticonvulsant effect of the high CBD extracts. These results suggest that not all cannabis extracts will be useful as a treatment for epilepsy and that the exact cannabinoid and terpenoid profiles are needed to evaluate the potential anticonvulsant properties of a cannabis extract.

BETTER THERAPEUTIC PROFILE OF CBD-ENRICHED EXTRACTS OVER PURIFIED CBD IN TREATMENT-RESISTANT EPILEPSY: OBSERVATIONAL DATA META-ANALYSIS

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Different therapies including cannabinoid compounds have become popular for treatment-resistant epilepsy in the recent years, particularly when it comes to hard-to-treat diseases, like Dravet and Lennox-Gastaut syndromes. Much of these were due to widely known clinical cases like the Charlotte Fiji and Sam Vogelstein, widely reported in media. This was the beginning of a rush of small-scale enterprises to produce "*Cannabis* oils", followed by independent initiatives of patients and patient associations, which decided to produce the "oils" by themselves. While parental reports of efficacy are available in the scientific literature, robust clinical data-driven pharmaceutical development is just recently being gathered and published. The current study systematically evaluated all the clinical data available in the scientific literature for cannabinoid treatment in treatment-resistant epilepsy in children. The aim is to shine some light on the currently controversial question *of whether "Cannabis-based extracts" or "purified/synthetic compounds" would be a preferential therapeutic approach.* Results: Systematic screening of papers in PubMed with the keywords "Dravet" "Lennox-Gastaut" and "epilepsy" combined with "Cannabis", "cannabinoid" and "child" revealed 30 papers. Out of these, 24 were not considered for the systematic review because: they didn't have abstract (13), were just opinion papers (6), reported non-clinical data (4), reported other subjects (1), ending up with 6 valid papers published from 2013 to 2016. Another recent *in press* paper was added manually (1), resulting in (7) valid references for the analysis, with mean impact factor 5.9 (2.3 - 21.8). Public data from partial RCT research reports (2) were also considered, when appropriate. Categorical data was analyzed with Fisher's exact test. The papers report clinical observational data from 442 patients treated with CBD-enriched extracts or "purified" CBD, daily dose of 1 to 50 mg/kg, with treatment duration ranging from 3 to 12 months (avg: 6.2 mo). Sixty-six percent (292/442) of the patients reported improvement in seizure frequency. There were more reports of "improvement in seizures frequency" in CBD-enriched extracts compared to purified CBD (224/285 vs. 68/157; $p<0.0001$). However, when a standard clinical relevant threshold of "reduction of 50% in seizures frequency" is considered, only 40% of the individuals are considered responders and there is no difference between treatments (64/168 vs. 65/157, $p=0.57$). Although either treatment yielded equivalent efficacy, CBD-enriched extracts patients reported significantly smaller daily oral doses, on average 7.1 mg/kg/day vs. 22.9 mg/kg/day for purified CBD. Curiously, as far as this data is considered, there was a superior efficacy (regardless of treatment) for Dravet syndrome (37/72, $p=0.01$), but not for Lennox-Gastaut syndrome (78/188, $p=0.18$), compared to the number of responders with refractory epilepsy in general (107/305). When it comes to adverse effects report, there is also a major advantage for CBD-enriched extract over purified CBD for both light (109/285 vs. 291/346, $p<0.0001$) and serious (23/285 vs. 77/346, $p<0.0001$) adverse effects. Importantly, these are all adverse effects reported and specific drug-related adverse effects could not be sorted out. In conclusion, the meta-analysis suggests that treatments using CBD-enriched extracts have higher potency and a better side effects profile (but not higher efficacy) than purified CBD, at least for this group of treatment-resistant epilepsy patients. The current lack of standardization of *Cannabis*-based extracts doesn't allow one to infer about the characteristics of the product that renders these therapeutic advantages, but they are probably related to additional compounds available in the extracts (other than CBD) that may interact synergistically. Well-controlled studies with standardized *Cannabis* extracts are needed to confirm these observations.

HOW SAFE ARE THC AND CBD FOR EPILEPSY? INSIGHTS FROM A PRECLINICAL SYSTEMATIC REVIEW

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The current rush for *Cannabis*-based anti-epileptic medicines has been preceded almost by a decade by preclinical data, showing that CB_1 receptors are important for the regulation of neuronal excitotoxicity, associated with epilepsy (Monory et al, 2006; Marsicano and Lutz, Science). Even before the putative mechanism of action was discovered, phytocannabinoids like CBD and THC were already considered potential candidates for anti-epileptic drugs, as far as animal data can predict therapeutic utility in humans (Consroe and Wolkin, 1976; Turkanis et al, Epilepsia 1979; Consroe et al, Carlini, 1982).

Recent evidence shows that CBD-based therapies can be useful in some forms of epilepsy, especially in treatment-resistant forms associated to syndromes, such as Dravet. For instance, there are reports of positive clinical outcomes ranging from 5 to 25 mg/kg, p.o. of CBD in treatment-resistant pediatric epilepsy. While THC is not considered an anti-epileptic drug on its own, it is often present in *Cannabis*-based medicines, at different ratios with CBD. The widest range available in Cannabis plants are 30:1 CBD:THC, such as Bedrolite® varieties from Bedrocan, that our company (Entourage Phytolab) is using as raw material to develop standardized extracts in Brazil. The objective of this review was to define whether the composition of CBD/THC exert a reasonable safety profile in determined therapeutic doses, as far as regulatory pre-clinical safety studies are regarded. Per recent meta-analysis of available clinical data (Pamplona and Coan, in press), the average therapeutic dose of oral *Cannabis*-based medicines is equivalent to 7mg/kg CBD (along with 0.2mg/kg of THC); as opposed to 22mg/kg p.o. CBD in purified form. Oral administration of these doses yields approximately 30ng/ml of CBD (95nM) and 4ng/ml of THC (13nM). To enter regulatory clinical studies in Brazil, the pharmaceutical product needs to conform with the following preclinical studies: acute and chronic toxicity (plus lethality), safety, pharmacology, genotoxicity, carcinogenicity, cytotoxicity and reproductive toxicity. Therefore, we reviewed all the preclinical data available in the scientific literature, yielding a total of 168 valid original references containing preclinical safety data for CBD and/or THC, to understand whether *Cannabis*-based product would present a suitable therapeutic index. (Jacob and Nair, 2016) was used for animal vs human dose conversion. Therefore, converting from our animal data we defined (84mg/kg p.o. of CBD and 2.8mg/kg p.o. of THC) as a "therapeutic-like" dose for mice (and half this value for rats, according to the conversion table). This range is among the valid range for positive results in epilepsy-like experimental models, confirming that the converted dose might be used for a direct comparison with potential toxicologic events. Per the literature, CBD was considered "safe", with potential mild adverse events occurring at doses higher than 100mg/kg p.o. and severe adverse events occurring at doses higher than 1000mg/kg p.o. Cytotoxicity would occur at 4000 to 32000nM (1258 to 10062 ng/ml), apart from immunological cells, which function could be dampened at lower than 100nM (31,45 ng/ml), presenting a potential risk (mouse doses). For THC, mild adverse events could occur at doses 7-18 mg/kg p.o. and reduced fertility at doses 10-100mg/kg p.o., while serious adverse events occur at 150mg/kg p.o. and higher doses, including potential testis/uterus atrophy. Cytotoxicity would occur at 5000 to 40000nM (1572 to 12578 ng/mL) and genotoxicity at 5000nM (1572 ng/mL). Both molecules are considered non-lethal, since many studies have difficulties defining a lethal dose in rodents and non-rodent species. The calculated therapeutic window for a product containing 30:1 CBD:THC is in the range of 3-10 times for mild adverse events and 30-50 times for serious adverse events without risk of lethality, considering average doses, as presented above. This therapeutic index is equal or superior than most of the drugs currently used for anti-epileptic treatment, like phenytoin, phenobarbital, and carbamazepine. Therefore, the proposed *Cannabis*-based treatment (30:1 CBD:THC as in Bedrolite) may be considered reasonable, considering that human beings would be exposed to a maximum of 300mg CBD + 10mg THC, when it comes to the expected treatment dose for epilepsy. This is considered a low dose of THC, devoid of relevant psychoactive effects when administered via oral dose (Wachtel et al, 2002). Benefits and potential risks shall be weighted, considering the neurodevelopmental cost of untreated epilepsy.

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THE PELICAN STUDY: THE PERCEIVED EFFICACY AND CANNABINOID CONTENT OF ARTISANAL CANNABIS OILS USED TO TREAT CHILDHOOD EPILEPSY IN THE AUSTRALIAN COMMUNITY

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Recent clinical and preclinical evidence indicates the efficacy of various cannabinoids in seizure reduction, with cannabidiol (CBD) notably effective in treating various forms of intractable childhood epilepsy. However, there are currently no registered cannabinoid medicines that can be prescribed in Australia for the treatment of epilepsy. This has driven desperate families to illegally source cannabis oils and tinctures to manage the seizures of family members, with a recent survey indicating that 13% of Australian families with a child with epilepsy are following this approach (Suraev et al., Epilepsy Behav. (2017)). Media coverage and other anecdotal reports suggest somewhat remarkable efficacy of these oils and tinctures. Interestingly, street cannabis in Australia tends to contain very little CBD, which invites the question of whether other cannabinoids present in these artisanal preparations are providing important antiepileptic effects.

The PELICAN study was established to liaise with families who are currently using cannabinoid preparations for their child with epilepsy, as well as those who have previously used and stopped, and those who have never used cannabis to treat their child's epilepsy. A semi-structured interview was utilised to probe issues around the safety and efficacy of the products being used, key issues facing families in their decisions to try cannabis, reasons for and against trying cannabis, and concerns surrounding disclosure to health professionals. Samples of the artisanal cannabis oils and tinctures were collected and analysed for 11 different cannabinoids using tandem liquid chromatography-mass spectrometry (LC-MS/MS).

Of the 39 families interviewed to date, 20 were currently using cannabis products, 3 had previously used and stopped, and 15 had never used cannabis to treat their child's epilepsy. Treatment-resistant epilepsy was reported in 83% (19/23) families who had tried cannabis products, but in only 40% (6/15) of the control families that had never used cannabis. A large proportion of currently using families reported beneficial effects of cannabis on their child's condition, with several reporting reduction or cessation of the use of conventional antiepileptic drugs after initiating cannabis.

A total of 37 cannabis products were collected, with some families supplying multiple products that had been used with the same child. A total of 95% (32/37) of products had been locally sourced in Australia. A wide variety of cannabinoid profiles were identified in these products, including THC dominant, THCA/THC combined, CBD only, and THCA/CBDA combined. The cannabinoid content of samples perceived as "effective" (>50% reduction in seizures) showed notable variability, ranging from 1.92 to 146.4 mg/g. The PELICAN study is continuing to recruit families and further, more comprehensive, results will be presented at the time of the conference.

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CHRONIC, BUT NOT ACUTE, INHIBITION OF ABHD6 RESCUES SELECT BEHAVIORAL SYMPTOMS IN THE HdhQ200/200 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Huntington's Disease (HD) is a devastating inherited autosomal dominant neurodegenerative disease characterized by progressive deterioration of motor and cognitive functions with no known cure and few palliative treatment options available. The HdhQ mouse model was developed by inserting pathogenic-sized CAG repeats into the *Hdh* gene, the mouse homolog of the HD gene. Our longitudinal studies of behavioral phenotypes and histology on the HdhQ200/200 model, a mouse line containing 200 CAG repeats, revealed disease onset as early as 6 months of age and end stage at approximately 12 months of age. We discovered that female and male HdhQ200/200 animals showed differential deficits in weight, grip strength, motor coordination, exploration, and circadian activity by 8 months of age. Analysis of pathology by semi-quantitative immunohistochemistry revealed reduced DARPP-32 and CB_1R expression at as early as 10 months of age. Using *in vivo* calcium imaging with GCaMP calcium indicator technologies, we found pronounced alterations in activity-dependent calcium dynamics in medium spiny neurons in 8- to 10-month-old HdhQ200/200 mice, indicating pronounced hyperexcitability in these neurons. Previous work suggests that chronic cannabinoid treatments may alleviate behavioral and pathological deficits seen in other HD models. We tested KT-182, a novel ABHD6 inhibitor, at the age of 8 months to examine if blocking this enzymatic activity and increasing select lipid signals, including 2-AG, was sufficient to rescue behavioral and pathological symptoms. While a single, acute treatment did not rescue any behavioral phenotypes, a one week chronic treatment of continuous infusion of KT-182 through mini-osmotic pumps did significantly improve motor coordination in HdhQ200/200 mice. Our next set of experiments will determine if chronic ABHD6 inhibition can also restore aberrant hyperexcitability as measured by calcium imaging. Our study will disclose detailed behavioral and pathological changes in the HdhQ200/200 mouse line and whether ABHD6 inhibition can attenuate these changes.

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RARE GENETIC VARIANTS IN ECS GENES ARE ASSOCIATED WITH NEUROLOGICAL PHENOTYPES

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Courtagen provides genetic testing services for patients with epilepsy, developmental, functional and mitochondrial disorders. Patients who receive such testing typically present with a wide variety of different phenotypes. We have tested over 6,500 such patients using comprehensive DNA sequencing panels representing up to ~1000 genes, which also include several genes encoding components of the endocannabinoid system. From this data set, we identified rare genetic variants in the core ECS genes: CNR1, CNR2, DAGLA, MGLL and FAAH, which encode CB1, CB2, diacylglycerol lipase alpha, monoglyceride lipase, and fatty acid amide hydrolase, respectively. Heterozygous rare variants in CNR1 were found to be significantly associated with headache or migraine, anxiety and sleep disorders compared to a set of 950 randomly selected patients without such CNR1 variants (P-value: 0.01, 0.04 and 0.01 respectively). Similarly, heterozygous rare variants in DAGLA were found to be significantly associated with seizures, developmental disorders and abnormalities of brain morphology (P-value: 0.04, 0.01 and 0.01, respectively). Rare variants in MGLL, FAAH and CNR2 were not found to be significantly associated with any neurological phenotypes in the patients tested.

The phenotypes associated with rare CNR1 variants are similar to those implicated in the theory of clinical endocannabinoid deficiency syndrome (migraine, fibromyalgia, stress induced anxiety and irritable bowel syndrome). This, in conjunction with previously published studies on the treatment of patients with such phenotypes, suggests that AEA, THC or other CB1 agonists may serve as effective therapeutic treatments for patients with such variants. While the individual variants may be rare, it is likely that patient populations with disorders of this nature will be enriched in them. The severe phenotypes associated with rare DAGLA variants underscore the critical role of rapid 2-AG synthesis and the endocannabinoid system in regulating neurological function and development. We anticipate that additional studies along these lines to investigate additional ECS-related genes and downstream effectors will lead to further insights into these fundamentally important pathways.

THE SELECTIVE CB2 AGONIST O-1966 ATTENUATES DAMAGE FOLLOWING TBI INDUCED BY BLAST INJURY

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The damage that occurs following traumatic brain injury (TBI) is the result of both the mechanical insult and the secondary injury that occurs in response to the mechanical insult. While CB2 agonists have been shown to be neuroprotective following multiple types of insult to the central nervous system, the mechanisms of action still remain unclear. Blast injury is a common cause of TBI in military personnel. The results of blast injury can be extremely devastating and can progress over days, weeks, months and even years. The goal of this investigation was to determine if administration of a selective CB2 agonist O-1966 could attenuate secondary injury following shock wave induced TBI and therefore minimize the progression of damage. The effect of administration of the CB2 agonist was tested on single and repeated exposure of rats to a pressure wave of 36 PSI to the head. O-1966 (2.5mg/kg) was administered immediately following, 24 hours after injury and on days 3 and 5 post-injury. Rats were euthanized on day 7 and their brains harvested for immunohistochemistry and Western blot analyses. O-1966 significantly inhibited hyperphosphorylated Tau in the rat cortex and in plasma, and significantly attenuated microglial activation. The attenuation of microglial activation may play an important role in mitigating the inflammatory contribution to secondary injury. The decrease in hyperphosphorylated Tau accumulation presents interesting implications for potential treatment of Chronic Traumatic Encephalopathy.

THE ACTIVITY OF CANNABINOID RECEPTORS MODULATES SYNAPTOGENESIS

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Due to their presence at the fetal and early postnatal periods, endocannabinoids (eCBs) have been proposed to play a major role in developmental processes. Through their two main receptors (CB1R and CB2R), eCBs participate to axon guidance (Argaw et al., 2011; Duff et al., 2013). Because several molecules that direct neurite extension also regulate synapse formation, we investigated a novel function for the eCBs and their receptors modulating synaptogenesis.

The present study was performed on mouse cortical neurons *in vitro,* a simple model convenient to assess a complex phenomenon such as synaptogenesis. CB1R and CB2R activity was modulated either with pharmacological agents or genetic tools (knockout mice). Cellular and molecular experiments such as immunocytochemistry were complemented with functional assays including patch clamp recordings.

A pharmacological approach combined with immunocytochemical analysis showed an increase of synaptic precursors and contacts on neurons treated with inverse agonists of CB1R, and conversely, the agonists induced a decrease. In the case of CB2R, our results suggest dual effects depending on short- or long-term modulation of receptor activity. To address whether CB1R and CB2R modulate the number of functional synapses, we used the FM1-43 assay and we recorded mini excitatory postsynaptic currents. Our results suggested that anatomical modulations of synaptic contact density are related with modifications of the synaptic function.

Identification of mechanisms that underlie synapse formation remains a fundamental question in neuroscience and will help in developing cures aiming at the treatment of neurodegenerative diseases. Otherwise, the effects of cannabis consumption on the developing nervous system are not fully understood, and the present work could provide potential mechanisms of action.

IMPROVED SYNTHETIC APPROACH OF PM226, A SELECTIVE CB2 CANNABINOID AGONIST WITH A NEUROPROTECTIVE PROFILE

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The chromenoisoxazole PM226 was previously discovered by us in studies that combined molecular modeling and structure-activity relationships of a series of chromenopyrazoles.[1] Bioisosteric replacement of the pyrazole by an isoxazole led to the most promising selective CB2 ligand (PM226) of the series. PM226 showed to be efficient in reducing microglia activation in an animal model of primary progressive multiple sclerosis (TMEV-IDD).[1] Its neuroprotective profile was also confirmed in an in vivo model of mitochondrial damage generated by intrastriatal application of malonate in rats.[2]

Although the preparation of PM226 has been described, considerable synthetic problems could be encountered along the synthetic route. Considering the therapeutic value of PM226, we decided to elucidate the key features of every synthetic step for an improved preparation of PM226. Reaction conditions for α–formylation of a chromanone, for condensation of a β-ketoaldehyde with hydroxylamine, and for *O*-methylation of the chromenoisoxazole revealed to be crucial for an efficient synthesis of the chromenoisoxazole PM226 and its derivatives.

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NON-STEROIDAL PREGNENOLONE ANALOGS AS SIGNAL SPECIFIC ALLOSTERIC MODULATORS OF THE CB1 RECEPTOR

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Pregnenolone, an endogenous negative allosteric modulator of the CB1 receptor, modulates BArr-mediated signaling, but does not affect the G-protein mediated pathway. The proposed binding site (TMH1/Hx8 exosite), previously identified through a fragment based MMC approach and receptor mutation experiments, is in an elbow region reported to move during BArr signaling. Pregnenolone has also been shown to protect the brain from cannabis intoxication. Further investigations regarding this endogenous ligand and its binding site are of particular interest.

We have designed a series of non-steroidal pregnenolone analogs with improved Lipinski parameters that can fit the pregnenolone binding site. A synthetic route lending access to four ligands with modified hydrogen bond donor and acceptor positions was developed. The analogs should decrease ERK signaling while having a minimal cAMP effect and they should reduce effects of Δ^9-THC if they are indeed acting as pregnenolone analogs.

Computational studies, the synthesis of these ligands and their pharmacological characterization will be presented.

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THE ENDOGENOUS HORMONE H4(99-103) IS THE ONLY KNOWN PEPTIDE THAT SIGNALS VIA CANNABINOID RECEPTOR CB2

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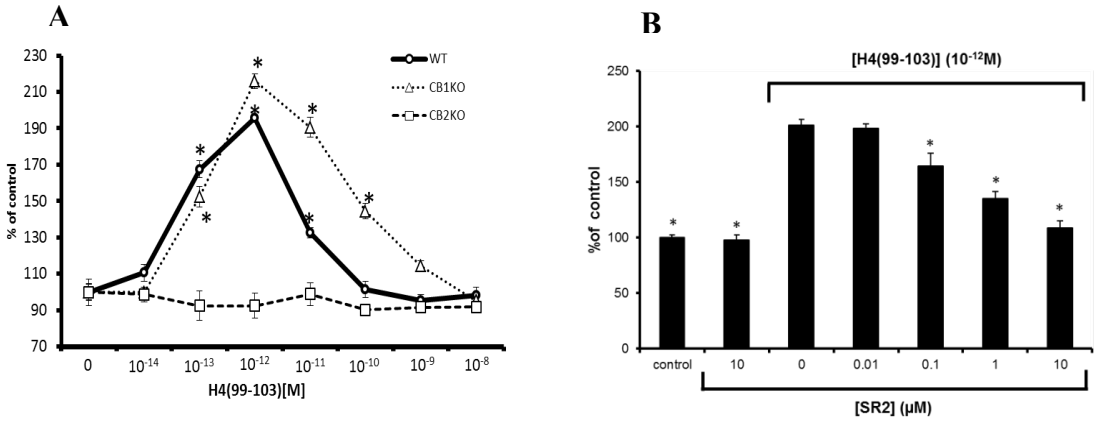
The endocannabinoid (EC) system consists of anandamide and 2-arachidonoylglycerol, their metabolizing enzymes, and CB1 and CB2 cannabinoid receptors. Studies of the skeletal EC system in mice and humans suggest the occurrence of a CB2 tone, protecting the skeleton against age-related bone loss. Because fatty acid derived ECs are short-lived neurotransmitter-like compounds synthesized 'on demand', we propose for the first time that an endogenous circulating peptide activates CB2. H4(99-103) is a pentapeptide hormone present in the circulation at significant concentrations. We tested its activity on WT and CB2-deficient cells and mouse models, and in human-derived osteoblasts. Like selective CB2 agonists, H4(99-103) triggers a proliferative dose-dependent response in osteoblasts, inhibitable by genetic or pharmacological ablation of CB2. In addition, H4(99-103) restrains *ex vivo* osteoclastogenesis and induces osteoclast apoptosis in WT but not in CB2-deficient cells. *In vivo*, H4(99-103) and CB2 agonists markedly decreased xylene-induced ear swelling measured manually and histologically. This effect on acute inflammation was completely absent in $CB2^{-/-}$ mice. H4(99-103) also demonstrated a CB2-dependent inhibition of macrophage inflammatory response. To demonstrate binding, we show that H4(99-103) suppressed forskolyn-induced cAMP levels in CHO cells expressing only CB2. Docking simulation suggests that H4(99-103) binds to CB2 at an extracellular allosteric site, in line with our [^{35}S]GTPgammaS binding assay showing that H4(99-103) modulates the response to classical CB2 agonists. These findings are the first demonstration of an endogenous peptide that signals via CB2. Ongoing studies aim to assess the role of H4(99-103) in maintaining the CB2 tone and attenuating age-related bone loss.



A. Mitogenic dose-response activity in mouse osteoblasts. H4 (99-103) stimulates number of WT and *CB1*$^{-/-}$, but not *CB2*$^{-/-}$ mouse calvarial osteoblasts. *p<0.05 vs. untreated. **B**, **CB2 agonistic activity of H4(99-103) in human osteoblasts**. SR144528 (SR2), a CB2 selective antagonist inhibits dose-dependently H4(99-103) mitogenic activity. *p<0.05 vs. H4(99-103)-treated (no SR2) cultures. Mean±SE, n=3

SEX DIFFERENCE IN THE EFFECT OF RIMONABANT ON COCAINE MEMORY IN MICE

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Cannabinoid CB_1 receptors are implicated in various forms of learning and memory, including cocaine-associated memory. We previously found that systemic or intra-medial prefrontal administration of the CB_1 receptor antagonist/inverse agonist rimonabant facilitates the consolidation of conditioned place preference (CPP) induced by a low dose (2.5, 5, or 10 mg/kg) of cocaine in male wild-type mice. The current study aimed to investigate the receptor and hormone mechanisms underlying the facilitating effect of rimonabant on cocaine-induced CPP. We first examined whether CB_1 receptors mediates rimonabant's effect by using CB_1 knockout mice. We found that rimonabant did not facilitate memory consolidation of a low-dose (10 mg/kg) cocaine-induced CPP in both genders of the CB_1 knockout mice. Moreover, rimonabant did not enhance the low-dose cocaine-induced CPP in female wild-type mice. Intriguingly, the facilitating effect of rimonabant on cocaine-induced CPP was found to be mediated by increase of the plasma corticosterone levels in male wild-type mice. Next, to test whether estrogen is involved in this sex difference effect, we examined rimonabant's effects on a low-dose cocaine-induced CPP in vehicle-treated *vs.* estrogen-treated ovariectomized (OVX) female wild-type mice and found two interesting results. First, estrogen replacement in the OVX mice *per se* enhanced the low-dose cocaine-induced CPP memory. Second, rimonabant facilitated cocaine-induced CPP memory in the OVX mice supplied with sesame oil, while impaired the same memory in the OVX mice supplied with estrogen. Taken together, our results indicate: 1) The facilitating effect of rimonabant on cocaine-induced CPP is mediated by CB_1 receptors and corticosterone in male wild-type mice; 2) Estrogen is involved in sex difference in the facilitating effect of rimonabant on cocaine-associated memory.

CB1-DEPENDENT LTD IN VENTRAL TEGMENTAL AREA GABA NEURONS: A NOVEL TARGET FOR MARIJUANA

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The ventral tegmental area (VTA) is necessary for reward behavior where dopamine cells signal reward, which are regulated by neighboring inhibitory GABA cells. Using whole cell electrophysiology in juvenile/adolescent GAD67-GFP mice we examined excitatory plasticity in fluorescent VTA GABA cells. A novel CB1-dependent long-term depression (LTD) was induced in GABA cells that was dependent on metabotropic glutamate receptor 5, and cannabinoid receptor 1 (CB1). LTD was absent in CB1 knock-out mice, but preserved in heterozygous littermates. Chronic injections of Δ^9-tetrahydrocannabinol occluded LTD compared to vehicle injections, however, a single exposure was insufficient. Because Δ^9-tetrahydrocannabinol depresses GABA cell activity, downstream dopamine cells will be disinhibited and thus this could potentially result in increased reward. As drug of abuse synaptic modifications are often tied to addiction, this data also suggest a possible mechanism for the rewarding and addictive effects of Δ^9-tetrahydrocannabinol, which is most commonly seen in adolescents, by potentially altering reward behavioral outcomes.

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Δ^9-TETRAHYDROCANNABINOL VAPOR INHALATION ATTENUATES OXYCODONE INTRAVENOUS SELF-ADMINISTRATION UNDER EXTENDED ACCESS CONDITIONS

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Prescription opioid abuse is a significant global problem, thus the use of non-opioid drugs has been investigated for the treatment of pain and the reduction of adverse effects such as abuse or overdose. The interaction between cannabinoids and opioids has been shown to modulate nociception and positive reinforcing effects in animals. In this study, Δ9-tetrahydrocannabinol (THC) and oxycodone were administered to rats and assessed for the possible enhancement of antinociceptive effects. We further investigated the effects of THC vapor inhalation, using electronic-cigarette technology, on oxycodone intravenous self-administration under extended access conditions.

Adult male and female Wistar rats were injected with THC (5.0 mg/kg, i.p.), oxycodone (2.0 mg/kg, s.c), THC/oxycodone combination or vehicle and then assessed for tail-withdrawal latency for nociception. Tail-withdrawal latency was increased by THC or oxycodone alone in both male and female rats, and latency was significantly higher in rats that were administered the THC/oxycodone combination compared to either drug alone. A separate group of male rats was trained to intravenously self-administer oxycodone (0.15 mg/kg/infusion) under a fixed-ratio 1 (FR1) response contingency during an 8 h session. Following acquisition, the rats were exposed to vaporized THC (100 mg/mL) or propylene glycol (PG) for 30 minutes prior to oxycodone self-administration. Self-administration in rats exposed to THC vapor was significantly decreased compared to PG-exposed control rats.

These data show the combined effects of THC and oxycodone exposure in rats and further demonstrate the interaction of cannabinoids and opioids on nociception and reward. Furthermore, these data suggest the potential use of cannabinoids in combination with opioids for the enhancement of antinociception, while mitigating opioid abuse and dependence.

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COMPARING AND CONTRASTING THE EFFECTS OF ACUTE THC ON THE TRANSCRIPTOME IN THE HIPPOCAMPUS OF ADULT AND ADOLESCENT MICE

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As the primary psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC) is hypothesized to produce most of its behavioral effects via activation of CB_1. Densely expressed in the hippocampus, CB_1 is a receptor target for endogenous cannabinoids (eCBs). Currently unpublished data from our lab revealed that the hippocampus had the highest levels of THC out of 8 brain areas after an acute 3 mg/kg injection in both adolescent and adult mice, suggesting that this brain region may be particularly sensitive to THC's effects. In addition, the eCB system undergoes dynamic changes during adolescence and may be more vulnerable to THC at this time. We previously demonstrated that acute THC induces age-dependent effects on levels of eCBs and related lipids in the hippocampus. To test the hypothesis that the acute, THC-induced changes in the hippocampal lipidome are accompanied by alterations in gene expression, post-natal day (PND) 35 adolescent and adult female mice were given 3 mg/kg THC or vehicle, then sacrificed 2 hours later. RNA was extracted from the hippocampus using the Agilent Absolutely RNA prep kit. RNA was sequenced using RNA-Seq on an Illumina sequencer. Utilizing DeSeq software, expression levels of transcripts in the THC-treated hippocampus were compared to vehicle at a 5% false discovery rate. To illuminate potential pathways impacted by acute THC and potential downstream consequences of the differentially expressed transcripts, the data set was further analyzed with Ingenuity Pathway Analysis (IPA) software (Qiagen).

Many genes were upregulated or downregulated in response to acute THC, with acute THC altering more genes in the adult hippocampus (189 genes; 109 upregulated, 80 downregulated) compared to the adolescent hippocampus (89 genes; 41 upregulated, 48 downregulated). Demonstrating that there are unique transcriptomic signatures of acute THC independent of developmental age, 31 genes were altered in both PND35 and adult mice. Changes in these 31 genes were in the same direction in the adult and PND35 hippocampus, but the change's magnitude was larger in the adult. Contrary to expectations, none of the genes classically associated with the eCB system were affected by acute THC. Many of the genes upregulated in both groups were involved in apoptosis, including PLEKHF1, which was the gene with the highest magnitude upregulation in both the PND35 and adult hippocampus. IPA predicted decreased seizure activity as a consequence of the set of differentially expressed transcripts in the adult hippocampus, but not in the PND35 hippocampus. Acute THC may affect cell survival, proliferation and differentiation in the hippocampus, with implications for neurogenesis, which likely has unique outcomes for the developing brain. The fact that THC affected more lipids and genes in adulthood may indicate increased "flexibility" in the adult brain that can better respond to challenges such as THC, which may ultimately drive a faster recovery from the exposure. It will be important to understand the wider metabolic costs and downstream effects of apoptotic signaling of THC at different developmental stages as this may represent a mechanism for the correlation between cannabis use in adolescence and psychopathology in adulthood.

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MARIJUANA EXTRACT VS DELTA-9-THC IN MICE

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The overwhelming majority of preclinical cannabinoid research on the CNS pharmacology of marijuana has utilized synthetic delta-9-tetrahydrocannabinol (THC) as a proxy for the plant. While invaluable knowledge has been gained through these studies, THC alone lacks the diversity of chemicals found in a typical marijuana flower most often used by humans. Hence, the degree to which other plant constituents may modulate the effects of THC (and the mechanism through which they may do so) has remained understudied. As human use of marijuana for medicinal and recreational purposes continues to increase, this issue becomes more critical to improvement of the translational validity of preclinical studies. To this end, we have begun a series of experiments to examine the effects of cannabis extracts and to compare these effects to those of THC in standard in vivo tests used to characterize cannabinoids.

An extract was created from marijuana plant material obtained from the NIDA Drug Supply program. The extract was analyzed and found to be approximately 70% delta-9-THC. Subsequently, doses of the extract were formulated such that the concentrations of THC to be delivered to the mouse in the extract matched the synthetic delta-9-THC dose delivered alone. Following intraperitoneal (IP) injection, male and female ICR (CD:1) mice were tested in a tetrad battery of tests in which psychoactive cannabinoids produce characteristic effects (e.g., locomotor activity, temperature, analgesia and catalepsy). Cannabimimetic effects of the marijuana extract will be compared to those of delta-9-THC alone (1, 10 and 100 mg/kg). In addition, male and female C57/Bl6 mice trained to discriminate 5.6 mg/kg delta-9-THC from vehicle are being tested with marijuana extract across a THC dose effect curve (0.3-10 mg/kg). Reversibility of the cannabimimetic effects after pre-treatment with CB1 antagonist rimonabant (SR141716) and CB2 antagonist SR144528 will be assessed. Finally, brain and plasma of mice dosed with delta-9-THC or marijuana extract (1, 10 and 100 mg/kg) will be collected at a single time point (30 min post injection) and analyzed for levels of: delta-9-THC, 11-OH-THC and COOH-THC.

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CASE REPORT: CLINICAL OUTCOME AND IMAGE RESPONSE OF TWO PATIENTS AFTER CHEMORADIATION TREATMENT IN ASSOCIATION WITH CANNABIDIOL

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Gliomas, the most common primary brain tumors, account for more than 40% of all CNS neoplasms and are highly resistant to the available therapeutic approaches (Hosli et al., 1998; Galanis and Buckner., 2000a,b). These tumors often have a poor prognosis, with a median survival time of approximately 1 year for patients with high grade gliomas (grade III/IV) (Ohgaki and Kleihues, 2005; Burnet et al., 2007). The Stupp protocol has become standard of care treatment of glioblastoma (GBM), and has led to significant survival improvements (van den Bent MJ et-al 2005). It consists of chemoradiation followed by adjuvant temozolomide, an alkylating agent.

Tumor pseudoprogression (PSD) can occur in up to 30% of the patients, manly MGTM methylated cases. It corresponds to a treatment-related increase of lesion size due to inflammatory responses that simulate the progression of the disease. In almost 60% of the cases, pseudoprogression occurs within the first 3 to 6 months after completing chemoradiation. (Domingues RC et-al 2011, van den Bent MJ et-al 2009, Chang S 2009). Pseudoprogression does not represent the progression of the disease, and is often a marker of longer survival, presumably because it represents a robust response to treatment (Brandes et al 2008). Cannabidiol is one of the most prevalent natural cannabinoids. It is a nonpsychoactive compound and presents a wide spectrum of pharmacological effects including anti-inflammatory, anti-proliferative, anti-invasive, anti-metastatic and pro-apoptotic effects (Grotenhermen 2005, Massi et al. 2004, Guzman 2003). Recent discussions also suggest a possible immunomodulation caused by cannabidiol.

The authors describe two patients with confirmed diagnosis of Glioblastoma Multiforme (WHO-IV), both presenting MGMT methylated and IDH-1 mutated who, after an incomplete surgical resection, were submitted to the Stupp protocol associated with cannabidiol (CBD). Both patients presented very satisfactory clinical and imaging responses in periodic evaluations. Right after the chemoradiation, one of the patients presented a very exacerbated and precocious PSD in the MRI, which was resolved in a short period of time. On the other hand, the other patient presented a marked remission of the altered areas in the MRI (figures 1 and 2).

Such aspects are not commonly observed in patients only treated with conventional modalities. This observation could highlight the potential effect of CBD increasing PSD response that could impact survival. Further investigation with more patients with critical molecular analyses should be done.

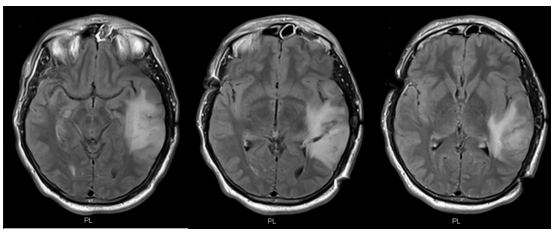

Figure 1: preoperative MRI.

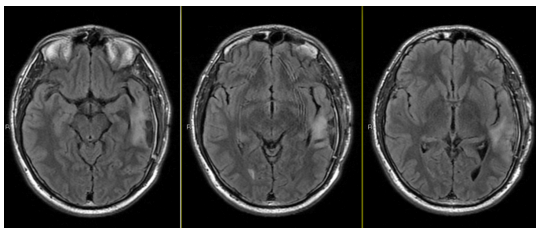

Figure 2: first follow up post-surgery, chemoradiation and CBD.

GPR3 AND GPR6, NOVEL MOLECULAR TARGETS FOR CANNABIDIOL

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GPR3 and GPR6 are members of a family of constitutively active, Gs protein-coupled receptors. Previously, it has been reported that GPR3 is involved in Alzheimer's disease whereas GPR6 plays potential roles in Parkinson's disease and Schizophrenia. GPR3 and GPR6 are considered orphan receptors because there are no confirmed agonists for them. However, GPR3 and GPR6 are phylogenetically related to the cannabinoid receptors. In this study, the activities of cannabinoids were screened on GPR3 and GPR6 using a β-arrestin2 recruitment assay.

Our results show that both GPR3 and GPR6 are constitutively active in β-arrestin2 recruitment assays. Among the variety of phytocannabinoids, synthetic cannabinoids, and endocannabinoids that we tested, cannabidiol (CBD), the major non-psychoactive component of marijuana, concentration-dependently reduced β-arrestin2 recruitment to both GPR3 and GPR6. These data demonstrate that CBD acts as an inverse agonist at both GPR3 and GPR6 receptors. In addition, our structure-activity relationship study of CBD revealed that the aliphatic side chain, as well as the free hydroxyl groups of CBD is essential for the inverse agonism of CBD on both GPR3 and GPR6.

These data demonstrate for the first time that both GPR3 and GPR6 are novel molecular targets for CBD. Our discovery that CBD acts as novel inverse agonist on both GPR3 and GPR6 indicates that some of the potential therapeutic effects of CBD (e.g. treatment of Alzheimer's disease, Parkinson's disease and Schizophrenia) may be mediated through these important receptors.

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CANNABIDIOL ACTS AS ALLOSTERIC MODULATOR OF CANNABINOID CB_2 RECEPTORS

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The mode of action of the main non-psychotropic component of *Cannabis sativa* L., cannabidiol (CBD), is not yet understood. At first it was assumed that it might act via CB_2 receptors (CB_2Rs) but the binding to the orthosteric center requires micromolar concentrations of the compound. More recently CBD is being considered as a ligand of serotonin 5-HT_{1A} receptors. In a heterologous expression system consisting of membrane preparations of cells (HEK-293) expressing the CB_2R human version, we have confirmed using a synthetic radiolabeled agonist, [^{3}H]-WIN 55,212-2, that CBD does not bind with high affinity to the orthosteric site of human CB_2Rs. The natural cannabinoid was, in contrast, able to negatively modulate in forskolin-induced intracellular cAMP determination assays the effect of JWH133, a selective CB_2R agonist. These results were consistent with CBD-mediated significant modification in the dissociation kinetics of the binding of a fluorophore-conjugated CB_2R-selective compound, detected using the HTRF (homogenous time-resolved fluorescence resonance energy transfer) methodology. In conclusion, some of the actions of CBD may be mediated by interaction with an allosteric site in CB_2R that allows modulation of the action of receptor agonists.

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PHARMACOLOGICAL TARGET PROFILING OF CANNABIDIOL

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Cannabidiol (CBD) is a phytocannabinoid that has no demonstrated cannabinoid-psychoactivity, but does exert pharmacological effects. Evidence of CBD's efficacy is accumulating for the treatment of pediatric epilepsy and anxiety. However, the underlying mechanism for these effects remains to be unclear. The anxiolytic effect of CBD can be blocked *in vivo* with a selective serotonin receptor (5HT)1A antagonist, but the exact mechanism of action remains to be established *in vitro*. The mechanism of CBD's action in epilepsy is also poorly understood; electrophysiological studies have demonstrated CBD can inhibit certain sodium channels (NaV), but CBD is active in animal models considered insensitive to NaV inhibitors. In some pediatric epilepsy sufferers, the large CBD doses required for clinical efficacy inhibits metabolism of other anti-seizure medications, resulting in sedative side effects. If the target(s) of CBD were known, perhaps more potent and selective congeners might be developed to reduce drug interaction problems. During drug development, the potential liabilities of lead compounds is generally assessed by examining their activity at a range of pharmacological and toxicologically significant "off-targets". Therefore, we examined the interactions of CBD with such a range of receptor/enzyme targets to provide insight into the mechanisms underlying CBD's pharmacology and its potential undesirable effects. The initial screen was conducted in duplicate at 100 nM and 10 μM CBD, and certain "hits" were then evaluated twice over a greater range of CBD concentrations (up to 100 μM), with duplicates at each concentration.

The results demonstrated that CBD displaces [3H] Batrachotoxin from rat cerebral cortical membranes with a Ki of 120 μM and [3H] CP55940 from recombinant hCB1 receptors with a Ki of 95 μM. The interaction of CBD with batrachotoxin sensitive NaVs is consistent with previous electrophysiology studies suggesting NaVs may be mechanism through which CBD exerts its anti-seizure effects. The initial screen also suggested that CBD may increase agonist binding at 5HT 1A, 2B and to a lesser extent 2A receptors. Dose response curves demonstrated that CBD enhances the binding of [3H]8-OH-DPAT to recombinant h5HT1A receptors with Kd of ~8 μM and enhances the binding of [3H](6)-2,5-dimethoxy-4 iodoamphetamine to recombinant h5HT2b receptors with a Kd of ~ 20 μM. The potentiation of orthosteric agonist binding to 5HT1A is consistent with the literature showing 5HT1A antagonist blockade of CBD's anxiolytic effects, but the current data suggest that CBD is an allosteric modulator at 5HT1A. The significance of CBD enhancement of 5HT 2B agonist binding is unclear but again suggestive of an allosteric effect. It should be noted that 5HT2B agonists like fenfluramine have been linked with cardiac valvulopathy, but whether allosteric modulators would exert a similar toxicological profile is unclear.

Acknowledgements

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FUNCTIONAL SELECTIVITY OF CBD IN THE ORPHAN RECEPTOR GPR3: A STRUCTURAL FOCUS

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GPR3 is an orphan receptor that belongs to the Class A family of G-Protein Coupled Receptors. It shares high sequence similarity with GPR6, GPR12 (64 and 61%), the lysophospholipid receptors S1P1 and LPA1 (34 and 33%), and the cannabinoid receptors CB_1 and CB_2 (21 and 26%). GPR3 is largely expressed in mammalian brain and oocytes and it is known as a Gs-coupled receptor activated constitutively in cells. GPR3 has been shown to be a possible target for the treatment of pathological conditions such as Alzheimer´s disease (AD), oocyte maturation or neuropathic pain. However, the lack of potent and selective GPR3 modulators is delaying the exploitation of this promising therapeutic target. Very few ligands, including the only inverse agonist, AF64394, have been identified for GPR3 so far (Jensen et al, *Bioorg. Med Chem. Lett.* 2014). AF64394 is a [1,2,4]triazolo[1,5-a]pyrimidine that acts as inverse agonist of both the GPR3 G-protein Dependent (GprotDep) and G-protein Independent β–arrestin (GprotIndepBarr) signaling pathways. Interestingly, we have recently discovered that cannabidiol (CBD) acts as a GPR3 inverse agonist of the GprotIndepBarr signaling pathway, but has no effect on GPR3 GprotDep signaling. Such GPR3 GprotIndepBarr signaling biased inverse agonists have great therapeutic potential in AD.

In this context, we have developed a GPR3 homology model that may help us to elucidate the structural determinants governing ligand-receptor interactions. Our GPR3 model is based on the crystallized S1P1 receptor structure (Hanson et al, *Science* 2012). Sequence divergences in transmembrane helixes 1, 4, 6 and 7 have been explored using the Monte Carlo/simulated annealing program, Conformational Memories (Whitnell et al, *J. Comput. Chem.* 2007). The extracellular and intracellular loop geometries were calculated using Modeller v9.1. The GPR3 inactive state model is being used to elucidate the molecular interactions of AF64394 and CBD with this orphan receptor rationalizing the structural basis of GprotDep and GprotIndepBarr signaling pathways. This homology model will enable the design of more potent and selective GprotDep and GprotIndepBarr-biased GPR3 ligands to further understand its biological role and its possible relation with the endocannabinoid system. [Support: NIDA KO5 DA021358 PHR]

DISCOVERY OF SIDE-CHAIN MODIFIED CANNABIDIOL-DERIVED NEUROPROTECTIVE AGENTS WITH IMPROVED "DRUG LIKENESS"

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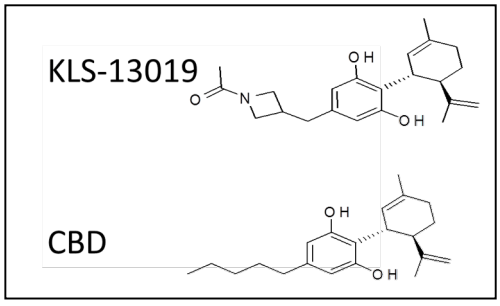
Cannabidiol (CBD) is the non-psychoactive component of *C. sativa* that has been shown to be neuroprotective in multiple animal models. Our interest is to advance a therapeutic candidate for the indication of Chemotherapy Induced Pain (CIP). CIP is a serious neurological disorder that occurs in a large number of oncology patients. Although cannabidiol is effective in models of CIP and reduces opiate drug dependence, it has limitations in terms of safety and oral bioavailability. We will describe a series of side chain modified resorcinols that were designed for greater hydrophilicity and "drug likeness", while varying hydrogen bond donors, acceptors, architecture, basicity, neutrality, acidity, and polar surface area within the pendent group. Our primary screen evaluated the ability of the test agents to prevent damage to hippocampal neurons induced by clinically relevant toxins, however this was followed up with evaluation in dorsal root ganglia for our best compound. Notably, out of thirteen new molecules synthesized, three were found with equal or superior potency to CBD. The best three new molecules were evaluated for any decrease in neuronal viability at concentrations above their EC_{50}. Whereas CBD is toxic at 30 μM, none of the KLS compounds exhibited toxicity up to 100 μM and KLS-13019 was only toxic at 300 μM giving it a therapeutic index of 7,500. None of the new molecules exhibited CB1 affinity at 10 μM. Kinetic aqueous solubility was improved for two of the new molecules. All three new molecules showed great improvements in permeability in both CACO and MDCK MDR1 cell lines with no efflux potential. Consistent with this improved in vitro ADME data, KLS-13019 (F% = 67) was found to have superior oral bioavailability versus CBD (F% = 8.5%) in mice. A non-provisional patent application has been filed globally for KLS-13019 and related molecules, and a U.S. patent allowance on composition of matter has been issued. Further evaluation of KLS-13019 in vitro and in vivo will be described in abstracts from Drs. Brenneman and Ward.



METABOLIC INTERACTIONS OF MAJOR PHYTOCANNABINOIDS WITH HUMAN CYP 2J2 ENZYME

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We have reported the metabolic interactions of major phytocannabinoids with human hepatic CYP enzymes (*Biochem. Pharmacol*. 79: 1691 (2010); *Chem. Biol.-Int*., 216: 62 (2014); *Drug Metab. Dispos*. 39: 2049 (2011); *Foren. Toxicol*. 29: 117 (2011); 31: 70 (2013); *Life Sci.* 80: 1415 (2007); 88: 730 (2011); 89: 165 (2012); 136: 87 (2015) *etc*). However, the metabolic interactions of cannabinoids with human extrahepatic CYP enzymes are not fully examined. CYP2J2 is a unique CYP enzyme, which is predominantly expressed in extrahepatic tissues including heart, small intestine, placenta, brain *etc*. CYP2J2 catalyzes the epoxidation of arachidonic acid and endocannabinoids (anandamide, 2-arachidonoylglycerol *etc.*). Some epoxy metabolites of physiological substrates may have important roles in the modulation of physiological functions such as cardiovascular and inflammatory system. In the present study, we examined the metabolic interactions of major phytocannabinoids (Δ^9-THC, CBD and CBN) with recombinant human CYP2J2 enzyme.

Δ^9-THC and CBN were selectively biotransformed by recombinant CYP2J2 to pentyl side chain hydroxylated metabolites at the 4'- and 5'-positions. The 4'-hydroxylated metabolite was 5~10 times more abundant in both cannabinoids. CBD was also metabolized on pentyl side chain at the 4''- and 5''-positions together with allylic positions at the 6- and 7-positions. The metabolism of these cannabinoids by CYP2J2 was potently inhibited by arachidonic acid, a physiological substrate of the CYP enzyme. The metabolic profile of major phytocannabinoids by CYP2J2 was quite different from those by other human hepatic CYP enzymes examined so far.

Δ^9-THC, CBD and CBN strongly inhibited the Luciferin-2J2/4F12 *O*-dealkylase activity of recombinant CYP2J2 with the IC_{50} values of 2.86, 3.12, and 1.19 μM, respectively. Kinetic analysis for the inhibition showed that Δ^9-THC and CBN inhibited the CYP2J2 activity in a mixed manner with the apparent K_i values of 1.06 and 0.229 μM, respectively, whereas CBD did in a competitive manner with the apparent K_i value of 0.705 μM. Furthermore, our results suggest that at least one of free phenolic hydroxyl groups and the pentyl side chain of CBD may play important roles in CBD-mediated CYP2J2 inhibition.

The present study demonstrates that Δ^9-THC, CBD, and CBN are potent inhibitors for CYP2J2, which metabolizes these cannabinoids mainly on the pentyl side chain at the 4'-position.

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PHYTOCANNABINOIDS CANNABICHROMENE (CBC) AND CANNABIDIVARIN (CBDV) MODULATE MITOCHONDRIAL COMPLEX PROTEINS IN PRIMARY HUMAN ASTROCYTES

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BACKGROUND

Cannabidiol (CBD) has a rich pharmacology, modulating several targets in both physiological and pathological conditions. However, little is known about the effect of CBD and other phytocannabinoids on mitochondrial function, which involves pathways that modulate cell signalling, metabolism and cell death. Thus, the aim of this study was to investigate the effects of CBD and other novel phytocannabinoids (CBC and CBDV) on mitochondrial complex proteins, in both normoxic (control) and oxygen glucose deprivation (OGD) conditions.

METHODS

In control experiments (normoxia), fully confluent human astrocytes (P3 and P4, from two separate experiments) were treated with CBD, CBC, and CBDV (GW Research Ltd, Cambridge, UK) at 10 nM, 100 nM, 1 μM and 10 μM (alongside a vehicle control) for 24 hrs. In oxygen glucose deprivation (OGD) experiments, astrocytes were treated with CBD, CBC, and CBDV (10 nM to 10 μM (alongside a vehicle control), but placed in glucose-free medium with 0% O_2 (using a BD GasPak™ pouch) conditions for 4hrs. After 4hrs, the medium was replaced with normal astrocyte medium containing fresh phytocannabinoids, the cells were then returned to the incubator for a 20 hr reperfusion period. After 24 h, cells were lysed (mitochondrial lysis buffer) and analysed for mitochondrial proteins (complex I, complex II, complex III, complex IV, complex V) according to the manufacturer's instructions using multiplex technology (#H0XPSMAG-16K, Merck Millipore).

RESULTS

In control conditions, treatments with 10 μM CBD resulted in a decrease in complexes I, II, III, IV and V ($P< 0.0001$, $P<0.001$, $P<0.01$, $P<0.0001$ and $P<0.0001$ respectively). CBC and CBDV treated cells showed a decrease in complex protein IV at 10 μM ($P<0.01$, $P<0.05$ and $P<0.05$ respectively).

After exposure to an OGD protocol, there was a significant reduction in the following mitochondrial complex proteins; I ($P<0.05$), II ($P<0.001$), III ($P<0.0001$) and IV ($P<0.05$) compared to normoxic conditions.

With previous exposure to OGD, cells treated with CBD (10 μM) had a reduction in complex I ($P<0.001$), complex II ($P<0.01$) and complex IV ($P<0.0001$). CBDV caused a slight reduction in complex I at 10 μM. CBC (10 μM) treated cells showed a reduction in complex II ($P<0.001$) and complex V ($P<0.01$).

CONCLUSIONS

In control conditions mitochondrial complex IV, which is responsible for the reduction of oxygen into water in the last stage of the electron transport chain (ETC), was decreased by all phytocannabinoids. Following OGD, CBD caused a decrease in complex IV. In similar studies, inhibition of complex IV by nitric oxide (NO) increases O_2 bioavailability deeper in tissue, thus a decrease in complex IV may be a mechanism by which phytocannabinoids are beneficial in ischaemia-reperfusion injury. In addition, complexes II is a major point of reactive oxygen species (ROS) generation in the respiratory chain, therefore its modulation by CBD and CBC could influence the amount of ROS produced during hypoxia.

EFFECTS OF PHYTOCANNABINOIDS ON ASTROCYTIC METABOLISM DURING NORMOXIA AND OXYGEN-GLUCOSE DEPRIVATION

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Besides THC and CBD, few data exists on the effects of the other phytocannabinoids in normal and pathological settings. Our group has previously shown that CBD is effective at reducing the permeability decreases at the blood brain barrier (BBB) associated with oxygen glucose deprivation (OGD). Therefore the aim of the present study was to establish the effects of CBD, CBDV and CBC on cellular metabolism in astrocytes in a model of ischemia/reperfusion, to provide further insights into the molecular actions of phytocannabinoids in stroke.

Fully confluent human astrocytes (P6, n=6, from two separate experiments) were exposed to oxygen glucose deprivation (OGD) conditions (0% oxygen & 0% glucose) for 4 h using GasPak EZ Anaerobic pouches (BD, UK) with RPMI (no glucose) medium. Cells were treated with phytocannabinoids (GW Research Ltd, Cambridge, UK) at various concentrations (10nM, 100nM, 1μM and 10μM) pre-OGD and post OGD. Following OGD, cells were returned to normoxic conditions (glucose containing media + 20% O_2, 5% CO_2) and incubated for a further 20 h before measuring cellular metabolism using the CellTiter 96® AQueous One Solution Cell Proliferation Assay. Data were analysed using a one way ANOVA and compared to control vehicle using Dunnett's *post hoc* analysis.

Under control (normoxic) conditions, there was no significant effect on cellular metabolism following treatment with phytocannabinoids, however there was a trend for a concentration-dependent increase. The 4 h OGD protocol produced a significant increase in cellular metabolism shown by the MTS assay ($P<0.01$). Following exposure to OGD conditions, a concentration-dependent increase in cellular metabolism was seen after CBD treatment (10 μM, $P<0.01$) and CBDV treatment (10 μM, $P<0.05$).

4 h OGD caused an increase in the production of formazan, reflecting an increase in the activity of NAD (P) H-dependent oxidoreductase enzymes. CBD and CBDV at 10μm further increased metabolism, potentially reflecting an increase in cell proliferation and/or an increase in anti-oxidant capabilities. Further experiments should look to identify the consequences of increased metabolism, mechanisms of action, as well as establishing the effects of phytocannabinoids in more severe OGD protocols.

SYSTEM PHARMACOGNOSY MAPPING OF PHYTOCANNABINOIDS FOR CB1 AND CB2 ACTIVITY AND SPECIFICITY

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Cannabis medical therapies are becoming a reality, but the complexities of phytochemicals and their pharmacological effects on human systems continue to be challenges to make therapeutic decisions or study molecular mechanism of actions. Therefore, we used system biology approaches to integrate the molecular pharmacognosy information of phytocannabinoids and their effects on human endocannabinoid system. About one hundred phytocannabinoids have been classified by their metabolic/chemical nature as well as their pharmacological effects. In this study, we focused on the pharmacological effects and specificities on Cannabinoid receptors, CB1 and CB2 of representative phytocannabinoids (including cannabigerol, cannabichromene, cannabidivarin, tetrahydrocannabinoids, cannabicycol, cannabinol, cannabinodiol and cannabitriol).

Available data was collected on natural cannabinoids such as their botanical compositions, stabilities, receptor binding affinities and pharmacological effects. We used computer modeling to align and normalize their individual pharmacological data on the receptors based on structure-activity relationships between ligands and targets. Thus, a database was compiled to analyze and compare the relationships and effects of phytochemicals on multiple targets of the Endocannabinoid systems. Multiple components' effects on CB1 and CB2 respectively were analyzed, and their complex relationships were visualized and effects via mapping and plots. These system analysis enable us to understand the pharmacological effects of phytocannabinoid mixtures and identify receptor specific formulations. In the future, this system pharmacognosy approach can be used to develop optimized formulations of complex and natural cannabinoids as botanical drug products.

A CHEMICAL COMPARISON OF CANADIAN MEDICAL CANNABIS: PRE- AND POST-DECARBOXYLATION

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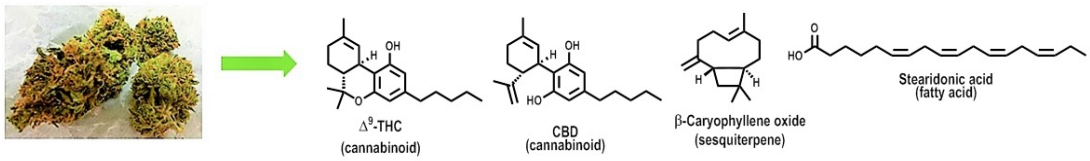
Medicinal cannabis has been available to patients for the treatment of various clinical conditions in Canada since 2000. Several chemical constituents in this plant exhibit anti-inflammatory, antibacterial, antiviral, anticancer activities as well as have shown efficacy in neuropsychological disorders. 564 compounds were characterized in *Cannabis sativa* L. including the unique pharmacologically-active cannabinoids, which act primarily through the CB1 and CB2 cannabinoid receptors. In their natural state within the cannabis plant, the cannabinoids carry a carboxylic acid moiety, and are physiologically almost inactive. In their decarboxylated forms, these phytocannabinoids such as Δ^9-THC and CBD, either alone or in combination with other phytocannabinoids, exhibit a variety of biological activities. We have been interested in characterizing Canadian medical cannabis and understand its chemical composition to comprehend the differences between different commercially available cannabis strains, and their potential therapeutic benefits.

Using Supercritical Fluid Extraction, cannabis resin was extracted from a commercial sample of medical cannabis and was subsequently subjected to decarboxylation. We employed UPLC-MS to analyze the extracted chemicals prior to and post-decarboxylation. Within the pre-decarboxylated extract, 63 compounds including the characteristic phytocannabinoids, sesquiterpenes and plant fatty acids were observed. Changes in the chemical composition of the post-decarboxylated cannabis extract were revealed through the disappearance of 26 compounds and the emergence of 22 new ones found, including the neutral cannabinoids. Thus, the decarboxylated cannabis extract, possessing the neutral cannabinoids and other plant components may serve as a starting point for development of potential lead compounds. This serves as a stepping stone to consider the analyses of commercial cannabis samples available in the country, and understand their chemical composition, hence potential pharmacological profiles.



Acknowledgements: Funded by University Health Network (Toronto, Canada), Canada Foundation for Innovation/Ontario Research Fund (#CFI 32350), and CannScience Innovations Inc. (now known as Scientus Pharma, Inc.) (#UHN-2014-0738).

DISCOVERY OF MAJOR PHYTOCANNABINOIDS IN HEMP SEEDS

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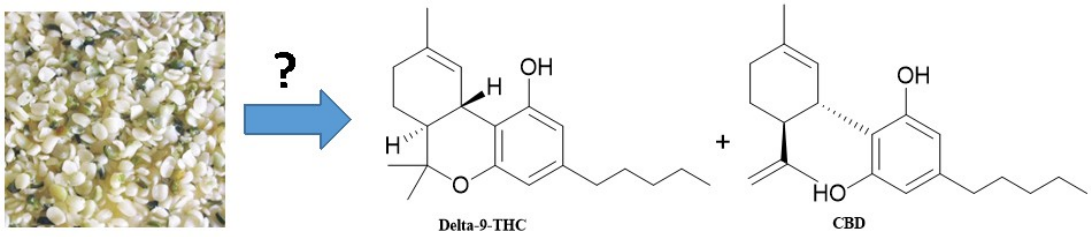
Aside from usage as fiber and medicine, hemp (*Cannabis spp.*) is also prized as a food source. Particularly, hemp germplasm is known for its high nutrient content, with a 100 g serving of seeds meeting up to 63% of the recommended daily value for protein. However, the presence of the psychoactive cannabinoid Δ^9-tertrahydrocannabinol (THC) in seeds is highly regulated to eliminate any possibility of exposing consumers to narcotic substances. In fact, recently a case of THC poisoning was reported in a pre-school child consuming hemp seed oil to strengthen immunity.

Under Canada's Industrial Hemp Regulation (IHR) Program, the sale and provision of cannabis seed derivatives are strictly limited to products containing no more than 10 µg of THC per gram of food-grade hemp. We were interested in evaluating consumer-grade hemp seeds obtained from local grocery stores. We extracted resins from 3 commercial brands of hemp seeds using different extraction methods and quantified THC and cannabidiol (CBD). Results show that THC content in the resins varied from 19% - 770% of the legal limit, with all brands having at least 1 set of resins that surpassed the 10 µg/g threshold. Extraction methods influenced the quantity of THC and CBD quantified. Given the lack of a standardized testing protocol for hemp seed derivatives, the yields from various extraction procedures were also compared to determine an optimal method for the testing of such products.



Acknowledgements: Funded by University Health Network (Toronto, Canada), Canada Foundation for Innovation/Ontario Research Fund (#CFI 32350) and CannScience Innovations, Inc. (now known as Scientus Pharma, Inc.) (#UHN-2014-0738).

CHEMICAL PROFILE UTILITY OF CANNABIS PRODUCTS FROM A CONNECTICUT (US) CANNABIS DISPENSARY

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The rapidly expanding use of Cannabis as a medicine in countries worldwide has led to efforts to discriminate cannabis varieties based on chemical profiles of cannabinoids and terpenoids which serve as the active ingredients in the cannabis drug product. Substantial research over the last two decades has established the utility of distinguishing different Cannabis varieties based on their complex chemical profile with the implication that different varieties of cannabis may have different clinical utility due to relevant differences in chemical constituents. These studies typically examined *Cannabis sp.* flowers and employed rigid methods for controlling the genetic background, growing and processing conditions, and chemical analysis. If this approach is applied to medical Cannabis products in medical distribution systems where Cannabis products include a wide variety of dosage forms, production methods, and analysis by different laboratories; the utility of the chemical profiling is less certain. This study sought to determine if the chemical profiles of Cannabis products from a Connecticut state dispensary could establish reliable, relevant differences and are thus useful as discriminatory tools.

This study analyzed the chemical profiles from state required certificates of analysis (COA), of the Cannabis products from a Connecticut state Cannabis dispensary in the US. The results presented here examine a six month period of time, which is half of the overall yearlong study. Cannabis product COAs represent products from four different producers, analyzed in two different laboratories. Three machine learning approaches were used to train and then predict the Cannabis product descriptors of: *Type* (Flower, Extract, Kief, Edible, Topical), *Strain* (Hybrid, Indica, Sativa, "CBD"), or *Method* (Hydro or Organic). The learning was based on up to 51 assay results in the chemical profile for each of 96 products. Using ten-fold cross-validation, the decision tree (J48) correctly predicted 78% of instances of *Type*; corresponding numbers for a support vector machine (SMO) and a neural network (Multilayer Perceptron [MLP]) were 77% and 73% respectively. Specifically, the true positive rate (TP) for "Flower" was highest at 97% (Precision=1; Recall=0.969) using J48. Similar to *Type*, *Method* was highly predictable on the basis of the data. J48, SMO and MLP correctly classified 80%, 84% and 88% of instances, respectively. However, on the basis of these approaches, *Strain* (with the exception of "CBD") was only poorly predicted.

These results suggest that chemical profiles of Cannabis products are useful in discriminating the method of growing and type of product, but overall poor predictability in discriminating the strain of Cannabis. This poor predictability could be due to several factors stemming from the limited strain information reporting under Connecticut law. Despite the limited predictability for strain, the results from this approach suggest that chemical profiles can reliably predict useful discriminatory variables about a wide variety of Cannabis products in a system with diverse Cannabis dosage forms, strains and methods of production.

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

[1]Rik Kline, [1]Robert Walsh, [1]Steven Gust, [2] Brian Thomas and [3]Mahmoud ElSohly

[1] National Institute on Drug Abuse
[2] Research Triangle Institute
[3] University of Mississippi

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

BREEDING AND DEVELOPMENT OF INDICATION SPECIFIC CANNABIS CHEMOVARS TO IMPROVE EFFICACY AND SAFETY

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Introduction: A sophisticated breeding program was developed utilizing chemical markers to maximize the yield of phytocannabinoids and terpenoids. The aim of the research is to expand the toolbox of plant-based medicines, while improving therapeutic efficacy and overall safety of cannabis products. Cannabis is often divided into several categories based on cannabinoid content: Type I, THC-predominant, is currently the prevalent offering both medical and recreational marketplaces. In recent years, the therapeutic benefits of CBD have been better recognized, leading to the promotion of additional chemovars that are Type II cannabis that contains both THC and CBD, and Type III cannabis is comprised mostly of CBD. While high-THC and high-myrcene chemovars dominate the markets today, it is clear that these may not be therapeutically optimal for patients who require distinct chemical profiles to achieve relief for specific conditions. Furthermore, the development of type II cannabis that contain terpenoid-rich profiles and CBD have the potential to improve both efficacy of the medicine and potentially minimize the adverse effects associated with chronic high-THC cannabis use.

Methods: Cannabis samples were analyzed for cannabinoid and terpenoid content using the techniques of Giese et al., 2015. Reporting of analytical results was effected with PhytoFacts®, a patented method of graphically displaying phytocannabinoid and terpenoid content, as well as scent, taste and therapeutic effect data: https://phytofacts.info .

Results: Various examples from the breeding program will be highlighted, and include examples from Type I, II and III cannabis chemovars, as well as ones that are highly potent in terpenoid content in general, or with specific single components: limonene, pinene, terpinolene, linalool, et al. Additionally, it will be demonstrated how Type I, II and III plants have been developed with identical terpenoid proportions. Various chemovars and their intended therapeutic applications will be highlighted to illustrate properties such as enhanced analgesia, anti-inflammatory and anti-convulsant effects, treatment of depression and anxiety, or to reduce adverse events attributable to THC such as panic, toxic psychosis, and short-term memory impairment.

Conclusions: Advanced Mendelian breeding techniques and analytical assays have resulted in the ability to produce desired cannabinoid and terpenoid profiles that portent to provide far more effective and safer treatment of pain, inflammatory and psychiatric conditions.

WHOLE GENOME SEQUENCING OF SEVERAL CANNABIS DERIVED POWDERY MILDEW SAMPLES AND THE DEVELOPMENT OF FIELD PORTABLE COLORIMETRIC DETECTION TOOLS FOR INFECTING FUNGI

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Powdery Mildew is a frequent and destructive fungi infecting Cannabis crops. It is believed to be vascularized in plant tissue and may remain visually undetected during cloning or other vegetative stages of growth[1]. It usually expresses conidia late into flowering causing more severe economic loss. McPartland, Clarke and Watson[2] have suggested P. macularis and L. taurica may be responsible for cannabis derived powdery mildew. Despite this, no molecular evidence for the speciation of this fungi has been published to date.

In an effort to design a simple field portable test to detect the vascularized dormant presence of powdery mildew prior to conidia propagation, we whole genome shotgun sequenced several powdery mildew samples from multiple geographic locations in the Northeastern United States. Assembled contigs were shown to have ITS similarity to Golovinomyces ambrosiae and orontii. However, significant divergence across large 100 kilobase contigs suggest a novel and diverged species infecting Cannabis.

Using these sequence references we designed an assay compatible with an eight well USB portable amplification system designed by miniPCR. This $700 amplification system is combined with a novel colorimetric DNA amplification assay that affords iPhone based detection of the presence of powdery mildew DNA.

1. Wessling R, Panstruga R. Rapid quantification of plant-powdery mildew interactions by qPCR and conidiospore counts. Plant methods. 2012 Aug 31;8(1):35. PubMed PMID: 22937820. Pubmed Central PMCID: 3522566.
2. John McPartland RC, David Watson. Hemp Diseases and Pests: Management and Biological Control.

MIGRATION WHILE ROOTED: INVESTIGATING THE PROLIFERATION OF THE *CANNABIS* SPECIES BY MAMMALIAN VECTORS

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INTRODUCTION: The cultivation of *Cannabis sativa* L. (*Cannabaceae*) is as old as agriculture itself, as one of the first domesticated plants for fiber, oilseed and marijuana. Hempseed has been an important nutritional source, being more than half comprised of oils and protein. Biological dispersal is the movement of individuals away from the population into which they were born. Dispersal has consequences for individual fitness, gene flow, population genetics, and species distribution. Plants have limited mobility, and rely on seed dispersal vectors—wind, water, and animals. The possible dispersal vectors of *Cannabis sativa* (prior to humans) have received little attention. Charles Darwin investigated hemp seed dispersal by pigeons—some seeds passed through the avian digestive system and germinated. We initiated an investigation of hemp seed survival through the mammalian digestive system.

METHODS: The effects of gastrointestinal passage on seed germination were tested in an *in vitro* digestion model. ~ 10 grams of seeds (Finola) were fed into a simulated gastrointestinal system (DRUID, Ryerson University), subjected to sequential digestive enzymes (pancreatin, pepsin, trypsin, chymotrypsin, peptidase, α-amylase, and lipase), bile salts, and mucin at 38.5ºC for 1+3 hours (gastric + intestinal respectively). Seeds were planted in sterile potting soil (NPK = 0.03-0.03-0.03), each seed had individual cell in the planting tray as to avoid competition and facilitate counting. Grow room conditions were kept at 20-25ºC and relative humidity of 50-60% during 16 h of light (250 W/m^2). Emergent seedlings were counted as soon as identification was possible. Seedling emergence was monitored over a period of 2 weeks. The percentage of germinating seeds were compared among the 72 control seeds, not subjected to *in vitro* digestion, 72 that had undergone gastric digestion and 72 seeds that were subject to full gastrointestinal digestion.

RESULTS: Of the 72 seeds in each trial, the control has 42 seeds germinated, 30.6% (22/72) of gastric seeds emerged, and 25/72, 34.7% of the fully (gastrointestinal) digested seeds produced a viable seedling.

DISCUSSION: This study demonstrated the ability of *Cannabis* seeds to survive passage through an *in vitro* digestion model (mammalian). The total emergence of a seedling (germination) only drop off 52% and 59.5% for gastric and intestinal digestion respectively (when compared to a control), and may in fact increase the rate of germination. This would suggest that mammal could have played a crucial role in dispersing the Cannabis plant. A single variable remains to be added to the protocol, that of mastication. Our next experiment will test full mammalian digestion with *in vivo* feeding experiments.

NON-TARGETED SCREENING AND QUANTITATION USING LIQUID CHROMATOGRAPHY COMBINED WITH HIGH-RESOLUTION MASS SPECTROMETRY (LC-HRMS) IN THE ANALYSIS OF MEDICINAL CANNABIS AND SYNTHETIC CANNABINOIDS

Lingyun Li, Lei Li, Mark Dittmar, Lorie Durocher, Kenneth Aldous and David Spink

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The evolution of accurate-mass high-resolution mass spectrometry (HRMS) coupled with gas or liquid chromatography (GC or LC) has provided powerful analytical tools for cannabinoid researchers. Targeted and non-targeted analyses of phytocannabinoids (PCs), synthetic cannabinoids (SCs) and their metabolites require high sensitivity, high specificity and rapid throughput analyses, which are afforded by LC-HRMS and tandem MS (MS/MS) for both screening and quantitative methods. Medicinal cannabis is widely available for patients in 28 states in the U.S.; however, the lack of rigorous regulations and their enforcement in some states allows inconsistent quality of the medicinal marijuana products, which may be a concern for patients. For example, a laboratory in California reported that 85% of the medicinal cannabis products on the market contained measurable levels of pesticides. Pesticide screening and analysis remain challenging, since hundreds of pesticides could have been used and only trace residues are left in the complex sample matrices. Another public health problem that can be investigated by using LC-HRMS and MS/MS analyses is the increasing use of SCs known as K2 or spice. Recently Adams et al. (New Engl. J. Med. 2017, 376:235-242) reported on what they refer to as the "zombie" outbreak due to illicit use of the very potent synthetic cannabinoid AMB-FUBINACA in New York City. While there are increasing challenges faced by emergency and critical care physicians, psychiatrists and substance abuse professionals, from the analytical perspective it is still very difficult to confidently detect and identify the new SCs and related metabolites, since the SCs that are found on the street are constantly changing and new SCs and other drugs of abuse are constantly emerging. Therefore, methods that target only known PCs and SCs for detection and identification are of limited utility. It is necessary to develop untargeted methods for screening purposes that are capable of detecting an array of drugs of abuse, whether they are known or unknown at the time of testing. In order to solve these analytical problems, we report here on our development of a screening and quantitation method using LC in combination with quadrupole time-of-flight (QTOF) HRMS that employs sequential window acquisition of all theoretical fragment-ion spectra (SWATH). With this LC QTOF SWATH platform, we were able to screen for pesticides, PCs, SCs and other drugs using an accurate-mass database. For the identified compounds, we use the MS/MS fragments to perform quantitative analysis. We envision that this universal method will be easily applied to the analysis of biological samples for non-targeted metabolite analysis and for biomarker screening and quantitation.

SURVEY OF AUSTRALIANS KNOWLEDGE, PERCEPTION AND USE OF CANNABIS FOR MEDICINAL PURPOSES

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Several State governments of Australia have proposed changes allowing the legalization of cannabis for medicinal purposes without consultation of the Australian public. This project aims to provide the most detailed survey of the Australian publics' knowledge, perception and patterns of cannabis use for medicinal and non-medicinal purposes in the context of possible policy change.

Design and Methods: An online self-complete survey was published using the 'Survey Monkey' service. Cannabis users and non-users aged over 18 years were recruited solely through the Facebook Advertisement Platform. Analysis to date was limited to simple Chi-Square comparisons and analysis of variance between users and non-users.

Key Findings: Preliminary results from 963 completers showed 33% had used recreationally, 55% medicinally, and 12% were non-users. Of those who identified as medicinal users, only 12% reported no recreational use. Recreational users were younger than non-users while purely medicinal users were older and more likely to have a health condition. Medicinal users had more entrenched use and greater likelihood of police involvement. As frequency of medicinal use increased, belief in the evidence base and opposition of further clinical trials was more prevalent. Perceptions of policy and use of pharmaceutical preparations will be discussed.

Discussions and Conclusions: There is a need to better communicate the current evidence for medicinal cannabis use and harm minimisation practices. The use of cannabis for purely medicinal reasons is uncommon, however; this important minority report particularly heavy cannabis use in methods which are otherwise thought to be a health risk.

Acknowledgements: The authors had received funding from the Australian Government Department of Health

THE MEDICAL CANNABIS RECOMMENDATION: AN INTEGRAL EXPLORATION OF DOCTOR-PATIENT EXPERIENCES

Regina Nelson

Union Institute & University
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This presentation shares data from Nelson's dissertation study which aid in understanding the experiences of doctors and patients as participants in medical cannabis compassionate care programs by means of the medical cannabis recommendation process. An improved understanding of experiences related to the medical cannabis recommendation process expand scholarly knowledge pertinent to future research in healthcare, leadership, public policy, as well it may have other academic applications. In the U.S., when engaging with compassionate care programs, physicians and patients must both contend with the polarities of state policies that conflict with the federal Controlled Substances Act that bleed into other life experiences. An integral methodology was applied to a narrative inquiry of 32 physician and patient participants. By assessing common and differing narratives through an integral lens (Wilber, 2000a), while grounding experiences in the All-Quadrants, All-Levels (AQAL) theoretical frame, a developed understanding emerges. Participants describe how the interobjectified public and institutional policies (Lower Right Quadrant) affect the cultural (Lower Left Quadrant), relational (Upper Right Quadrant), and subjective consciousness (Upper Left Quadrant). Findings will help guide future research, educational initiatives, and assist with normalizing the use of cannabis.

DEMOGRAPHIC PILOT STUDY OF 300 CANNABIS PATIENTS

Kim Judson, David Bearman, Deborah Malka,
Jeffrey Hergenrather and Christine Paoletti

American Academy of Cannabinoid Medicine

This study looks at the demographic characteristics of our patient study population. The study grown was a randomly drawn sample of one hundred patients from each of three cannabinoid medicine specialists. Over 50 data points were collected and analyzed. We looked at such parameters as age, sex, diagnosis and conventional prescriptions.

Some sample demographics:

•Gender: nearly evenly split; with slightly more males @ 55%.
•Age: about 64% between 35-64; about 22% over 65 years old.
•Education: 30% completed only high school degree; the rest college 12% Master's, 4% Doctorate

The range of conditions included ADD/ADHD, PTSD, Crohn's Disease, depression, bipolar disorder, fibromyalgia, migraines, epilepsy, nausea, appetite stimulation and cancer. About three quarters of patients have a history of musculoskeletal disorders. About three quarters of patients are seeking medical cannabis for relief from pain. Some of these conditions have been studied in animals and tissue culture, few have been studied in humans.

We look forward to future studies that address specific strains for different medical conditions. Data from this study combined with earlier studies such as those done by Amanda Reiman, MPH, Ph.D. at Berkeley patients Group and Deborah Malka, M.D., Ph.D. at Medi-Cann provide a basis for and suggests further more focused in-depth studies.

The AACM received funding to do demographic study of 300 patients (100 chosen at random from three cannabinoid medicine specialists). The data was analyzed by Kim Judson, P.D., Professor at CSU Monterey Bay. We looked at such data as diagnoses, age, sex, occupation and other demographic data. This information helps provide a roadmap for what to look at next. Most common condition treatment was analgesia.

CHARACTERIZING CURRENT AND POTENTIAL CANNABINOID THERAPY USERS IN A PATIENT REGISTRY OBSERVATIONAL SURVEY STUDY

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

Methods: Participants (N = 703) who were either adult patients or caregivers of patients with a health condition requiring medical treatment completed an online survey hosted on Qualtrics. The patients had a mean age of 36.91; 61% were female, and 82% were Caucasian. 424 patients currently used cannabinoid products as therapeutic interventions to assist with at least one health condition. 279 patients were considering use of cannabinoid products as a treatment, but were not using cannabinoids at the time the survey was completed. Cannabinoid product users and non-users were assessed for age, gender, race, education level, primary medical condition, number of emergency room (ER) visits, patient quality of life (QOL), and current number of over-the-counter (OTC) and prescription medications currently used. Number of sick days, quality of sleep, and average pain over the past 30 days were also assessed. Independent samples t-tests and chi-square analyses were used to assess differences between cannabis users and non-users on study outcomes.

Results: The demographics of the cannabinoid users versus non-users were comparable; groups did not differ on gender, race or educational achievement. On average, current users of cannabinoid therapies were older than non-users (M = 38.58, SD = 21.28 vs. M = 34.38, SD = 20.11; t(701)= -2.61, p = 0.009), were more likely to have a primary medical condition of chronic pain $\chi^2(1)= 4.40$, p = 0.045), and less likely to have a neuropsychiatric condition as the primary medical concern $\chi^2(1)= 4.31$, p = 0.038). With regards to health-related outcomes, current users of cannabinoid products reported fewer ER visits (M = 0.4, SD = 0.77 vs. M = 0.74, SD = 1.31; t(172) = 2.49, p = 0.014) and fewer sick days taken from work/school (M = 4.18, SD = 8.15 vs. M = 5.81, SD =9.65; t(447)= 2.14, p = 0.033) in the past month compared with non-users. The differences in ER visits and sick days was not due to the disparity between groups on the rate of chronic pain or neuropsychiatric disorder as the primary medical condition. No significant differences between cannabinoid users and non-users were observed for the number of OTC or prescription medications, or for individual item ratings of patient QOL, quality of sleep, or average pain rating in the 30 days prior to survey completion.

Conclusions: In a convenience sample of individuals with significant health problems, current users of cannabinoid products for therapeutic purposes had fewer emergency room visits and fewer sick days on average compared with demographically matched individuals who were contemplating use of cannabinoids for therapeutic purposes, but who had not initiated use. Those using cannabinoid products were also older, more likely to have chronic pain and less likely to have a neuropsychiatric condition as their primary medical concern. This suggests that there may be some added benefit of cannabinoid use on current health care utilization and outcomes for certain health conditions. Additional research is needed to prospectively evaluate the impact of cannabinoid use on health-related outcomes and to identify specific health conditions and cannabinoid product characteristics are associated with greater therapeutic benefit.

THE COMPOSITE CANNABIS ASSESSMENT TOOL (CCAT): DEVELOPMENT AND VALIDATION OF A NEW MEASURE FOR THE CONCURRENT ASSESSMENT OF MEDICAL AND NONMEDICAL CANNABIS USE

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Humans use cannabis for a variety of purposes and across diverse contexts. Whereas some cannabis use may be problematic, it may also serve a legitimate therapeutic purpose, or be a benign recreation. Adapting assessment tools to the evolving contexts of cannabis use is a research priority, as current approaches to cannabis use assessment focus primarily on problematic use, and thus provide an impression that may be incomplete in ways that are clinically important. The Composite Cannabis Assessment Tool (CCAT) was developed to provide a brief measure to better identify and differentiate the diverse motivations and consequences associated with cannabis use.

A preliminary set of 25 candidate items was generated through collaboration with experts in the field and a thorough examination of the research literature on medical and non-medical cannabis use. The 25-item set was administered to 482 adult cannabis users in the community. Using principle components analysis, we identified a three-factor structure characterized by factors reflecting therapeutic, negative, and positive facets of cannabis use. The three items from each factor with the highest item-to-factor correlations were selected to create a 9-item measure consisting of three subscales. The 9-item set was administered to 2133 medical cannabis users, and the three-factor solution was replicated. Subscales demonstrated adequate internal validity (α= .61 - .76). As predicted, medical users evinced substantially higher scores on the therapeutic subscale ($F(1, 2634) = 1725.27$, $p<.01$). Medical users also scored slightly higher on the problematic subscale ($F(1, 2778) = 12.70$, $p<.01$). An aggregate score that incorporated all subscales using a simple formula (*therapeutic* – (*negative* – *positive*)) was also able to discriminate among samples ($F(1, 2597) = 636.67$, $p<.01$). These preliminary results suggest that the CCAT represents a potentially useful addition to the cannabis assessment armamentarium with potential utility for clinical and research contexts.

THE CURRENT REGULATORY LANDSCAPE OF MEDICINAL CANNABIS IN BRAZIL

Antonio A. Vieira Neto

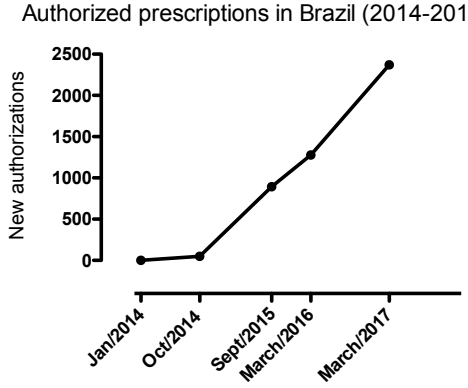
Entourage Phytolab, Brazil

In the last 2 years, the National Health Surveillance Agency (ANVISA) promoted a series of changes in its legal system in order to facilitate the access of Brazilian patients to the *Cannabis*-based products, regardless of its concentration of cannabidiol (CBD) and/or delta-9-tetrahydrocannabinol (THC), turning medical *Cannabis* into a reality in the country. *Cannabis* was already supported by the Federal Council of Medicine (CFM), which in 2014 defined the prescription guidelines for CBD-containing products. On May 6, 2015 and on March 18, 2016 ANVISA enacted Resolutions of its Collegiate Board No. 17 and No. 66, respectively, as amended, which established the criteria and procedures for the importation of CBD and or THC-based products for individuals and for personal use upon the presentation of a registered medical prescription.

Since the implementation of this normative system, Brazilian patients have sought in *Cannabis* a relief to a large variety of ailments, provided that nearly (i) 17% of the registered patients import such products for purposes of mental and behavioural disorders; (ii) 55% for the treatment of diseases of the nervous system; (iii) 13% for the treatment of neoplasms; (iv) 1% for skin and subcutaneous tissues ailments; and (v) 1% for the treatment of infectious and parasitic diseases(source: CGPCON/Anvisa). Ever since ANVISA issued the aforementioned regulations, the number of registered patients has been increasing monthly and has reached the sum of nearly 2370 registered patients up to date. There were 1279 between April 2014 and March 2016 (total of 1.8 new authorizations/day) and it grew 67% to 3 new authorizations per day in 2017. Therefore, at least 1000 new import authorizations are expected for the current year.

Although ANVISA has created the right to *Cannabis*-based products, rapid mechanisms that allow access to such products are still needed, given that the whole import process may take up to 45 days.

As a suggestion, such procedures could be improved and shortened by means of (i) granting a Brazilian company a licence to store such products in Brazil; (ii) promote the distribution of such products in the Brazilian territory, and/or, (iii) granting manufacture license to registered products in industrial scale. Such measures would assure Brazilian patients immediate availability of the necessary products and facilitate ANVISA's supervision. There is a favorable environment for such measures, and as the number of patients is increasing rapidly, it is undeniable that ANVISA shall be able to appropriately regulate this market to meet Brazilian patients' expectations and necessities.



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

Jahan Marcu[1], Steph Sherer[2] and Pavel Kubů[3]

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

Recently, PFC and international partner groups have started incorporating European Union standards for hemp and cannabis. This presentation will provide an update on cannabis regulations in the US and Europe, data on cannabis dispensary staff training in the US, as well as a data grading all US states, with a medical cannabis program, under a 400 point assessment with a 5 general categories: Patient rights and civil protections, access to medicine, ease of navigation, functionality, and consumer safety

(see example of the analysis below).

MEDICAL CANNABIS ACCESS STATE REPORT CARD **2017**

CONNECTICUT

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AREAS FOR IMPROVEMENT

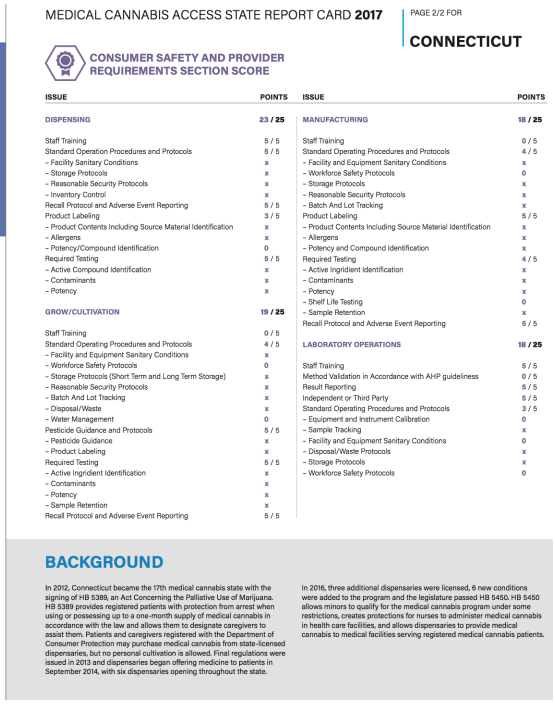
Patients would benefit from lower prices and a greater variety of products by lifting the single-dispensary designation requirement. The lack of civil discrimination protections and parental rights protections put patients in jeopardy of discrimination. Connecticut regulators should also consider adding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

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

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

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

FINAL GRADE B-



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

CONSUMER SAFETY AND PROVIDER REQUIREMENTS SECTION SCORE

ISSUE	POINTS
DISPENSING	**23 / 25**
Staff Training	5 / 5
Standard Operation Procedures and Protocols	5 / 5
– Facility Sanitary Conditions	x
– Storage Protocols	x
– Reasonable Security Protocols	x
– Inventory Control	x
Recall Protocol and Adverse Event Reporting	5 / 5
Product Labeling	3 / 5
– Product Contents Including Source Material Identification	x
– Allergens	x
– Potency/Compound Identification	0
Required Testing	5 / 5
– Active Compound Identification	x
– Contaminants	x
– Potency	x
GROW/CULTIVATION	**19 / 25**
Staff Training	0 / 5
Standard Operating Procedures and Protocols	4 / 5
– Facility and Equipment Sanitary Conditions	x
– Workforce Safety Protocols	0
– Storage Protocols (Short Term and Long Term Storage)	x
– Reasonable Security Protocols	x
– Batch And Lot Tracking	0 / 2
– Disposal/Waste	x
– Water Management	0
Pesticide Guidance and Protocols	5 / 5
– Pesticide Guidance	x
– Product Labeling	x
Required Testing	5 / 5
– Active Ingridient Identification	x
– Contaminants	x
– Potency	x
– Sample Retention	x
Recall Protocol and Adverse Event Reporting	5 / 5

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

BACKGROUND

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

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

MEDICINAL CANNABIS USE IN AUSTRALIA: A CONSUMER SURVEY

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The Australian medical cannabis landscape is currently in a state of flux. Recent legislative changes have introduced a federal licensing system for local cultivation and manufacturing, expedited the importation of cannabis products from oversease companies, and revised the scheduling classification of cannabis. These changes have the potential improve patient access to legal medicinal cannabis across Australia. However, the reality is that most users still currently rely on illicit sources for access to cannabis. Given its continuing illegality, little is known about how the Australian community accesses and uses medicinal cannabis. An improved understanding of the current situation could help inform policy and shape future regulatory and clinical frameworks and medication development in Australia.

The CAMS (Cannabis As Medicine Survey) study is the first large scale survey of medicinal cannabis users in Australia in more than a decade. The intention of the survey was to create a national snapshot of the demographics of medical cannabis consumers, the conditions that are currently being treating with cannabis, the patterns of cannabis use, perceived efficacy of cannabis products, as well the physical and mental health of consumers. The survey was conducted on-line with recruitment facilitated via social media and consumer advocacy groups.

There was a total of 1749 respondents with 68% of respondent males. Survey participants were spread evenly across the age groups (18-25=23%, 26-35=25%, 36–45=22%, 46-60=25%). The majority of participants had used cannabis everyday in the past month (52%). A total of 45% of those surveyed had never used cannabis recreationally prior to commencing their medicinal use. Most people (80%) used less than 5 grams of cannabis per day. The most popular route of administration was via a water pipe or bong (42%) followed by smoking a joint (20%) and using a vaporiser (13%).

Chronic pain was the primary condition being treated by medical cannabis users (34%) followed by anxiety disorders (15%), depression (11%) and sleep disorders (7%). Of those using cannabis to treat pain, back pain was the most common ailment (48%), followed by arthritis (16%) and neuropathy (14%). Respondents reported overwhelmingly positive changes in their primary health condition being treated as a result of cannabis use, with over 90% of respondents reporting that their primary condition was much improved, or very much improved, across pain, anxiety, depression and sleep disorders.

Interestingly, in terms of potential harms, the proportion of people meeting DSM-IV criteria for cannabis dependence was 11.8% if they had never used cannabis prior to their use for medical reasons. In contrast, 24.7% of those who had used cannabis recreationally before shifting into medicinal use met DSM-IV dependence criteria. Dependence on essential medicines is an active area of research in our group and it should be noted that standard definitions of dependence do not always fit comfortably with medicinal use of drugs.

It is hoped that data generated from the CAMS survey will help inform Australian policy makers and medical professionals and avoid a disconnect with consumers of medical cannabis. We plan to run the CAMS survey annually to track trends in medicinal cannabis use across time in Australia.

Acknowledgements: Funded by the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney.

MEDICAL CANNABIS USE IS ASSOCIATED WITH DECREASED USE OF PRESCRIPTION AND OVER-THE-COUNTER SLEEP MEDICATIONS

Gwen Wurm, Julia Arnsten and Marcus Bachhuber

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Introduction: Cannabis may improve the quality and duration of sleep and help treat various sleeping disorders, but no states have approved medical cannabis for treating sleep disorders.

Understanding whether medical cannabis users are using for sleep is important to inform policy.

Methods: From 8/16-10/16, customers at two cannabis dispensaries in Colorado completed a survey about their use. Customers who had provided a cell phone or email contact received a survey link.

Results: 240 individuals responded as users of medical cannabis. 59% were male, 25% were <30 years old, 53% were 30-49, and 22% were ≥50. 41% had completed college or more, and 30% had attended some college. Two-thirds (64%) were white, 12% Black, and 5% other races, with 19% identifying as Hispanic. 84% (n=202) used cannabis for sleep. Among those who used for sleep, 73% reported using cannabis nightly for sleep and 20% reported using 2-3 times per week for sleep; the vast majority (86%) stated that cannabis was very or extremely helpful. Among those who previously used prescription or over-the-counter sleep medications, 73% (53/73) reported decreasing or stopping prescription sleep medication since using cannabis, and 83% (76/92) reported decreasing or stopping over-the-counter sleep medications since using cannabis. Over 90% (188/202) of medical cannabis users for sleep, also used for pain.

Conclusions: Among medical cannabis users, many use cannabis regularly to help with sleep. Users report decreased use of both prescription and non-prescription sleep medications. There is a strong relationship between use of medical cannabis for sleep and for pain.

USING MEDICAL CANNABIS TO TREAT PAIN AND IMPACT ON PAIN MEDICATION USE

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Introduction: Despite increased medical cannabis availability, little is known about the impact of use on both prescription and over the counter pain medication from the perspective of patients that visit a dispensary. Understanding how medical cannabis users are self-treating pain is important to inform policy.

Methods: From 8/16-10/16, customers at two cannabis dispensaries in Colorado completed a survey about their use. Customers who had provided a cell phone or email contact received a survey link.

Results: 240 individuals responded as users of medical cannabis. 59% were male, 25% were <30 years old, 53% were 30-49, and 22% were ≥50. 41% had completed college or more, and 30% had attended some college. Two-thirds (64%) were white, 12% Black, and 5% other races, with 19% identifying as Hispanic. Of the medical cannabis group 92% (221/240) of respondents used cannabis to treat pain, including back pain (57%), headache (32%), chronic daily pain (43%), musculoskeletal pain (25%), injury-related pain (35%), and menses (8%), and most (87%) used daily to treat pain. The vast majority (90%) found cannabis very or extremely helpful for treating their pain. Among those who previously used prescription opioid pain medication in the last 6 months (149/220), 82% reported decreasing or stopping prescription pain medication since using cannabis. Among those that used over-the-counter analgesics in the last 6 months over 80% reported decreasing or stopping over-the-counter pain medications. Of the group that used for pain 85% (188/221) also used for sleep.

Conclusions: Among medical cannabis users, many use cannabis regularly to treat a wide variety of pain syndromes. Users report decreased use of both prescription opioid and non-prescription analgesics in management of their pain. There is a strong relationship between use for pain and sleep in this population.

USING RECREATIONAL CANNABIS TO TREAT PAIN AND IMPACT ON PAIN MEDICATION USE

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Introduction: Despite increased medical cannabis availability, marijuana is available without a prescription in only eight states and remains the most commonly used federally illicit drug. This suggests substantial overlap between medical and recreational use, especially in treating pain. Understanding how recreational cannabis users are self-treating pain is important to inform policy.

Methods: From 8/16-10/16, customers at two recreational cannabis stores in Colorado completed a survey about their use. Customers who had provided a cell phone or email contact received a survey link.

Results: Among 1,492 respondents, 23% identified as medical cannabis users and were excluded from the sample. Of the remaining 1,146 recreational user respondents, 58% were male, 40% were <30 years old, 48% were 30-49, and 11% were ≥50. One-third had completed college or more, and 35% had attended some college. Two-thirds (67%) were white, 11% Black, and 15% other races, with 17% identifying as Hispanic. 65% (n=742) of respondents used cannabis to treat pain, including back pain (53%), headache (39%), chronic pain (33%), musculoskeletal pain (23%), injury-related pain (22%), and menses (14%), and most (72%) used daily to treat pain. Among those who previously used prescription or over-the-counter pain medication, 88% (310/351) reported decreasing or stopping prescription pain medication since using cannabis, and 83% (473/585) reported decreasing or stopping over-the-counter pain medications.

Conclusions: Among recreational cannabis users, many use cannabis regularly to treat a wide variety of pain syndromes. Users report decreased use of both prescription and non-prescription analgesics associated with recreational cannabis use.

THE EFFECTS OF MEDICAL CANNABIS USE ON CHRONIC PAIN AND SLEEP

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Sunnybrook Health Science Centre, Toronto, Ontario, Canada
Allevio Pain Management Clinic, Toronto, Ontario, Canada

Reports of sleep disturbances are particularly high in chronic pain patients (Menefee et al., 2000). In this patient population, the prevalence of sleep disorders is estimated to be between 50-80% (Artner, 2003). A large body of evidence suggests that the relationship between pain and sleep is bidirectional (Koffel et al., 2016). Along these lines, addressing sleep disturbances in chronic pain patients may improve treatment outcomes in this population. Medical cannabis has demonstrated efficacy in treating both chronic pain symptoms (Abrams et al., 2007; Wilsey et al., 2008) and reducing sleep disturbances (Gates et al., 2014). The current prospective observational trial aimed to evaluate the effectiveness of medical cannabis to relieve chronic pain symptoms and improve sleep in a sample of 120 chronic pain patients following one month of treatment. Chronic pain was assessed using the Brief Pain Inventory (BPI). Sleep was assessed using 3 items derived from the Pittsburgh Sleep Quality Index (PSQI) and the sleep interference item from the BPI.

Following one month of medical cannabis use, patients reported a 19.7% reduction in the Pain Severity Subscale ($p<0.001$) and a 21.8% reduction in the Pain Interference Subscale ($p<0.001$) of the BPI. With regards to sleep, patients reported a 32.1% reduction of pain interference with sleep ($p<0.001$), based on the BPI. They also reported a 44.9% reduction in the time it takes to fall asleep ($p<0.001$), a 15.8 % increase in the duration of sleep ($p<0.001$) and a 40.4% increase in sleep quality ($p<0.001$), based on items from the PSQI. Results from this study suggest that medical cannabis use reduces chronic pain symptoms and improves sleep in chronic pain patients. Given the previously reported evidence that improvements in sleep can reduce sensitivity to pain, medical cannabis use may improve outcomes for chronic pain patients directly by acting on the pain pathway and indirectly by improving sleep.

SHORT-TERM EFFICACY OF CBD-ENRICHED HEMP OIL IN GIRLS WITH DYSAUTONOMIC SYNDROME AFTER HUMAN PAPILLOMA VIRUS VACCINATION

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Background: Cannabidiol (CBD)-based treatments for several diseases, including Tourette's syndrome, multiple sclerosis, epilepsy, movement disorders and glaucoma, are proving to be beneficial and the scientific clinical background of the drug is continuously evolving.

Objectives: To investigate the short-term effect of CBD enriched hemp oil for relieving symptoms and improving the life quality (QOL) in young girls with adverse drug effects (ADRs) following human papillomavirus (HPV) vaccine.

Methods: In this spontaneous spontaneous anecdotal, retrospective, "compassionate-use," observational, open-label study, 12 females (age 12–24 years) with severe somatoform and dysautonomic syndrome following HPV vaccination were given sublingual CBD-rich hemp oil drops, 25 mg/kg per day supplemented by 2–5 mg/ml CBD once a week until a maximum dose of 150 mg/ml CBD per day was reached over a 3 month period. Patients' quality of life was evaluated using the medical outcome short-form health survey questionnaire (Sf-36).

Results: Two patients dropped out due to iatrogenic adverse events and another two patients stopped the treatment early due to lack of any improvement. Sf-36 showed significant benefits in the physical component score ($P < 0.02$), vitality ($P < 0.03$) and social role functioning ($P < 0.02$) after the treatment. The administration of hemp oil also significantly reduced body pain according to the Sf-36 assessment. No significant differences from the start of treatment to several months post-treatment were detected in role limitations due to emotional reactions ($P = 0.02$).

Conclusions: This study demonstrated the safety and tolerability of CBD-rich hemp oil and the primary efficacy endpoint. Randomized controlled trials are warranted to characterize the safety profile and efficacy of this compound

ACUTE MARIJUANA USE: RETINAL GANGLION CELL DYSFUNCTION

Denise A. Valenti

IMMAD-Impairment Measurement Marijuana and Driving
A company for the responsible use of marijuana.
Quincy, Massachusetts, United States of American

Background: There are cannabinoid receptors throughout the retina with substantial density within the ganglion cell layers. Marijuana use has been demonstrated to cause dysfunction in the retinal ganglion cells with chronic use and photoreceptor and retinal ganglion cell dysfunction with acute use. Other research has shown decreased recovery of glare function and reduced central visual perceptions with acute use of marijuana. Studies have demonstrated enhanced peripheral detection in dark adapted participants. One of the more common diseases that causes retinal ganglion cell dysfunction is glaucoma. Neurologic disease that impacts retinal ganglion cells include Alzheimer's disease and Parkinson's disease. Tests of retinal ganglion cell function include visual field tests. This study identified functional impairment of the retina with acute marijuana use, utilizing a specialized visual field test that has identified retinal ganglion cell impairment in glaucoma, Alzheimer's disease and Parkinson's disease.

Methods: The retinal dysfunctions were identified during visual screenings. A visual field screening instrument, Frequency Doubling Technology (FDT) was in use to evaluate functional vision during simple vision screenings during events where attendees had potentially previously legally consumed marijuana. The screening consisted of standard eye tests of near and far acuity and basic motility tests. It is not out of the ordinary for screenings for diabetes or glaucoma to utilize a FDT during the screening and we included this test in the screenings. FDT tests seventeen ten degree squares within the central forty-degree field of each eye. The target is an alternating pattern of stripes of .25 cycles per degree that vary in contrast. The data produced is in two forms; the contrast in decibels and for each of the seventeen ten degree fields and a deviation percentage from a normative data base specific to age. Self-reports of dosing, quality of product and time of dosing were used. We also relied on self-reports of general health. The information obtained specific to the visual system did not differ from other traditional health screenings. Generally, a question about medications and recreational drug use is part of a vision eye screening also.

Results: The majority of those screened demonstrated significant dysfunction in many of the field areas compared to the age matched data base. There was considerable variability in the inferior field, but the deficits were present. Superior regions were impacted significantly.

Conclusion: Acute use of marijuana causes visual impairment in retinal ganglion cell function and this was demonstrated with a complex visual target that measures visual function. Further research is warranted. Such work would include correlations with biologic measures of cannabinoids and correlations with other functional test of vision such as electro diagnostic evaluations and brain imaging.

MEDICAL CANNABIS IN THE TREATMENT OF POST-TRAUMATIC STRESS DISORDER AND ITS ASSOCIATED SYMPTOMS

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Post-traumatic stress disorder (PTSD) is a psychiatric disorder characterized by chronic, irregular activation of the stress response following a major traumatic event. Many PTSD patients report using cannabis to cope with their symptoms (Bremner et al., 1996). A few studies have supported the use of cannabis and its active components in the treatment of PTSD (Roitman et al., 2014; Greer et al., 2014). However, there is a lack of literature on the use of medical cannabis in the treatment of PTSD symptoms. The aim of the current study was to examine the effectiveness of medical cannabis treatment to alleviate symptoms of PTSD and improve quality of life in PTSD patients in a prospective observational trial. The effect of 1 month of daily medical cannabis use on PTSD symptoms and quality of life was assessed in a sample of 37 PTSD patients, including veterans, first responders and civilians seeking treatment at Apollo Marijuana Clinics. PTSD symptoms were assessed using the PCL-5 (PTSD Checklist for DSM-5). The effects of medical cannabis use on health-related quality of life was explored using the 36-item short-form survey (SF-36).

Following medical cannabis treatment for 1 month, patients reported a statistically significant 22% reduction in PTSD symptoms as assessed by the PCL-5 ($p<0.001$). This reduction in symptom was accompanied by a 54% improvement in Emotional Well-Being Subscale of the SF-36 ($p<0.001$). Patients also reported an increase in 77% increase in the Social Functioning Subscale of the SF-36 ($p<0.001$). Results from this study suggest that medical cannabis use provides symptom relief for patients suffering from PTSD. Medical cannabis use also improves measures of quality of life in patients, through improvements in emotional and social functioning. Improvements in these areas are of interest as emotional and social functioning is often impoverished in PTSD patients. Together, these findings suggest that medical cannabis holds promise as a treatment for PTSD.

MEDICAL CANNABIS FOR ANXIETY AND DEPRESSION: EARLY LONGITUDINAL CLINICAL DATA

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While an emerging evidence-based literature suggests that cannabinoid medicines shows promise as a therapeutic agent in mood and anxiety disorders, robust data continue to be needed, both to characterize relevant symptomatic changes and chemovariants, allowing for optimized and personalized treatment. We have previously reported on an emerging database recorded by our clinic group since the inception of Canada's expanded medical cannabis program, (MMPR, Gov of Canada 2013, Saynor et al, 2016). Our methodology employs a standardized electronic questionnaire and data visualization instrument providing standardized severity self-ratings (5-point Likert Scale) of key common symptoms of Pain, Sleep Quality, Mood, Relaxation and Stress Perception, and overall Composite Wellness Score (CWS, range of 5-25).

We present data of a naturalistic observational cohort of 58 fully informed and consenting patients (M, n=46, mean age=38; F, n=12, mean age=41) *specifically reporting anxiety and/or depression as a primary presenting concern*, in format of fresh dried cannabis flora or fresh cannabis oil extracts (M, mean daily max authorized M= 5.7+/-3.2 g/day, F=4.5g+/-1.8g/day max). In this consecutive sample, only those patients with a minimum of follow-up visits of between 3 and 7 self-report tablet-based surveys were included. Standardized systematic inquiry of key symptoms was made at 6-month intervals, using our symptom rating tool and a 95% confidence interval for symptom rating comparisons. At time of initial consultation, (t=0), the full sample reported a mean of 2.1 ± 0.3 for Sleep ("Somewhat Poor"), 2.1 ± 0.2 for Stress ("Somewhat High"). 2.4 ± 0.3 for Relaxation ("Somewhat Tense" to "Neutral"), 2.7 ± 0.3 for Mood (close to "Neutral") and 3.0 ± 0.3 for Pain ("Neutral"). Baseline CWS was 12.2 ± 1.0.

At 12 months (t=12), the patient group (n=58) had achieved a 3.5 ± 0.3 score in Relaxation, a 1.1 point improvement since t=0; a 3.1 ± 0.3 score in Sleep Quality and in Stress, (1.0 point improvement in each). Improvement in Mood was not statistically significant, even less so in Pain. CWS was 16.7 ± 1.2, an improvement of 4.4 points since t=0. At 24 months (t=24), symptom self-report for a now reduced sample size (n=15) indicated that those participants who persist realized the greatest improvement in Relaxation at 3.7 ± 0.5, Stress at 3.5 ± 0.5, and Sleep at 3.5 ± 0.6; each up by 1.4 points since intake (t=0). Mood was now significant at 3.9 ± 0.4 points, an improvement of 1.2. Pain remained consistent. CWS was 18.1 ± 1.9, an improvement of 5.8 points since t=0. We suggest that despite early promising symptomatic relief with cannabis, for patients reporting primary mood/anxiety symptoms, mood improvements may require more persistent clinical follow-up, while capacity to cope with stressors improves earlier, and may mediate mood response. Complete data demographics are described and graphically represented.

References: 1. Government of Canada, Marihuana for Medical Purposes Regulations (2013). http://www.laws-lois.justice.gc.ca/eng/regulations/SOR-2013-119/
2. Saynor L, Sudan K, Moller HJ: Toward a naturalistic characterization of a medical cannabis population: emerging Canadian data. ICRS 2016 20th Annual Symposium of the International Cannabinoid Research Society, June 26- July 1, Bukovina, PL

BEDROCAN CLINICAL TRIALS – FIBROMYALGIA AND PALLIATIVE CARE

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Bedrocan has recently started its first two double-blind, placebo-controlled clinical trials with the use of standardized, pharmaceutical-grade medicinal cannabis and cannabis placebo, produced under Good Manufacturing Practice guidelines. The first trial is conducted in Leiden, The Netherlands, under the lead of Prof. Albert Dahan of the department of Anesthesiology of the Leiden University Medical Center. It is an investigation into how the symptoms of fibromyalgia are affected by the two major cannabinoids of the cannabis plant: delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Research suggests that cannabinoids have a beneficial effect on fibromyalgia symptoms by reducing pain and improving sleep quality. Although cannabis has not been established as an approved medicine for this medical condition, surveys indicate that fibromyalgia patients already self-medicate themselves using cannabis. Moreover, some patients have reported cannabis to produce fewer side-effects than the available conventional treatments and, most importantly, to be effective.

The second clinical study is being conducted at the Calvary Mater Newcastle Hospital and Sacred Heart Health Service in New South Wales, Australia, by the research team of Prof. Meera Agar. The trial inquires into the capacity of cannabis to improve the quality of life for terminally ill cancer patients in their final stages of life. Specifically, the study focuses on the effects of cannabis on appetite stimulation and related symptoms. In addition, it addresses questions about the safety, efficacy, dosage and frequency, side-effects and optimal delivery methods of cannabis, when administering it to terminally ill patients.

The presentation will provide an overview of both studies, including their design and the short- and long-term goals. Moreover, the two clinical studies will be presented in a broader context, which is the aim of Bedrocan to establish herbal cannabis as a fully approved medicine.

CHRONIC PAIN TREATMENT WITH CANNABIDIOL IN KIDNEY TRANSPLANT PATIENTS IN URUGUAY

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Chronic pain is a major therapeutic problem in kidney transplant patients due to the limitation of using NSAID because its nephrotoxicity. There is benefit of the modulation of the endocannabinoid system in the treatment of chronic pain. The use of cannabidiol (CBD) in kidney transplant patients has not been communicated previously.

Objective: We aimed to assess the effect, safety and possible drug interactions in kidney transplant patients treated with CBD for chronic pain.

Methods: We assessed patients who receive 50 mg twice a day of CBD for treatment of chronic pain. We included kidney transplant patients suffering from uncontrolled chronic pain with stable tacrolimus levels for at least two months.

Results: We assessed 4 patients mean age 63.5, who had asked for CBD pain treatment.

Table1. Base line characteristics and assessment by day of treatment per patient

	Patient 1			Patient 2			Patient 3			Patient 4		
Day	**1**	**4**	**7**	**1**	**4**	**7**	**1**	**4**	**7**	**1**	**4**	**7**
Age (years)	75			**58**			**61**			**60**		
Sex	**F**			**M**			**F**			**M**		
Pain cause	**FM**			**OA**			**FM**			**OA**		
Adv Reaction		nausea						Itch	Itch		APE	
Creatinine (mg/dl)	1.10	1.13	1.07	1.03	1.24	1.09	0.92	0.93	0.92	1.14	1.17	1.15
Hemoglobin (g/dl)	11.4	11.6	11.7	13.4	13.8	13.6	12.4	12.9	12.3	15	14.3	14.2
Leucocytes (mm3)	3990	4420	4340	7080	7360	7570	4480	4770	5470	8830	9140	9780
Platelets (10^3 mm3)	185	186	179	215	216	238	182	224	232	248	227	237
TGO/TGP (mg/dl)	14/11	16/12	16/12	14/18	29/24	16/19	20/12	17/12	16/12	16/9	17/10	18/10
Tacrolimus(5-15ng/ml)	10.1	8.2	10.7	7.4	9.2	7.9	14.4	14.5	17.4	9.7	10.1	10.3

FM=Fibromyalgia; OA=Osteoarticular disease; APE=Abdominal pain episode

Table 2. Individual pain assessment and physical limitation perception

Pain Score Index (1-10) / Limitation perception (none, mild, moderate, severe)								
Patient	**Previous week**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
1	6/moderate	5/moderate	5/moderate	5/mild	2/mild	2/mild	2/mild	3/mild
2	2/mild	2/none	2/none	2/none	2/none	2/none	2/none	2/none
3	4/mild/	1/none	1/none	1/none	3/mild	3/none	4/none	2/none
4	7/moderate	4/mild	4/mild	4/mild	4/mild	3/mild	4/mild	3/mild

CBD dose reduction to 50mg/day has been done on day 4 in patient n°1 due to persisting nausea. Tacrolimus dose reduction in patient nº3 has been done in day 4 and 7 due to persisting elevated levels (before CBD) and itching. No other intervention was made.

Conclusion: During this period of follow up, we didn't find any severe adverse effect. It is necessary to continue the follow up to assess effect, safety and possible drug interactions.

MEDICAL CANNABIS FOR THE TREATMENT OF CHRONIC HEADACHE

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Background: Chronic headache is a major public health issue and is a great burden for the person, the health care system, and society. Although conventional treatment options are effective, patients constantly seek alternatives. In 2007, the Israeli Ministry of Health began providing registrations for medical cannabis. Today there are roughly 27,000 medical cannabis patients in Israel, 8,000 (30%) receive treatment at Tikun-Olam (TO), almost 2% of them suffer from chronic headache. The aim of this study is to evaluate the safety and efficacy of the medical cannabis treatment in chronic headache patients.

Methods: We analyzed the data routinely collected as part of the treatment program on 70 chronic headache patients of TO.

Results: The average age was 47.1±14.9 years, 39.4% were males, 46.5% were employed, the median number of different medications per day was three and 47.9% of the patients reported previous experience with cannabis. The median pain intensity was 9/10 (IQR 8-10). In addition to pain, the main symptoms requiring therapy were: sleep problems (80.3%), vomiting (57.7%), weakness (39.4%) and anxiety (39.4%). At the six months follow-up, four patients discontinued the treatment and 31 patients (44.3%) responded to the questionnaire, 93.5% of them reported an improvement in their condition and 6.5% reported no change in their medical condition. The median pain intensity was reduced to 5/10 (IQR 3-6) ($p<0.001$). Twelve patients (38.7%) reported suffering from side effects. The most common side effects were nausea and dry mouth.

Conclusions: The treatment appears to be safe and efficacious. A larger cohort of patients are required in order to evaluate the effectiveness of cannabis for the treatment or prevention of migraine, tension-type headache and chronic headache disorders.

CANNABINOID-INDUCED HYPEREMESIS SYNDROME

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Cannabis is a recreational drug most widely used in the United States and worldwide. It has been used in the medical profession as an antiemetic and an appetite stimulant; however, chronic, heavy prolonged use has been shown to cause the poorly understood paradoxical Cannabinoid Hyperemesis Syndrome (CHS). CHS is a medical condition which was identified for the first time in 2004 case series of nine patients in Australia. CHS is characterized by cyclic episodes of uncontrollable vomiting as well as compulsive bathing in hot water. CHS progresses into three distinct phases: prodromal, hyper-emetic, and recovery.

The pathophysiology of CHS remains unclear with a dearth of research dedicated to investigating its underlying mechanism. Supportive care with intravenous fluids and cannabis cessation appears to be the widely-used treatment options. Increased awareness of CHS allows for earlier recognition by emergency departments, leading to prompter treatment and the prevention of future recurrence.

The aim of this study was to summarize the available evidence on CHS diagnosis, pathophysiology, and treatment. A PubMed search was performed using search terms like cannabinoid hyperemesis and compulsive bathing. A total of 89 case reports were included in this study of patients with CHS admitted to the emergency department that were published in the literature from 2004 to 2014. The average age was 28.9 years and majority of them (77.6%) are males. The average onset age of cannabis use was 16.69 and the average age when vomiting began was 24.05. The average period of time between the onset of symptoms and diagnosis of CHS was 4.56 years. Bathing in hot water was present in 87% of cases.

Acknowledgements: Funded by PhytoSciences.

A NOVEL PORTABLE CANNABINOID DETECTION DEVICE UTILIZING DIFFERENTIAL ION MOBILITY SPECTROSCOPY

Steven Knight[1*] and Pritesh Kumar[2]

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With recreational and medicinal marijuana legalization on the rise and documented research stating that consumption can result in certain cognitive and psychomotor impairment, law enforcement agencies will require a non-invasive, on-site, portable, precision instrument to determine if a motorist has consumed marijuana prior to, or while, operating a motor vehicle. A breathalyzer requires separation of complex organic matrices using a thermal desorption device following the analysis of the concentrated sample via a hybrid differential mobility spectrometer. We have designed an instrument which can be fine-tuned to target metabolites of cannabis, 11-hydroxy $^{\Delta}$9-THC, traces of the parent molecule $^{\Delta}$9-tetrahydrocannnabinol ($^{\Delta}$9-THC), or any cannabinoid of interest to determine if an individual has consumed cannabis within six hours, via smoking, or twelve hours, if orally ingested. This device requires significant experimentation to maximize the efficiency of detection for each cannabinoid. Furthermore, our device can be installed and uninstalled in a vehicle applying an ignition switch device, only allowing the ignition of the vehicle when the operators' breath sample has cannabinoids present in concentrations within the defied and pre-programed parameters.

Acknowledgements: Funded by PhytoSciences.

BEYOND SCHEDULE I OR II: ON THE DEVELOPMENT OF CANNABINOID-BASED DRUGS APPROPRIATE FOR LESS RESTRICTIVE SCHEDULING UNDER THE CONTROLLED SUBSTANCE ACT

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As medicines derived from cannabis move from clinical development and towards regulatory review in the United States, the influence of the U.S. Controlled Substance Act (CSA) on ultimate scheduling decisions for these drugs will have a profound impact on patients' access to these therapies, as well as their commercial viability. In developing CSA scheduling recommendations, the Drug Enforcement Administration, Food and Drug Administration, and National Institute on Drug Abuse, must consider eight factors listed in the CSA to develop their recommendations for new medicinal products. Although unusual, that can lead to products with the same active ingredient being scheduled differently. For example, FDA-approved codeine-containing products vary in their scheduling from Schedule (II) to the least restrictive (V) depending on the dose, the formulation and other ingredients. Thus, oral dronabinol products (e.g., Marinol®), in which the active ingredient is mixed sesame oil in capsules is placed in Schedule III, whereas a product with a synthetic version of the same substance and with the same pharmacological effects is placed in schedule II.

CSA scheduling determinations are very important in patient care and public health because the more restrictive scheduling, by design, increases the barriers to prescribing and use. For the most dangerous substances, such as morphine, hydrocodone, and fentanyl, which cause widespread addiction and more than 35,000 overdose deaths per year, Schedule II placement is well supported by the data. However, for cannabinoids, which rarely result in overdose death and whose overall profile of abuse-related effects is much weaker (National Academies, 2017), Schedule II is not only unsupported by the evidence, it can also produce the unintended consequence of reducing the likelihood that people in need will turn to cannabinoid-based medicines when natural cannabis products can be legally obtained in many states.

Lessons learned in the development and regulation of cannabinoids and more recently opioid medications have important implications for the development of cannabis-based pharmaceuticals. These lessons can not only increase their chances of approval for therapeutic use, but also to support recommendations for placement in schedules that are less restrictive than II. This poster will summarize the types of testing and outcomes that would likely be required to support placement in schedules less restrictive than II.

27TH Annual Symposium of the International Cannabinoid Research Society
June 22 - 27, 2017 Montréal, QC, CANADA

SCHEDULE OF EVENTS

Thursday, 22 June

16:00 – 18:00 **REGISTRATION** (Le Centre, Sheraton, Montréal)
18:30 – 20:00 **WELCOME RECEPTION**

DAY 1: Friday, 23 June

08:30 - 08:45 **WELCOME AND OPENING REMARKS**
08:45 – 10:30 **ORAL SESSION 1:** Inflammation and Autoimmunity
10:30 – 11:00 **COFFEE BREAK**
11:00 – 12:00 **ORAL SESSION 2:** Drug Development and Medicinal Chemistry
12:00 – 13:00 **LUNCH**
13:00 – 15:00 **POSTER SESSION 1 (P1)**
15:00 – 15:30 **ICRS YOUNG INVESTIGATOR PRESENTATION:** Cardiovascular Effects of Cannabidiol
15:30 – 16:45 **ORAL SESSION 3:** Endocannabinoids, Related Lipids and Cannabinoid Receptors
16:45 – 17:15 **COFFEE BREAK**
17:15 – 18:00 **ORAL SESSION 3** (continued)
18:00 – 19:00 **ICRS PRESIDENTIAL PLENARY SPEAKER:** Accomplices to Murder: Endocannabinoids Direct Microglia to Kill Newborn Neurons

DAY 2: Saturday, 24 June

08:30 – 10:30 **ORAL SESSION 4:** Pain
10:30 – 11:00 **COFFEE BREAK**
11:00 – 12:00 **ORAL SESSION 5:** Cannabis Use, Stress and Psychiatry
12:00 – 13:00 **LUNCH**
13:00 – 15:00 **POSTER SESSION 2 (P2)**
15:00 – 16:00 **ORAL SESSION 6:** Epilepsy
16:00 – 17:00 **ICRS PRESIDENTIAL PLENARY SPEAKER:** Sex-Dependent Synaptic Modulation in the Hippocampus
17:00 – 17:30 **COFFEE BREAK**
17:30 – 19:00 **ORAL SESSION 7:** Feeding, Metabolism and Obesity
19:00 **IN MEMORIAM**

DAY 3: Sunday, 25 June

08:30 – 10:00 **ORAL SESSION 8:** Fear, Anxiety and PTSD
10:00 – 10:30 **COFFEE BREAK**
10:30 – 11:00 **ORAL SESSION 8** (continued)
11:00 – 12:00 **KANG TSOU MEMORIAL LECTURE:** The Role of Genetic Variation in the Endocannabinoid System in Adolescent Brain Development
12:00 – **FREE TIME**
12:00 – 13:00 **ICRS BUSINESS MEETING**

DAY 4: Monday, 26 June

08:30 – 10:00 **ORAL SESSION 9:** Phytocannabinoids
10:00 – 10:30 **COFFEE BREAK**
10:30 – 11:00 **ICRS LIFETIME ACHIEVEMENT AWARD:** Cannabinoid / Serotonin Interactions in the Regulation of Nausea
11:00 – 12:00 **ORAL SESSION 10:** Human Studies
12:00 – 13:00 **LUNCH**
12:15 – 13:00 **NIDA Career Infosession**
13:00 – 15:00 **POSTER SESSION 3 (P3)**
15:00 – 16:00 **ORAL SESSION 10** (continued)
16:00 – 16:15 **COFFEE BREAK**
16:15 – 18:00 **ORAL SESSION 11:** Reward / Addiction
20:00 – **AWARDS CEREMONY & ICRS BANQUET**

Tuesday, 27 June **DEPARTURE**

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