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conference 2015

7th European Workshop on Cannabinoid Research and IACM 8th Conference on Cannabinoids in Medicine

17-19 September 2015
Sestri Levante, Italy

PROGRAM AND ABSTRACTS

7th European Workshop on Cannabinoid Research & IACM 8th Conference on Cannabinoids in Medicine
17—19 September 2015 in Sestri Levante, Italy

Place
Convento dell'Annunziata
Via Portobello
16039 Sestri Levante (GE)
Italy

Registration Fee
The registration fee is 300 Euros. Students pay a reduced fee of 150 Euros. The registration fees include daily rates (lunch for all days, coffee during the breaks) and an evening dinner on Saturday.

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GENERAL INFORMATION

Badges

Please wear your badge at all times during the conference. You will also need to wear it for the evening dinner.

Conference Dinner

On Saturday evening we will have our gala dinner at the conference place. Please tell us during registration if you would like to participate in the gala dinner.

Poster Sessions

There will be three poster sessions at the conference:

Session 1: Thursday, September 17, 18:15 - 19:30 after the last talk.

Session 2: Friday, September 18, 12:30 - 14:30 during and after the lunch break.

Session 3: Saturday, September 19, 12:15 - 14:00 during and after the lunch break

All posters will be available until the end of Poster Session 3 on Saturday. Presenters of posters with even numbers will be present at Poster Session 2, presenters of posters with odd numbers will be present at Poster Session 3. All presenters of posters will be present at Poster Session 1.

Thursday, September 17

Registration

13:30-13:45   Opening

13:45-14:25   **Plenary Lecture I**
Chair: Giovanni Marsicano

Daniele Piomelli (USA)
Unhealthy skepticism: the future of endocannabinoid-based therapeutics

14:25-18:15   **Session 1: Neural Circuits (physiology and anatomy)**
Chair: Istvan Katona

14:25-14:50   **Jaideep Bains** (Canada)
Non-canonical endocannabinoid signaling at hypothalamic synapses

14:50-15:15   **Raffaella Tonini** (Italy)
Synaptic endocannabinoid signaling at striatal projection pathways in the pathophysiology of motor control

15:15-15:40   **Istvan Katona** (Hungary)
Cell type-specific STORM superresolution imaging of cannabinoid signaling in brain circuits

15:40-16:05   **Daniela Cota** (France)
Novel insights on the role of the endocannabinoid system in energy balance regulation

16:05-16:30   **Tamas Horvath** (USA)
POMC neurons become orexigenic in response to CB1R activation in fed mice

16:30-17:00   **Coffee Break**

17:00-18:15   **Selected Talks**
Chair: Tiziana Rubino

17:00-17:15   **Jürg Gertsch** (Switzerland)
Peptide endocannabinoids (pepcans) – Their emerging role as endogenous allosteric CB receptor modulators

17:15-17:30 **Marc P. Baggelaar** (The Netherlands
A chemical biology approach to discover selective endocannabinoid biosynthesis inhibitors

17:30-17:45 **Zsolt Lenkei** (France)
Actomyosin contraction mediates presynaptic long-term synaptic plasticity

17:45-18:00 **Arnau Busquets-Garcia** (Spain/France)
Central and peripheral cannabinoid CB1 receptors control stress-induced amnesia

18:00-18:15 **Carolina Muguruza** (France)
Motivated to run: the role of cannabinoid type-1 (CB1) receptors

18:15-19:30 **Poster session I**

Friday, September 18

8:30-10:10 **Session 2: Neurons, Glia and Brain Dysfunctions**
Chair: Giovanni Marsicano

8:30-8:55 **Thomas Nevian** (Switzerland)
Endocannabinoid mediated long-term depression at cortical synapses

8:55-9:20 **Alfonso Araque** (USA)
Synaptic regulation by endocannabinoids through astrocyte signaling

9:20-9:45 **Silvia Marinelli** (Italy)
Bimodal regulation of GABAergic signalling by cortical cannabinoid type-2 receptors

9:45-10:10 **Olivier Manzoni** (France)
Prefrontal endocannabinoid synaptopathies: bad food, bad mood and beyond.

10:10-10:40 **Coffee Break**

10:40-11:30	**Session 3: Pain**
	Chair: Daniele Piomelli

10:40-11:05	**David Finn** (Ireland)
	Pain modulation by negative affective state: role of the endocannabinoid system

11:05-11:30	**Victoria Chapman** (UK)
	Endocannabinoid modulation of chronic pain states

11:30-12:30	**Selected Talks**
	Chair: Miriam Melis

11:30-11:45	**Andrea Ruiz-Calvo** (Spain)
	Potential neuroprotective role of specific CB1 receptor subpopulations in the mouse corticostriatal circuitry

11:45-12:00	**Tifany Desprez** (France)
	Mitochondrial CB1 receptors are required for amnesic effects of cannabinoids

12:00-12:15	**Francesco Papaleo** (Italy)
	COMT modulation of long-term memory through dysregulation of the endocannabinoid system

12:15-12:30	**Clémentine Bosch-Bouju** (France)
	Endocannabinoid-dependent plasticity is a synaptic correlate for social defeat-induced anxiety

12:30-14:30   Lunch & Poster Session II

14:30-16:10	**Session 4: Immune and metabolic control**
	Chair: Francisco Molina-Holgado

14:30-14:55	**Beat Lutz** (Germany)
	To be fat or not - the many faces of the CB1 receptor

14:55-15:20	**Valerio Chiurchiu** (Italy)
	Endocannabinoid signaling in Innate and Adaptive Immunity: from basic immune control to potential treatment of chronic inflammatory diseases

15:20-15:45	**Sophie Lotersztajn** (France)
	The endocannnabinoid system in the liver: novel perspectives

15:45-17:55	**Session 5: Stress, addiction and memory (Sponsored by Aelis Farma)**
	Chair: Joseph Cheer

15:45-16:10	**Marco Pistis** (Italy)
	Emerging role of N-acylethanolamines and their receptors in neuropsychiatric disorders

16:10-16:35	**Daniela Parolaro** (Italy)
	Cannabis and adolescence in rodents: from behavior to epigenetics

16:35-17:05	**Coffee Break**

17:05-17:30	**Andres Ozaita** (Spain)
	Targeting the endocannabinoid system for intellectual disability: clues from mouse models

17:30-17:55	**Pier-Vincenzo Piazza** (France)
	From an endogenous defense to a new therapy of cannabis abuse

17:55-18:55	**IACM General Meeting**

Saturday, September 19

8:15-9:00	**Business Meeting of the European Workshop on Cannabinoid Research**

9:00-9:45	**Plenary Lecture II**
	Chair: Giovanni Marsicano
	Vincenzo di Marzo (Italy)
	Endocannabinoids and cannabinoids: not just brainy but also brawny

9:45-12:15	**Session 6: Cannabis-based medicines: some potential applications**
	Chair: Manuel Guzman

9:45-10:15	**Roger Pertwee** (UK)
	New potential therapeutic applications for certain phytocannabinoids

revealed by pharmacological discoveries

10:15-10:45 **Ethan Russo** (USA)
Under-investigated Indications in Cannabis Therapeutics

10:45-11:15 **Coffee Break**

11:15-11:45 **Guillermo Velasco** (Spain)
Towards the utilization of cannabinoids as anticancer agents

11:45-12:15 **Mauro Maccarrone** (Italy)
Endocannabinoid signalling at the skin/immune cell interface

12:15-14:00 **Lunch & Poster Session III**

14:00-15:00 **Selected Talks**
Chair: Kirsten Müller-Vahl

14:00-14:15 **David Baker** (UK)
VSN16R a safe, new drug to treat spasticity

14:15-14:30 **Michelle Sexton** (USA)
An international survey of medical cannabis use: use patterns and health effects

14:30-14:45 **Yuval Zolotov** (Israel)
Adherence to medical cannabis among licensed patients in Israel

14:45-15:00 **Attila Oláh** (Hungary)
Investigation of the anti-acne effects of fatty acid amide hydrolase inhibitors on human sebocytes

15:00-15:50 **Session 7: Pharmaceutical perspectives of cannabinoid-based medicines**
Chair: Mark Ware

15:00-15:25 **James Brodie** (UK)
Pharma perspective 1: GW Pharmaceuticals

15:25-15:50 **Tjalling Erkelens / Arno Hazekamp** (The Netherlands)
Pharma perspective 2: Bedrocan

16:20-18:20	**Session 8: Clinical and patient perspective of cannabinoid-based medicines**

Chair: Roger Pertwee

16:20-16:50   **William Notcutt** (UK)
Clinical perspective

16:50-17:20   **Mark Ware** (Canada)
The Canadian experience

17:20-17:40   **Ilya Reznik** (Israel)
The Israeli experience

17:40-18:00   **Franjo Grotenhermen** (Germany)
Perspectives from a doctor's office in Germany

18:00-18:20   **Alberto Sciolari** (Italy)
Perspectives from an Italian patient

20:30	**Gala Dinner with IACM- and Poster Award Ceremony**

IACM Conference 2017

Save the date: The IACM 9th Conference on Cannabinoids in Medicine will be hold on 15-16 September 2017 in Cologne, Germany.

Oral Presentations

NON-CANONICAL ENDOCANNABINOID SIGNALING AT HYPOTHALAMIC SYNAPSES

Jaideep S. Bains and Jaclyn Wamsteeker Cusulin, Hotchkiss Brain Institute,
University of Calgary, Calgary, AB, Canada T2N 4N1

Introduction: Endocannabinoids (eCBs) are nearly ubiquitous retrograde messengers at synapses throughout the nervous system. eCB signaling, and particularly presynaptic CB1 receptor (CB1R) function is labile, exhibiting bidirectional plasticity following many *in vivo* experiences such as stress. What specifically about these experiences drives changes in synaptic eCB signaling, however, remains unclear. One possibility is that the activity levels of the presynaptic axons and terminals that express CB1Rs is deterministic for eCB signaling efficacy. Since activity in neural circuits is driven by experience, we asked whether presynaptic CB1R function reflects the representation of an experience in discrete neural circuits.

Methods: In order to test this idea, we examined GABA synapses onto parvocellular neuroendocrine cells (PNCs) in the paraventricular nucleus (PVN) of the hypothalamus. These cells serve as a critical node for the integration of stress circuit activity by coordinating the output of the hypothalamic-pituitary-adrenal (HPA) axis.

Results: We report that exposing male rats (p21-35) to repeated homotypic immobilization stress, results in functional loss of eCB signaling at these synapses. This loss of eCB signaling recovers spontaneously within five days, but exposure to a novel stress caused an immediate restoration of the eCB signaling at GABA synapses. Furthermore, this recovery was mimicked by the general recruitment of limbic circuits by electroconvulsive seizure (ECS) in vivo, or by manipulations that increase neural activity in vitro.

Discussion: The present results demonstrate that an experience with high salience, specifically novel stress, can erase the synaptic effects of repeated stress load, suggesting that shifts in the state of synaptic eCB signaling are controlled by the relative value of an experience. In addition, our data demonstrate that artificial representation of experience, through manipulation of neural and synaptic activity, is sufficient to gate the synaptic availability of the eCB system. Together these findings suggest that the relative salience an experience may impact information processing in a neural circuit by moving synapses in and out of an optimal working range.

CELL TYPE-SPECIFIC STORM SUPER-RESOLUTION IMAGING OF CANNABINOID SIGNALING IN BRAIN CIRCUITS

István Katona

Institute of Experimental Medicine, Hungarian Academy of Sciences,
Budapest, H-1083; Hungary

Pathophysiological activity of synaptic endocannabinoid signaling is associated with several brain disorders. For example, cell type-specific quantitative molecular changes in the density and nanoscale location of key endocannabinoid signaling molecules at chemical synapses have been described in temporal lobe epilepsy and in Fragile X Syndrome. In the present lecture, I will introduce a new imaging approach based on the combination of patch-clamp electrophysiology with correlated confocal and Stochastic Optical Reconstruction Microscopy (STORM) super-resolution imaging, which makes cell-type specific molecular investigations possible at the nanoscale level together with the direct measurements of related physiological and anatomical parameters. In addition, a new open-source software called VividSTORM will also be presented. VividSTORM enables the efficient visualization and measurement of the nanoscale density of any target protein in a cell type-specific manner in association with any physiological and pathophysiological processes. This new approach allowed us to decipher some key molecular parameters, which contribute to the cell-type specific differences in the strength of synaptic endocannabinoid signaling under physiological conditions in intact brain circuits. Moreover, this methodology could visualize robust, but reversible downregulation of presynaptic CB_1 receptors on GABAergic axon terminals after chronic high-dose THC treatment demonstrating its usefulness to monitor molecular changes in a cell-type-specific manner. Interestingly, highly elevated tissue concentration of the endocannabinoid 2-AG did not evoke a similar adaptive response, but resulted in a strong tonic decrease in GABAergic transmission at perisomatic, but not at dendritic inhibitory synapses. These findings show a surprising cell-type specificity of tonic endocannabinoid signaling and also suggest that the molecular tolerance mechanisms of presynaptic CB_1 receptors may be different for exogenous and endogenous cannabinoid ligands.

NOVEL INSIGHTS ON THE ROLE OF THE ENDOCANNABINOID SYSTEM IN ENERGY BALANCE REGULATION

D. Cota[1,2]

[1] INSERM U862, Neurocentre Magendie, Physiopathalogie de la plasticité neuronale, Bordeaux F-33000, France. [2] Neurocentre Magendie, Physiopathalogie de la plasticité neuronale, Université de Bordeaux, U862 Bordeaux F-33000, France

The melanocortin system is perhaps the best characterized hypothalamic neuronal circuit involved in the regulation of energy balance. This system includes pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP) neurons, which project to several structures, including the hypothalamic paraventricular nucleus (PVN), and exert opposite effects on food intake and body weight. The endocannabinoid system and the intracellular mechanistic target of rapamycin (mTOR) have been both implicated in the hypothalamic regulation of energy balance, however their function within the melanocortin system is poorly understood.

Methods: *In vivo,* studies evaluating acute food intake responses to the mTOR inhibitor rapamycin in mice lacking components of the mTOR pathway or cannabinoid –type receptor 1 (CB1R) in POMC neurons as well as in mice undergoing specific and reversible activation of POMC neurons through pharmaco-genetic DREADD approach. *In vitro,* electrophysiology in (i) POMC-YFP mice to assess POMC neuron excitability and in (ii) WT and POMC-CB1–KO mice to assess implication of mTOR, melanocortin and endocannabinoid signaling in the control of neurotransmitter release on parvocellular neurons of the PVN. *Ex vivo*, assessment of hypothalamic POMC-derived melanocortins and endocannabinoid levels respectively by RIA and liquid-mass spectrometry.

Results: Central inhibition of mTOR by intracerebroventricular administration of rapamycin induced strong hyperphagia in previously fasted mice. This was due to specific inhibition of the mTOR complex 1 (mTORC1) pathway in POMC neurons and consequent decreased hypothalamic levels of the POMC-derived anorectic peptide α-MSH, leading to increased hypothalamic levels of the endocannabinoid anandamide. Rapamycin rapidly induced the firing of POMC, GABAergic-like, neurons while inhibiting POMC, glutamatergic-like, neurons. In parvocellular neurons from WT mice, rapamycin or CB1R agonist reduced miniatures excitatory postsynaptic currents (mEPSCs) frequency, an effect that was reversed by the activation of melanocortin receptors by α-MSH, pharmacologic CB1R blockade or deletion of CB1R from POMC neurons. CB1R on gabaergic POMC neurons participates to the rapamycin-induced hyperphagia as suggested by the ability of the GABA inhibitor picrotoxin to blunt rapamycin effects in POMC-CB1-KO but not WT mice. Accordingly, activation of CB1R reduced miniatures inhibitory postsynaptic currents (mIPSC) frequency in parvocellular neurons of WT but not in POMC-CB1-KO mice. Finally, *vivo* acute pharmaco-genetic activation of POMC neurons induced mTORC1 signaling in POMC neurons, prevented the orexigenic action of rapamycin and inhibited rapamycin-induced increase in hypothalamic anandamide content.

Conclusions: These studies unveil the role of mTORC1 and CB1R-dependent endocannabinoid signaling in the regulation of POMC neuronal function and food intake.

POMC NEURONS BECOME OREXIGENIC IN RESPONSE TO CB1R ACTIVATION IN FED MICE

Tamas L. Horvath,[1] and Marco Koch[1]

[1]Program in Integrative Cell Signaling and Neurobiology of Metabolism, Section of Comparative Medicine, Yale University School of Medicine, New Haven CT 06520 USA.

Hypothalamic arcuate nucleus pro-opiomelanocortin (POMC) cells promote gradual onset of satiety. Cannabinoid receptor 1 (CB_1R) is critical for central regulation of food intake. We interrogated whether CB_1R-controlled feeding is paralleled by altered activity of POMC neurons.

Methods and Results: Chemical promotion of CB_1R activity increased feeding, and strikingly, CB_1R activation also promoted neuronal activity of POMC cells. This paradoxical increase in POMC activity was crucial for CB_1R-induced feeding, because Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-mediated inhibition of POMC neurons blocked CB_1R-triggered food intake while DREADD-mediated activation of POMC neurons enhanced CB_1R-driven feeding. The *Pomc* gene encodes both the anorexigenic peptide, melanocyte-stimulating hormone, and the orexigenic peptide, beta-endorphin. CB_1R activation selectively increased beta-endorphin but not in the hypothalamus, and, administration of the opioid receptor antagonist naloxone blocked CB_1R-induced feeding.

Conclusions: Taken together, these results unmasked a previously unsuspected role of POMC neurons in promotion of feeding by cannabinoids.

PEPTIDE ENDOCANNABINOIDS (PEPCANS) – THEIR EMERGING ROLE AS ENDOGENOUS ALLOSTERIC CB RECEPTOR MODULATORS

Jürg Gertsch[1], Vanessa Petrucci[1], Stefanie C. Hofer[1], Maria Salome Gachet[1], Pal Pacher[2], and Jürg Gertsch[1]

[1]Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland [2]NIH, NIAAA, Rockville, MD 20852, USA.

Endocannabinoids (ECs) have differential roles in different tissues where they activate CB1 and CB2 receptors in the context of distinct metabolic, immune, neuromodulation and cellular stress responses. Depending on the tissue and context, CB1 activation can both be positive or negative. Following the initial report about hemopressin as novel peptide CB1 receptor ligand (Heimann et al., 2007, PNAS) we have identified a class of endogenously produced N-terminally extended hemopressin peptide endocannabinoids, which we called pepcans. Some of these peptides (Pepcan-12) showed pronounced negative allosteric modulation at CB1 receptors (Bauer et al., 2012, J. Biol. Chem. 287, 36944-67). Using monoclonal Abs and LC-MS we did not detect hemopressin (now suspected to be an acidic extraction artefact) in brain or plasma, but pepcan-12, which appears to be the major bioactive species. In the CNS, we have identified the major noradrenergic nuclei as source for pepcan production, as well as the adrenal medulla (Hofer et al., 2015, Neuropharmacol, S0028-3908(15)00113-6). Here we investigated the effect of pepcans on CB2 receptors and the context of their peripheral release in physiological and pathophysiological conditions.

Methods: CB2 receptor binding and functional assays were both performed on stably transfected cell lines and primary cells using endogenous agonists, synthetic ligands and pepcans alone or in combination. We measured GTPgammaS, cAMP, beta-arrestin and CB receptor-dependent functional effects on monocytes/macrophages and in differentially stimulated whole blood. Pepcans were quantified by ELISA and LC-MS/MS. Endotoxemia, liver and kidney ischemia reperfusion injury, and cisplatin nephrotoxicity mouse models were used to analyze pepcan production.

Results: Our study shows that pepcan-12 is a potent negative allosteric modulator at CB1 receptors and a potent and efficient positive allosteric modulator at CB2 receptors, making it an ideal natural modulator of the ECS, significantly inhibiting the effects of ECs at CB1 receptors but potently increasing their effects at CB2 receptors. In the periphery, pepcan-12 is generated from precursor pepcans upon LPS stimulation and in the context of liver and kidney ischemia reperfusion, similarly to ECs. It is released from the adrenal glands into the blood where it is able to modulate the immune response via CB receptors.

Conclusions: Endocannabinoids are typically released under cellular stress and act to different degrees in peripheral tissues. Continuous and strong peripheral CB1 activation can enhance metabolic problems and tissue damage, including obesity, liver fibrosis, kidney damage and cardiomyopathy, but CB2 receptor activation exerts protective effects. Pepcan-12, being the ideal CB allosteric modulator in pathophysiological conditions, may tune the EC signaling to protect tissues from CB1 receptor overstimulation and CB2 receptor understimulation.

A CHEMICAL BIOLOGY APPROACH TO DISCOVER SELECTIVE ENDOCANNABINOID BIOSYNTHESIS INHIBITORS

Marc P. Baggelaar[1], Pascal J. P. Chameau[2], Vasudev Kantae[3], Jessica Hummel[1], Ku-Lung Hsu[4], Freek Janssen[1], Tom van der Wel[1], Marjolein Soethoudt[1], Hui Deng[1], Hans den Dulk[1], Marco Allarà[5], Bogdan I. Florea[1], Vincenzo Di Marzo[5], Wytse J. Wadman[2], Chris G. Kruse[2], Herman S. Overkleeft[1], Thomas Hankemeier[3], Taco R. Werkman[2], Benjamin F. Cravatt[4] & Mario van der Stelt[1]

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Diacylglycerol lipase (DAGL)-α and -β are enzymes responsible for the biosynthesis of the endocannabinoid 2-arachidonoylglycerol (2-AG). Selective and reversible inhibitors that could provide a critical counterpart for DAGL KO models are required to study the function of DAGLs in neuronal cells in an acute and temporal fashion, but these inhibitors are currently lacking.

Methods and Results: Here, we describe the identification of a highly selective DAGL inhibitor using structure-based design and a chemoproteomics strategy to characterize the selectivity of the inhibitor in complex proteomes. Key to the success of this approach is the use of comparative and competitive activity-based proteome profiling (ABPP), in which broad-spectrum and tailor-made activity-based probes are combined to report on the inhibition of a protein family in its native environment (mouse brain). Competitive ABPP with broad-spectrum fluorophosphonate-based probes and specific β-lactone-based probes led to the discovery of α-ketoheterocycle LEI105 as a potent, highly selective dual DAGL-α/DAGL-β inhibitor. LEI105 did not affect other enzymes involved in endocannabinoid metabolism including abhydrolase domain-containing protein 6, abhydrolase domain-containing protein 12, monoacylglycerol lipase and fatty acid amide hydrolase and did not display affinity for the cannabinoid CB_1 receptor. Targeted lipidomics revealed that LEI105 concentration-dependently reduced 2-AG levels, but not anandamide levels, in Neuro2A cells. We show that cannabinoid CB_1-receptor-mediated short-term synaptic plasticity in a mouse hippocampal slice model can be reduced by LEI105.

Conclusions: We have developed and characterized a highly selective reversible DAGL inhibitor using an advanced chemoproteomics assay. We apply this newly discovered inhibitor to provide new pharmacological evidence to support the hypothesis that 'on demand biosynthesis' of 2-AG is responsible for retrograde signaling in a mouse hippocampal slice model.

ACTOMYOSIN CONTRACTION MEDIATES PRESYNAPTIC LONG-TERM SYNAPTIC PLASTICITY

Maureen H. McFadden[1,2], Hao Xu[3,4], Yihui Cui[3,4], Rebecca A. Piskorowski[5], Laurent Venance[·], Vivien Chevaleyre[5] and Zsolt Lenkei[1,2,]

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Long-term synaptic plasticity is critical for adaptive function of the brain, but presynaptic mechanisms of functional plasticity remain poorly documented. Here, we show that changes in synaptic efficacy during one of the most widespread forms of long-term presynaptic plasticity, i.e. endocannabinoid-mediated long-term depression (eCB-LTD), require contractility of the neuronal actomyosin cytoskeleton.

First, we measured the effect of CB1R activation on synaptic vesicle release at individual axonal boutons by expressing synaptophysin-pHluorin (SpH) in rat hippocampal cultures. While CB1R activation led to a marked reduction in vesicle release as compared to control conditions, pharmacological inhibition of either non-muscular myosin II (NMII) or of its major activating kinase, Rho-associated protein kinase (ROCK), prevented this effect, as well as the increased number of silent boutons observed under CB1R activation. We then tested whether activity-dependent forms of plasticity mediated by endogenous cannabinoids (eCB) could engage similar mechanisms, by investigating two well-described eCB-mediated forms of plasticity both at inhibitory synapses in the hippocampus and at excitatory corticostriatal synapses. At both types of synapse, both NMII and ROCK inhibition prevented long-term forms of eCB-mediated synaptic depression, while short-term forms, such as depolarization-induced suppression of inhibition or of excitation, i. e. DSI or DSE, remained unaffected.

Collectively, these results show that the long-term, but not short-term, decrease in neurotransmitter release under CB1R activation relies on ROCK-mediated actomyosin contraction, providing a novel mechanistic link in the presynaptic regulation of synaptic function.

PREFRONTAL ENDOCANNABINOID SYNAPTOPATHIES: BAD FOOD, BAD MOOD AND BEYOND

O.J. Manzoni [1,2], A. Manduca[1,2], A. Bara[1,2], T. Larrieu[3], O. Lassalle[1,2], S. Layé[3]

[1] INSERM U901, Marseille 13009, France. [2] INMED, Marseille, France. Université Aix-Marseille, UMR S901, France. [3] Laboratoire NutriNeurO UMR INRA 1286 - Université de Bordeaux, 146 rue Léo-Saignat 33076 Bordeaux Cedex, France.

Major neuropsychiatric disorders including mental retardation, autism, schizophrenia, depression and addiction are accompanied by alterations of the neural code. At the synaptic level, postsynaptic molecular machines made of macromolecular complexes organized into a scale-free network process the neural code.

Aberrant synaptic transmission and/or plasticity participate in the etiology of neuropsychiatric diseases: disruption of a molecular cog in the synaptic machine causes or result in deficits in synaptic plasticity and leads to abnormal information processing.

An increased rate of depression has been measured in westernized countries in the last 50 years. Determinants of this increase could involve genetic factors and/or social and environmental factors, notably nutrition. In the last century, the rapid expansion of western countries has been paralleled by drastic dietary changes in diet: n-3 Poly-Unsaturated-Fatty-Acids (PUFA, omega-3), from fish and plants have been replaced by saturated fats from domestic animal and n-6 PUFA (omega-6), from common vegetable oils and animal sources. The risk of developing depression has been associated to low n-3 PUFA content of the diet and the estimated ratio of n-6:n-3 PUFA in a typical Western diet is around 20:1, in marked contrast with the optimal 1:4 ratio.

How dietary PUFA modify behavior and synaptic activity in the neuronal circuits controlling emotion and cognition remain to be fully elucidated.

We combined electrophysiological, biochemical and behavioral methods to describe how endocannabinoid lipids and associated supramolecular complexes participate to neuronal integration, emotional behaviors and cognition in mice exposed to dietary PUFA imbalance at different times of life (i.e. during in-utero development or adulthood). We specifically explore the effects on prefrontal cortex synaptic functions and associated behaviors. Our results reveal that adult PUFA imbalance disrupts multiple forms of synaptic plasticity and impedes not only emotional behavior but also cognitive functions. We show that pharmacological modulation of either the input or the outputs of the endocannabinoid macromolecular complex corrected both behavioral and synaptic phenotypes.

PAIN MODULATION BY NEGATIVE AFFECTIVE STATE: ROLE OF THE ENDOCANNABINOID SYSTEM

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Introduction: Pain shares a bidirectional, reciprocal relationship with negative affective state whereby the latter can both influence, and be influenced by, the pain experience. In particular, acute stress/fear tends to suppress pain through the phenomenon of stress/fear-induced analgesia, while chronic anxiety and depression are often associated with enhanced pain perception/hyperalgesia. Our work aims to elucidate the role of the endocannabinoid system in discrete brain regions in this complex, bidirectional modulation of pain by negative affective state.

Methods: We study fear-induced analgesia by assessing formalin-evoked nociceptive behaviour in an arena previously paired with aversive footshock. Anxiety/depression-related hyperalgesia is modelled using the stress-hypersensitive Wistar-Kyoto rat strain which exhibits hyper-responsivity to noxious stimuli. Site-specific intracerebral microinjection of pharmacological agents is combined with analysis of gene and protein expression and mass spectrometry to investigate the role of the endocannabinoid system in discrete brain regions regulating pain and affect.

Results: Our results demonstrate that pharmacological blockade of the cannabinoid CB_1 receptor prevents, whereas pharmacological inhibition of endocannabinoid degradation enhances, fear-induced analgesia in rats. Our data suggest a key role for the endocannabinoid system in the dorsolateral periaqueductal grey, basolateral amygdala and ventral hippocampus in mediating fear-induced analgesia. Recent data also suggest a key role for the endocannabinoid system in subregions of the medial prefrontal cortex in both fear-induced analgesia and in modulation of inflammatory pain in the absence of fear (including peroxisome proliferator activated receptors [PPARs] and GPR55). We have demonstrated that anxiety/depression-related hyperalgesia in the stress-hypersensitive Wistar-Kyoto rat strain is associated with alterations in levels of endocannabinoids and altered expression of genes coding for components of the endocannabinoid system in key brain regions regulating pain and affect. Pharmacological blockade of the CB_1 receptor exacerbates hyperalgesia in Wistar-Kyoto rats, while pharmacological blockade of endocannabinoid degradation attenuates hyperalgesia. Additional data suggest an important role for the endocannabinoid system in the rostral ventromedial medulla and periaqueductal grey in regulating hyperalgesia in Wistar-Kyoto rats.

Conclusions: In conclusion, the brain's endocannabinoid system plays a key role in pain and its modulation by negative affect. Increased understanding of the neurobiological mechanisms underpinning stress-pain interactions may aid the development of novel endocannabinoid system-targeted therapeutic approaches for the improved treatment of pain, affective disorders, and their co-morbidity.

ENDOCANNABINOID MODULATION OF CHRONIC PAIN STATES

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Systemic administration of cannabinoid receptor ligands is well known to produce analgesia in animal models of acute and chronic pain. CB_1 receptors are present at presynaptic sites on sensory afferent fibres and central pathways activated by painful stimuli; stimulation of these receptors results in profound analgesic effects in models of acute and chronic pain. However, the undesirable effects of cannabinoids, caused by the global activation of CB_1 receptors, has resulted in research focused on the modulation of endogenous ligand activity. The endocannabinoid (EC) system is one of the key endogenous systems regulating pain processing at peripheral, spinal and supraspinal regions. Following noxious stimulation, levels of the EC ligands anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) are often altered at peripheral, spinal and supraspinal sites. Changes in levels of the ECs may be temporally segregated at least in a model of post-operative pain where AEA signalling is reduced in the spinal cord at the onset of a pain state, whilst enhanced 2-AG signalling may be involved in pain resolution. Selective inhibitors of the major catabolic enzymes (MAGL and FAAH) which regulate levels of AEA and 2-AG are a promising alternative analgesic strategy which appear to avoid the unwanted effects of global CB_1 receptor agonism. An unpublished study from our team demonstrated that the potent selective MAG lipase inhibitor MJN110 significantly reverses established pain behaviour in a rat model of osteoarthritis. However, sustained global elevation of 2-AG via genetic deletion of MAGL or persistent blockade of MAGL activity can produce functional antagonism of the brain EC system, resulting in profound downregulation and desensitisation of CB_1 receptors in nociception-associated regions and a loss of analgesic phenotype. These problems may not be insurmountable as chronic partial inhibition of MAGL produces sustained analgesia in the absence of cannabinoid side effects in mice. In addition, novel inhibitors with more attractive therapeutic profile have been developed which may avoid functional antagonism of the EC system upon chronic administration.

POTENTIAL NEUROPROTECTIVE ROLE OF SPECIFIC CB_1 RECEPTOR SUBPOPULATIONS IN THE MOUSE CORTICOSTRIATAL CIRCUITRY

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The CB_1 receptor exerts a protective role in many different animal models of acute brain damage and chronic neurodegeneration, which has raised hope about the possible clinical use of cannabinoids as neuroprotective drugs. However, the assessment of the physiological relevance and therapeutic potential of the CB_1 receptor in neurological diseases is hampered, at least in part, by the lack of knowledge of the cell-population specificity of CB_1 receptor action. In order to study the potential neuroprotective role of different CB_1 receptor pools in the cortico-striatal circuitry we used an adenoviral-vector delivery strategy based on the expression of CFP-tagged mutant huntingtin harboring a pathogenic polyQ repeat of 94 residues under the control of specific promoters.

In a first series of experiments we expressed mutant huntingtin under minimal neuronal (CaMKII) or astroglial (GFAP) promoters in the dorsal striatum in order to achieve its expression in medium-sized spiny neurons (MSNs) or striatal astrocytes, respectively. One day after infection with mutant huntingtin, animals were treated daily i.p. for 2 weeks with 1 mg/Kg of THC or 8 mg/Kg of JZL-184, a potent and selective inhibitor of monoacylglycerol lipase. Selective mutant huntingtin expression in astrocytes, as well as in MSNs, led to impairments in motor coordination and alterations of striatal markers. When mutant huntingtin was expressed solely in MSNs, none of the pharmacological treatments prevented striatal damage. In contrast, when mutant huntingtin expression was restricted to astrocytes, THC and JZL-184 normalized motor ability and striatal integrity. We are currently investigating in further detail the possible neuroprotective role of CB_1 receptors located on striatal astrocytes. Since astrocytes are crucial for neuronal physiology and their dysregulation occurs in many neurodegenerative diseases, this cell type may represent a relevant target to apply cannabinoid-based therapies.

Key words: CB_1 receptor, huntingtin, corticostriatal circuitry.

MITOCHONDRIAL CB1 RECEPTORS ARE REQUIRED FOR AMNESIC EFFECTS OF CANNABINOIDS

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Introduction: Brain mitochondria maintain cellular energy homeostasis, but their acute mobilization during behavior is unknown. The discovery that the cannabinoid type-1 (CB1) receptor, a G protein-coupled receptor crucial for behavioral adaptation, is present in brain mitochondria (mtCB1) suggests that acute regulation of mitochondrial activity participates in animal behavior. In this study, we investigate the contribution of mtCB1 receptor in the cannabinoid-induced memory impairment.

Methods: To address this question, we developed new tools to specifically investigate the functional roles of mtCB1 receptors both in vitro and in vivo. We found that intra-mitochondrial soluble-adenylyl cyclase (sAC) activity mediates the effects of mtCB1 receptor signaling on brain cellular respiration. Using pharmacological modulators of sAC activity, we could prevent the mtCB1-dependent decrease of respiration. In addition, we generated a functional mutant protein, DN22-CB1 (by deleting the first 22 amino-acids of the CB1 protein), lacking mitochondrial localization and whose stimulation does not alter mitochondrial respiration.

Results: 1) Inhibition of sAC in the hippocampus blocked cannabinoid-induced decrease of field excitatory postsynaptic potentials at hippocampal CA3-CA1 synapses and amnesic effect in the novel object-recognition task. **2)** Moreover, viral in vivo expression of DN22-CB1 in the hippocampus abolished cannabinoid-induced impairment of both synaptic transmission and memory performance in mice.

Conclusions: This study shows that hippocampal mtCB1 receptors are required for cannabinoid-induced memory impairment. By directly linking mitochondrial activity to behavioral performance, these data indicate bioenergetic processes as primary acute regulators of high brain functions.

COMT MODULATION OF LONG-TERM MEMORY THROUGH DYSREGULATION OF THE ENDOCANNABINOID SYSTEM

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Introduction: The catechol-O-methyltransferase (COMT) is one of the major enzymes involved in the catabolism of dopamine in the brain, especially in the prefrontal cortex (PFC). Converging evidence demonstrated that COMT genetic variations constitute a key modulator of PFC-dependent cognition, including working memory and executive functions. Despite this, the impact of COMT on long-term memory formation and recall is still poorly explored.
The cannabinoid system has a central role in formation and extinction of long-lasting aversive memories and clinical studies have suggested an interaction of this system with the human COMT Val genotype (that increases COMT activity). However, the impact of the COMT Val genotype on the endocannabinoid system and its potential link with memory functions is unknown.

Methods and Results: Here we report that transgenic mice overexpressing the human COMT-Val gene (COMT-Val-tg) present increased remote fear memories (>50 days), while having unaffected short-term (24-hour) conditioned memory. In particular, the retrieval of these memories was selectively modulated by COMT, as silencing the COMT Val overexpression starting from 30 days after the initial fear conditioning completely normalized remote memories in COMT-Val-tg mice.
Notably, increased remote fear memory in COMT-Val-tg mice was associated with an overdrive of the endocannabinoid system (i.e. increased NAPE-PLD, AEA, FAAH and decreased CB1R) in the PFC, but not in the hippocampus. Remarkably, COMT Val gene silencing completely normalized these abnormalities in the endocannabinoid system. Finally, acute pharmacological blockade of CB1R with AM251 in wild-type control mice at the 50-day retrieval phase recapitulated COMT-Val-tg mice remote memory alterations.

Conclusions: These results indicate that increased COMT enzymatic activity modulate PFC-dependent remote memory through the dysregulation of the endocannabinoid system in the PFC.

ENDOCANNABINOID-DEPENDENT PLASTICITY IS A SYNAPTIC CORRELATE FOR SOCIAL DEFEAT-INDUCED ANXIETY

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Introduction: Chronic stress is closely related to the development of common affective disorders such as anxiety. Interestingly, a proportion of humans exposed to chronic stress does not show signs of psychopathology and maintains physiological stability and thus are resilient to stress. The neurobiological mechanisms that underlie individual differences to stress and resilience are only partially understood. An increasing body of evidence links the endocannabinoid system to the regulation of emotional states, such as anxiety. Here, we investigated the relationship between endocannabinoid synaptic plasticity and the development of anxiety in mice submitted to chronic social defeat stress, a well-characterised model used for the induction of depressive and anxiety-like behaviours.

Methods: C57/Bl6j male mice were submitted to 10 days of social defeat (5 min of physical interaction followed by 3 hours of sensorial interaction) with a new aggressor every day (CD1 male mice). After the last session of stress, C57/Bl6j mice were tested for their anxiety-related behaviour in an open-field test, social interaction test, light/dark box and elevated plus maze test. Then, endocannabinoid-dependent plasticity was tested for each mouse with patch-clamp whole cell recordings in a sagittal brain slice at excitatory synapses in the nucleus accumbens. Plasticity protocol consists in pre-post spike-timing dependent plasticity (STDP). Drugs were used to characterise the plasticity in defeated and control animals: AM251, SR141716A and JZL184.

Results: After 10 days of social defeat, mice showed a strong deficit in social interaction and a high level of anxiety-like behaviour. This emotional alteration observed in defeated mice was associated to increased body weight and high corticosterone secretion. We found that STDP induced a long-term depression (LTD) in control mice that was abolished by prior bath application of CB1 antagonists AM251 or SR141716A. By contrast, STDP did not induce plasticity in defeated mice, but LTD could be restored by prior bath application of JZL184, an inhibitor of 2-AG degradation. Normal anxiety-like behaviour was also restored with i.p. injection of JZL184 prior to the elevated plus maze test. When segregating defeated mice in anxious and non anxious subgroups based on a cluster analysis of behavioural results, we found that endocannabinoid plasticity was abolished only in defeated anxious mice, whereas it was attenuated but still present in defeated non-anxious mice. Finally, we showed a significant correlation between the anxiety score of each animal and the endocannabinoid-dependent LTD.

Conclusions: These results suggest that endocannabinoid plasticity could be a synaptic correlate of stress-induced anxiety.

TO BE FAT OR NOT - THE MANY FACES OF THE CB1 RECEPTOR

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It has been well established that the CB1 receptor (CB1R) is involved in the regulation of energy balance and feeding. However, it is remarkable that CB1R is positioned at many different sites in the body in charge of the control of these physiological processes which are required for the organism's survival. In addition, in models of disturbed energy balance, i.e., in diet-induced obesity, CB1R present at multiple sites is responsible for the emergence of the pathology. In these processes, CB1R both in the central nervous system and in peripheral organs plays roles. During the last years, several mutant mouse lines lacking CB1R in specific cell types were generated and investigated in the context of energy balance. This presentation will cover CB1R in cortical glutamatergic neurons, central serotonergic neurons, adrenergic / noradrenergic cells, and adipocytes, and tries to provide an overview of the multi-faced roles of CB1R, aiming at understanding the context-specific and cell type-specific functions of CB1R in control of energy balance.

ENDOCANNABINOID SIGNALING IN INNATE AND ADAPTIVE IMMUNITY: FROM BASIC IMMUNE CONTROL TO POTENTIAL TREATMENT OF CHRONIC INFLAMMATORY DISEASES

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The immune system can be modulated and regulated not only by foreign antigens but also by other humoral factors and metabolic products, which are able to affect several quantitative and qualitative aspects of immunity. Among these, endocannabinoids are a group of bioactive lipids that might serve as secondary modulators, which when mobilized coincident with or shortly after first-line immune modulators, increase or decrease many immune functions. Most immune cells express these bioactive lipids, together with their set of receptors and of enzymes regulating their synthesis and degradation. These lipids exert indeed manifold immunomodulatory effects in the different cell populations of innate (monocytes/macrophages, granulocytes, dendritic cells) and adaptive immunity (T and B lymphocytes) in both mice and human immune system compartments. The most active endocannabinoids in immunoregulation are anandamide (AEA) and 2-arachidonoylgylcerol (2-AG) that exert distinctive immunoregulatory effects on specific innate and adaptive immune cells, such as CD4+ T-helper and regulatory T-lymphocytes, classically-activated M1 and alternatively-M2 macrophages, as well as different subsets of dendritic cells. In particular, AEA is able to strongly suppress T-helper-1 and T-helper-17 cytokines production either directly or by inhibiting dendritic cell-dependent T cell activation. On the other hand, 2-AG seems to be the only endocannabinoid able to induce Foxp3+ and IL-10 producing regulatory T cells. Furthermore, AEA inhibits the IL-1β producing NLP3-inflammasome pathway in human macrophages, whereas 2-AG acts as a danger signal by activating such signaling pathway. Additionally, AEA also affects the balance of M1 and M2 macrophages by switching M1 from a pro-inflammatory to an anti-inflammatory-like phenotype and by potentiating the anti-inflammatory responses of M2 cells. These immunomodulatory effects in the different immune cell subsets are significantly altered in several chronic inflammatory diseases, including neurodegenerative diseases and metabolic disorders and are due to a different reorganization of key elements of the endocannabinoid system, in particular CB_2 receptors and AEA-degrading enzyme FAAH. Hence, endocannabinoids are "master regulators" of a global immunomodulation of the "innate-adaptive immune axis", with an impact on both physiological and pathological processes, where these evidences may be of crucial importance for the rational design of new endocannabinoid-based immunotherapeutic approaches.

THE ENDOCANNNABINOID SYSTEM IN THE LIVER: NOVEL PERSPECTIVES

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Alcohol abuse, non-alcoholic fatty liver disease as hepatic manifestation of metabolic syndrome, and viral infections are prominent causes of chronic liver injury leading to endstage liver disease. Schematically, these conditions result in hepatocyte injury and inflammation carried by innate and adaptive immune cells, thereby activating liver fibrogenesis. Progression of fibrosis leads to cirrhosis, to the life-threatening complications of portal hypertension and liver failure, and potentially to hepatocellular carcinoma.

The endocannabinoid system is a central mediator of acute and chronic liver injury, with the description of the role of CB1 and CB2 receptors and their endogenous lipidic ligands in various aspects of liver pathophysiology. CB1 receptor antagonists represent an important therapeutic target for chronic liver disease, owing to beneficial effects on lipid metabolism and in light of their antifibrogenic properties. Moreover, the efficacy of peripherally-restricted CB1 antagonists with limited brain penetrance has now been validated in preclinical models of NAFLD, and our preliminary results demonstrate beneficial effects on liver fibrosis. In addition, we have described that CB2 receptors are expressed in hepatic macrophages, Th17 lymphocytes and hepatic fibrogenic cells and identified CB2 receptor as a promising anti-inflammatory, hepatoprotective and antifibrogenic target. Finally, our recent data demonstrate that MAGL inhibitors are potent anti-inflammatory and antifibrogenic molecules in the liver. We will discuss the latest advances on the impact and the therapeutic potential of molecules targeting the endocannabinoid system on key steps of chronic liver disease progression.

EMERGING ROLE OF N-ACYLETHANOLAMINES AND THEIR RECEPTORS IN NEUROPSYCHIATRIC DISORDERS

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In a clinical trial carried out to test the efficacy of off-label add-on fenofibrate in the treatment of nocturnal frontal lobe epilepsy, we observed that, besides reducing seizure frequency, this drug also improves mood and subjective quality of life. Fenofibrate is an agonist at peroxisome proliferator-associated receptor-α (PPARα), conventionally prescribed as lipid-lowering medication. PPARα are expressed in the CNS by neurons and glial cells and are activated by endocannabinoid-like N-acylethanolamines such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). We demonstrated that activation of these receptors triggers nicotinic acetylcholine receptors phosphorylation in ventral tegmental area (VTA) dopamine and cortical neurons and regulates their functional activity. This mechanism explains therapeutic potentials in nicotine addiction and epilepsies, but also in neuropsychiatric disorders such as depression. In fact, among the proposed pathophysiological mechanisms, the hypercholinergic hypothesis of depression is supported by preclinical and clinical observations.

Methods: in this study, we tested the hypothesis that PPARα activation exerts antidepressant properties. To this aim, we used an animal model of depression: rats exposed to unpredictable and unescapable mild stressors develop depressive-like symptoms, specifically escape deficit to aversive stimuli (a measure of learned helplessness) and anhedonia, indexed by deficit in self-administration of sucrose pellets. Sustained PPARα activation was achieved by administration of a diet enriched with fenofibrate (0.2 %w/w in food pellets, 14 days). Control rats received normal food.

Results: Fenofibrate completely prevented the development of escape deficit and counteracted stress-induced anhedonia by restoring normal responding for sucrose in stressed animals under both the fixed ratio 5 and the progressive ratio protocols.

We investigated the possible mechanisms of action of PPARα agonists by recording the electrophysiological activity of mesolimbic dopamine neurons in the VTA and of serotonin neurons in the dorsal raphe (DR). These neurons are involved in reward, motivation and mood regulation and play a relevant role in the neurobiology of depressive symptoms. Firing rate and pattern were analyzed and compared between controls and fenofibrate-treated rats. After fenofibrate treatment, dopamine neurons displayed enhanced burst firing, whereas DR neurons showed increased firing rate, suggestive of higher probability of dopamine and serotonin release in terminal areas, respectively. Enhanced dopamine release is in line with an increase in the DARPP-32 phosphorylation detected in the nucleus accumbens. These electrophysiological and neurochemical results are consistent with the antidepressant effects observed after chronic fenofibrate treatment.

Conclusions: The potential antidepressant effects of PPARα agonists open up innovative avenues in the treatment of depressive disorder. Translational studies in humans are predicted to be facilitated by the fact that fibrates as well as the endogenous PPARα agonist PEA are already approved for human use.

CANNABIS AND ADOLESCENCE IN RODENTS: FROM BEHAVIOR TO EPIGENETICS

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The increasing cannabis consumption among adolescents, together with studies correlating its early use with mental diseases, and the present political debate on cannabis legalization, strongly suggest the need of more research on the neurobiological basis of adolescent brain vulnerability. . However, despite their unquestionable value, epidemiological data are not conclusive. Thus modeling the adolescent phase in animals appears to be a useful approach to investigate the impact of Cannabis use on the adolescent brain. In line with this, work from our lab demonstrated that chronic THC exposure during adolescence has long-term influence on measures of depressive- and psychotic-like behaviors in female rats (Realini et al, 2011; Rubino et al, 2008; 2015; Zamberletti et al,2014).   The neurobiological basis of this association include the induction of alterations in the maturational events of the endocannabinoid system occurring in the adolescent brain. This, in turn, may profoundly dysregulate developmental processes in some neurotransmitter systems, such as GABA and glutamate, mainly in the prefrontal cortex . Interestingly adolescent THC exposure in female rats also induces a persistent neuroinflammatory state specifically localized within the adult prefrontal cortex (PFC), characterized by increased secretion of the pro-inflammatory markers, TNF-α, iNOS and COX-2, and reduction of the anti-inflammatory cytokine, IL-10. This neuroinflammatory phenotype is associated with down-regulation of CB1 receptor on neuronal cells and upregulation of CB2 on microglia cells. Conversely blocking microglia activation with ibudilast during THC treatment significantly attenuates short-term memory impairments in adulthood, simultaneously preventing the increases in TNF-α, iNOS, COX-2 levels as well as the up-regulation of CB2 receptors on microglia cells. In contrast, THCinduced depressive-like behaviors were unaffected by ibudilast treatment.

Though it is still unclear how microglia are actually activated by chronic THC treatment, these results suggest that chronic THC exposure during adolescence, at least in female rats, prompts immune dysfunctions in the PFC that co-exist with the detrimental effect on synaptic function and could potentially work together to cause the long-term cognitive impairments associated with the treatment

Finally the emerging role of epigenetic mechanisms in the development of psychiatric diseases (e.g. depression and drug addiction) led us to hypothesize that alterations in epigenetic modifications could play a part in the etiopathogenesis of the depressive/psychotic-like phenotype induced by adolescent, but not adult, THC exposure in female rats. Adolescent THC exposure induced alterations in selective histone modifications (mainly H3K9me3) that impacted the expression of a set of genes closely related to synaptic plasticity mechanisms. These findings join others present in literature (Rubino et al., 2015; Cass et al., 2014; Zamberletti et al., 2014) supporting the hypothesis that adolescent cannabinoid exposure might disrupt remodeling of cortical circuits, thus leading to behavioral disfunctionality. Changes in both histone modifications and gene expression were more widespread and intense after adolescent treatment in comparison with that of adults, further supporting the existence of adolescent vulnerability to THC effect.

TARGETING THE ENDOCANNABINOID SYSTEM FOR INTELLECTUAL DISABILITY: CLUES FROM MOUSE MODELS.

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Intellectual disability disorders encompass a number of genetically derived malfunctions without therapy. Some of these disorders have been translated to mouse models, where the behavioral, cellular and molecular characteristics of the different alterations can be readily analyzed under well-controlled conditions. Using the mouse models of fragile X syndrome, Down syndrome and Williams-Beuren syndrome, which display cognitive deficits resulting from different genetic alterations, we found that pharmacological and genetic approaches targeting the endocannabinoid system managed to improve cognitive performance in these models. In addition, we have observed that the mouse models of all three disorders show a de-regulated mammalian target of rapamycin (mTOR) signaling activity, crucial for proper cognitive performance, in brain areas such as the hippocampus or the cortex. In agreement with our previous research, targeting the endocannabinoid system normalized mTOR signaling in the brain. All together, our results point to the endocannabinoid system as a suitable target for the design of therapies that improve cognitive performance in different intellectual disability disorders independently of their genetic etiology.

ENDOCANNABINOIDS AND CANNABINOIDS: NOT JUST BRAINY BUT ALSO BRAWNY

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Until a few years back, very little was known of the involvement of endocannabinoids and cannabinoid receptors in skeletal muscle cell differentiation. My group recently reported (1) that, possibly due to changes in the expression of genes involved in its metabolism, the levels of the endocannabinoid, 2-arachidonoylglycerol (2-AG), are decreased both during myotube formation *in vitro* from murine C2C12 myoblasts, and during mouse muscle development *in vivo*. Furthermore, 2-AG, as well as the CB1 agonist arachidonoyl-2-chloroethylamide, prevented myotube formation in a manner antagonized by CB1 knockdown and CB1 antagonists, which, *per se*, instead stimulated differentiation. It was also found that muscle fascicles from CB1 knockout embryos contain more muscle fibers, while postnatal mice show muscle fibers of an increased diameter relative to wild-type littermates (1). From the mechanistic point of view, we showed that inhibition of Kv7.4 channel activity, which plays a permissive role in myogenesis and depends on phosphatidylinositol 4,5-bisphosphate (PIP2), underlies the effects of 2-AG. In fact, CB1 stimulation reduced both total and Kv7.4-bound PIP2 levels in C2C12 cells and inhibited Kv7.4 currents in transfected CHO cells. We, therefore, suggested that 2-AG and CB1 control not only neurotransmitter release in the brain via inhibition of voltage-activated Ca^{2+} channels, but also myoblast differentiation via inhibition of Kv7.4 channels (1).

We have now undertaken new studies to understand if: 1) CB1 also plays a role in skeletal muscle satellite cell differentiation – we have used primary human satellite cells to address this question; 2) CB1 is regulated by PAX7, a transcription factor and a major marker of satellite cells (2); 3) pharmacological manipulation of CB1 receptors ameliorates muscular function of Dmd^{mdx} mice, an animal model of Duchenne's muscular dystrophy (3); and 4) non-THC plant cannabinoids, via non-CB1 receptor-mediated, but still endocannabinoid-related mechanisms (4), can also be used to modulate mouse myotube formation.

I will present data suggesting that CB1 signalling is indeed regulated by PAX7 and controls satellite cell differentiation to myotubes, and that pharmacological blockade of CB1 receptors ameliorates behavioral scores of locomotor activity and muscular function in dystrophic mice. Preliminary data suggesting that some non-psychotropic cannabinoids also affect myotube formation *in vitro* will also be presented. Thus, endocannabinoid signaling at CB1 receptors, as well as plant cannabinoids, affect not only the brain, but also the skeletal muscle, i.e. they are both "brainy" and "brawny".

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NEW POTENTIAL THERAPEUTIC APPLICATIONS FOR CERTAIN PHYTOCANNABINOIDS REVEALED BY PHARMACOLOGICAL DISCOVERIES

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Cannabinoids constitute a broad and complex range of compounds just some of which interact with orthosteric sites on CB_1 and CB_2 receptors as direct agonists or antagonists. These orthosteric ligands include not only numerous synthetic compounds, but also (i) Δ^9-tetrahydrocannabinol (THC) and Δ^9-tetrahydrocannabivarin (THCV) that can be produced by cannabis, and are hence known as phytocannabinoids, and (ii) agonists such as anandamide and antagonists such as sphingosine that are produced by mammalian tissues, and hence known as endocannabinoids. It has been discovered too, first, that certain synthetic and endogenous cannabinoids, as well as one phytocannabinoid, target allosteric sites on CB_1 and/or CB_2 receptors as positive or negative allosteric modulators (PAMs or NAMs), and second, that at least some endocannabinoids are released "autoprotectively" in certain disorders, and in an "autoimpairing" manner in other disorders. Cannabis is also a source of cannabinoids that do not seem to display significant potency as CB_1 or CB_2 receptor ligands but do share the ability of THC and THCV, and of endocannabinoids, to interact potently with other pharmacological targets. These phytocannabinoids include cannabidiolic acid (CBDA), cannabidiol (CBD) and cannabigerol (CBG). In addition to approved therapeutic applications of (i) synthetic CB_1/CB_2 agonists both for nausea and vomiting, and for anorexia, and (ii) cannabis-derived THC and CBD (in Sativex®) for cancer pain and multiple sclerosis, numerous *potential* therapeutic clinical applications for cannabinoids have been identified in recent investigations. Examples include the potential use of (i) THCV for treating nicotine dependence, possibly by inducing CB_2 agonism and CB_1 antagonism, (ii) THCV for reducing positive and negative signs of schizophrenia, partly via its enhancement of 5-HT_{1A} receptor activation, (iii) CBDA and CBD for the suppression of nausea and vomiting resulting from their enhancement of 5-HT_{1A} receptor activation, (iv) CBG for the relief of inflammatory pain that depends on its ability to activate α_2-adrenoceptors, and (v) a synthetic CB_1 receptor PAM (GAT211) for the relief of neuropathic pain. Interestingly, the dose-response curves of CBDA and CBD for their 5-HT_{1A} receptor-mediated anti-nausea and anti-emetic effects are bell shaped, and CBDA enhances 5-HT_{1A} receptor activation and reduces vomiting and signs of nausea much more potently and over a broader dose range than CBD. How Δ^9-THCV, CBDA and CBD enhance 5-HT_{1A} receptor activation, and whether CBDA and CBD share the ability of Δ^9-THCV to induce apparent 5-HT_{1A}-mediated reductions in signs of schizophrenia in rats, remains to be established. Findings obtained in some of these investigations will be described.

UNDER-INVESTIGATED INDICATION IN CANNABIS THERAPEUTICS

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Introduction: Cannabis has been employed throughout the ages for many varied indications, as might be expected for an agent that affects the endocannabinoid system, a regulatory physiological mechanism for maintaining homeostasis in human health and disease. There are several conditions that figure prominently in the historical record, but that have not benefited from modern investigation of their efficacy. This presentation will focus on three: tetanus, tinnitus and burns.

Methods: Current relevant historical literature was reviewed, and supplemented with online database review.

Results: The ECS plays a key role in control of muscle tone. In the 19th century before the advent of immunization, tetanus was not a rare disease, and was virtually universally fatal. Some of the first cases of survival were reported after repeated administration of cannabis-based medicines. Tetanus remains a public health problems in Third World areas where vaccines have yet to reach, and tertiary care with mechanical ventilation is unavailable. Cannabis-based medicines may allow survival for patients affected in such cases.

Tinnitus is a prominent public health problem affecting up to 15% of individuals. It is very disturbing to patients and responsible for high suicide rates in those affected, especially due to the poor performance or failure of conventional treatments. Modern investigation has highlighted the role of CB_1, TRPV1 and TRPV4 in the pathophysiology of tinnitus and would support the application of tetrahydrocannabinol and cannabidiol in concert as a novel treatment for this recalcitrant disorder.

European Renaissance herbalists almost uniformly recommended hemp-based treatment for skin burns. Modern investigation supports the benefit of TRPV1 blockade or desensitization in its treatment, a profile that would suggest the benefits of cannabidiol. Certainly, its multi-modality anti-inflammatory mechanisms also may prove beneficial therapeutically.

Conclusions: Cannabis-based medicines have proven very versatile treatments in a wide variety of pathological conditions. Clinical trials of such agents in tetanus, tinnitus and burn treatment are three additional areas that deserve current investigation.

TOWARDS THE UTILIZATION OF CANNABINOIDS AS ANTI-CANCER AGENTS

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A large body of evidence shows that cannabinoids, in addition to their well-known palliative effects on some cancer-associated symptoms, can reduce tumour growth in animal models of cancer and specifically of gliomas. The mechanism of cannabinoid anticancer action relies, at least largely, on the ability of these agents to stimulate autophagy-mediated cancer cell death. Moreover, the combined administration of cannabinoids and temozolomide produces a strong anticancer effect, which correlates with an intense activation of the signalling route that triggers the activation of cytotoxic autophagy. Research conducted in our group has also led to the identification of mechanisms of resistance to cannabinoid anticancer action. For example, up-regulation of the growth factor Midlkine (MK) promotes resistance to cannabinoid anticancer action in gliomas via stimulation of the Anaplastic Lymphoma Kinase tyrosine kinase receptor (ALK); and could be a factor of bad prognosis in GBM patients. All these preclinical findings have facilitated the promotion of a clinical study to investigate the safety and efficacy of the combined administration of the cannabis-based medicine Sativex and temozolomide in recurrent GBM.

ENDOCANNABINOID SIGNALLING AT THE SKIN/IMMUNE CELL INTERFACE

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In recent years, the presence of a full and functional endocannabinoid system (ECS) in the skin and in its adnexal structures (*e.g*., hair follicle, sebaceous and sweat glands) has been clearly documented. Substantial evidence has been also accumulated indicating a local regulatory role of the ECS in skin physiology, including the regulation of proliferation, differentiation and survival of skin cells, of sebum production and melanogenesis, as well as of immune competence and/or tolerance of keratinocytes. In line with this, recent evidence has shown that cutaneous type-1 cannabinoid receptor (CB_1) limits secretion of proinflammatory chemokines, suggesting that it might also regulate T-cell dependent inflammatory diseases of the skin (*e.g*., psoriasis, contact dermatitis, and atopic dermatitis). Here, the relevance of endocannabinoid signalling at the skin/immune cell interface will be discussed, and new data on the impact of CB_1-dependent signal transduction on cytokine profile of interferon-γ-activated human keratinocytes will be presented. Further analysis identified mTOR as a proinflammatory signalling pathway regulated by CB_1, able to promote either interleukin (IL)-12 and IL-23 release from keratinocytes or Th1 and Th17 polarization. Taken together, these findings demonstrate that in human keratinocytes endocannabinoids may suppress highly pathogenic T cell subsets through CB_1-mediated mTOR inhibition.

VSN16R A SAFE, NEW DRUG TO TREAT SPASTICITY

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Introduction: Current symptomatic treatments for spasticity, including *Cannabis sativa* often exhibit unwanted side-effects that limit their early adoption and use following onset of spasticity in multiple sclerosis.

Methods: We synthesized a novel compound, similar to a cyclic anandamide, called VSN16R. This was tested for safety and efficacy in *in vitro* assays and *in vivo* assays in animals and healthy human volunteers and in animal models of spasticity.

Results: VSN16R was potent *in vitro* and its action was blocked by SR141617A. The compound exhibited anti-spastic activity in experimental autoimmune encephalomyelitis in mice and was as active as baclofen and cannabinoids, but lacked their sedative side-effect potential. The drug was found to be a novel, water-soluble modulator of the CBe receptor *in vitro* and did not bind to CB_1, CB_2, TRPV1, GPR18 or GPR55. VSN16R was orally active and remarkably well-tolerated (with over a thousand fold therapeutic window) despite administration of large doses and demonstrated no obvious, adverse neurobehavioural effects in mice. It was also well tolerated in other larger animal species and importantly in humans, where it was found that VSN16R produced high bioavailability with over ten-hundred fold higher plasma levels than achieved with comparable doses of VSN16R that were therapeutic in rodents. The drug was without any obvious adverse behavioural or other physiological events in humans.

Conclusions: This study identifies a novel target for control of spasticity and suggests that VSN16R may be a useful novel anti-spastic agent, which offers tolerability advantages over existing treatments. This may facilitate adoption of earlier treatment following to development of spasticity in MS.

AN INTERNATIONAL SURVEY OF MEDICAL CANNABIS USE: USE PATTERNS AND HEALTH EFFECTS

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Background: Few large-scale epidemiologic or medical ethnographic surveys have been performed to describe demographics, use patterns and health effects of individuals accessing cannabis as a botanical medicine. We conducted an international, online survey with cross sectional design. We report demographics, conditions for which responders used Cannabis, routes of administration, use patterns, patient-reported outcomes (PRO) for efficacy and global health from a 10-item short form for physical and mental health.

Methods Individuals from anywhere in the world could participate in the anonymous survey by recruitment at Washington State cannabis dispensing facilities and via networking using the internet. Individuals participated by providing direct entry of responses into a REDCap database**.** Patient Reported Outcomes Measurement Information System (PROMIS) Global reported physical and mental health.

Results: 2265 people responded to the survey from Dec 2013-Dec 2014. 1331 participants were "medical" users with the majority identifying as male (55%). The most common symptoms for which participants used cannabis were pain, anxiety, depression, headache/migraine, and arthritis. Inhalation was the preferred route of administration. The majority of participants consumed between 3 to 7 grams of cannabis per week. Individuals reported an average symptom improvement of 3.96 on a scale of -5 (worsening) to +5 (improving), across conditions. Global Health scores for mental health were on par with the general population and for physical health were one standard deviation below the general population.

Conclusions:
This natural history study of self-selected participants, describes symptoms, efficacy, use patterns and global health of subjects using cannabis as a botanical medicine. Self-reported symptom improvement was consistent across conditions, achieved primarily by inhaling cannabis. We found that participants' global health is similar to that of the general population. Survey methodology was easy and inexpensive to administer, is informative, participants are willing to participate.

INVESTIGATION OF THE ANTI-ACNE EFFECTS OF FATTY ACID AMIDE HYDROLASE INHIBITORS ON HUMAN SEBOCYTES

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Introduction: Acne vulgaris is one of the most common human skin diseases. We have previously shown that (-)-cannabidiol (CBD) exerted remarkable complex "anti-acne" effects (normalization of "pro-acne agents"-induced lipogenesis, anti-proliferative and anti-inflammatory actions; *Oláh et al., J. Clin. Invest. 2014;124(9):3713-3724*) on human sebocytes, whereas endocannabinoids promoted sebaceous lipid synthesis (*Dobrosi et al., FASEB J. 2008;22(10):3685-3695.*). We have also demonstrated that, quite surprisingly, inhibitors (URB597 and JP104) of fatty acid amide hydrolase (FAAH) exerted "CBD-like" anti-acne effects. Therefore, in the current study, we aimed at exploring the mechanism of these unexpected actions of the FAAH-inhibitors.

Methods: Lipid synthesis of human SZ95 sebocytes was investigated by Nile Red staining. Alterations in the gene expression were monitored by RT-qPCR or by Western blot.

Results: First, by using specific antagonists, we showed that, in contrast to CBD, neither transient receptor potential vanilloid (TRPV)-4, nor TRPV1 played a role in mediating lipostatic actions of FAAH-inhibitors. Importantly however, we found that URB597 and JP104 down-regulated c-Myc (a key positive regulator of sebocyte proliferation) and nuclear receptor interacting protein-1 (NRIP1), down-regulation of which was proven to mediate lipostatic actions of CBD. Although anti-inflammatory actions of CBD were mediated by the adenosine A2a receptor-dependent up-regulation of tribbles homolog 3 (TRIB3) and inhibition of P65-NF-κB signaling, FAAH-inhibitors did not influence either TRIB3 expression or lipopolysaccharide induced NF-κB-activation. Instead, antagonists of peroxisome proliferator-activated receptor (PPAR)-α, -γ and –δ were equally able to prevent their anti-inflammatory action. Our ongoing FAAH RNAi studies intend to unveil whether these actions are indeed directly coupled to the abrogation of FAAH activity or they are mediated by yet unknown off-targets.

Conclusions: Collectively, these results strongly argue for that administration of FAAH-inhibitors, leading to down-regulation of c-Myc and NRIP1 and activation of PPARs, exert complex anti-acne effects on human sebocytes; therefore clinical studies are invited to exploit their potency as promising, novel class anti-acne agents.

PHARMACEUTICAL PERSPECTIVES OF CANNABINOID-BASED MEDICINES: POLYPHARMACOLOGY SHAKES HANDS WITH COMPLEX AETIOPATHOLOGY

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There is historical recognition for the utility of the cannabis plant in human health and we now know that cannabinoids target endogenous systems in the body regulating homeostasis and auto-regulation. Modern development of pharmaceuticals involves screening of synthesised molecular libraries to identify those most potent and selective at a single receptor/disease target. However, chronic and complex diseases, particularly those with neurological/immune system involvement, are poorly treated with single focus agents, and the conventional "magic bullet" approach has been largely unsuccessful. Disease causation is multifactorial and a 'broadside' approach may be more successful in overcoming the redundancy and multi-functionality that are inherent properties of these conditions.

Initial presentations may be due to deviations of fundamental physiological systems, but often different pathologies can be characterised by similar malfunctioning biological networks. Furthermore, the ensuing compensatory mechanisms that the body uses to counteract these changes weaken its dynamic ability to respond to additional insults resulting in florid co-morbidities. Consequently, diseases should be considered at a systems level.

Despite structural similarities, individual cannabinoids have unique pharmacology. Furthermore, plants contain a hierarchy of constituents interacting synergistically; extracts are often more potent than equivalent doses of isolated compounds. The multitarget, systemic, and prohomeostatic actions emerging for plant cannabinoids exemplify what might be needed for future medicines. Indeed, two combined cannabis extracts were approved as a single medicine (Sativex®) for spasticity in multiple sclerosis, while pure cannabidiol (Epidiolex®), a multi-target cannabinoid, is emerging as a treatment for paediatric drug-resistant epilepsy with recently reported clinical results in ulcerative colitis. This talk revisits the concept of polypharmacology and describes a new empirical model, the 'therapeutic handshake', to predict efficacy and safety of compound combinations of either natural or synthetic origin.

This model pairs up the aetiopathology profile of a disease with the polypharmacological profile of one or more molecules and thus predicts the most appropriate combination of cannabinoids/extracts as a potential treatment. Profiles within our model are compiled using appropriately weighted information from extensive literature searches and online databases combined with proprietary in house data. A modular approach looks to include many components: receptors, enzymes, genes, organelles and more to create a system wide view rather than a selective drill down perspective.

Our model guides development of medicines with superior risk/benefit profiles compared to synthetic compounds whilst still meeting the stringent regulatory requirements expected of modern day pharmaceutical products.

A REVIEW OF BEDROCAN RESEARCH: PATIENT-INSPIRED AND SCIENCE-BASED

Arno Hazekamp[1], Tjalling Erkelens[1]

Bedrocan BV, The Netherlands

In 2003 The Netherlands became the first country to make standardized herbal cannabis available on prescription. Under this program, Bedrocan has acquired more than 13 years of experience, becoming an international producer of pharmaceutical-grade cannabis. Today, our products are used by patients in The Netherlands as well as Canada, Italy, Germany, Finland and the Czech Republic. We provide a range of fully standardized cannabis products to patients, each with a reproducible cannabinoid profile and independently analyzed for quality by experienced laboratories. As Bedrocan strains are used by patients, as well as qualified for use in clinical research, they have become a preferred choice for scientists and physicians who wish to study the pharmacological effects of cannabis.

Over the years, Bedrocan has grown from a small cultivator of medicinal cannabis into a leading organizer of cannabis research. By teaming up in 2005 with Leiden University - The Netherlands' oldest university - Bedrocan gained access to state-of-the-art research facilities and a network of experts in a wide range of research fields. Our current network includes over 15 professional institutes, academic departments, and pharmaceutical companies worldwide. This makes the city of Leiden an important hub for cannabis-related research, ranging from fundamental chemical research and receptor-binding studies, to performing fully compliant clinical trials with herbal cannabis.

Bedrocan strives to bridge the gap between patients' needs for cannabis and the quality standards of modern medicine. We do this through an active research program, a commitment to sharing our knowledge through scientific publications, and by developing research tools which provide a platform to further the independent study of cannabis. Standardization, accurate dosing, and strict safety standards are the cornerstones of medicine today. We believe herbal cannabis can, and should, meet these standards of modern medicine. Simultaneously, we use input from those who use cannabis for medical purposes to identify topics that are most important to address in this time of rapidly expanding availability of cannabis products. We call this patient-inspired research. In all we do, our challenge is to make the best choices based on patients' needs and preferences, while using scientific data to support our final decisions on research methodology and product development.

This presentation will give an overview of Bedrocan's research program so far, explain the philosophy behind it, and will explore ways for further development of herbal cannabis as a modern medicine. Topics include: Standardized cultivation (cultivation, processing, packaging), Quality control (QC lab), Chemical profiling (cannabinoids and terpenes), Administration forms (oil, vaporizing, tea), Botanical research (Sativa-Indica varieties), Social studies (patient preferences), and Clinical research (chronic pain).

CLINICAL PERSPECTIVES OF CANNABINOID-BASED MEDICINES

Willy Notcutt[1]

Great Yarmouth, UK

In this presentation I will discuss problem issues emerging from the normal clinical use of Medicinal Cannabinoids in the UK.

1. Issues in regular use
   a. The importance of a schedule of careful titration
   b. Establishing benefit
   c. The problems of the recreational MJ user, advising and titrating
   d. Advising the novice cannabis user wanting/needing to use street cannabis
   e. Long Term use
      i. Long Term Reviews with stable dosing
      ii. Alternate/new diagnoses
      iii. Managing the other medications and withdrawing the unnecessary
      iv. Deaths
   f. Nabilone study
   g. Driving
2. Economics
   a. Pressures on health Care budgets
   b. Varying results of separate evaluations
   c. UK – NICE and the AWMSG
   d. Germany, Spain, Italy
   e. The cost of disease modifiers
   f. Lack of neurologists; failure to adopt this medicine
3. The Spread of 'Medical Mj'
   a. Uruguay, USA changes in legislation
   b. Emerging Medical MJ in UK – Health product or Medicine
4. Emerging Uses
   a. Symptom Control
   b. Disease modifying
5. Lack of Education – undergraduates, post graduates

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THE CANADIAN EXPERIENCE

Ware MA

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Since April 1 2014, The Marihuana (sic) for Medical Purposes Regulations (MMPR) have been in effect as a federal program of medical cannabis production and access. While ongoing court challenges have prevented the elimination of personal production, originally planned under the MMPR, the regulations allow for private companies to apply for licenses to produce and distribute cannabis for medical purposes under tightly controlled safety and quality controlled standards. As of September 8th 2015 there are 26 licensed producers (LPs) across Canada. Data from July 2015 suggest that 23,930 patients are currently receiving cannabis under the MMPR; average daily doses authorized are 3.2g/day.

The MMPR make no provision for research support, but several research projects are underway funded wholly or in part by the LPs; these studies will be summarized in this presentation. These studies include the Quebec Cannabis Registry and several proof of concept clinical trials.

Medical education of health professionals regarding medical cannabis and the MMPR remains a significant challenge, met in part by several online accredited programs and conferences. This session will discuss some of the challenges faced by developing and implementing quality medical cannabis education efforts.

The presentation will conclude with some reflections on recent developments to allow the preparation and use of derivatives (oils, tinctures etc) and the implications this will have on clinical research.

MEDICINAL CANNABIS IN ISRAEL

Ilya Reznik[1,2]

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In recent years, medicinal cannabis (MC) was reintroduced as a legitimate treatment in several countries. Now, the National MC program in Israel has the largest number of patients per population in the world. Also, Israel is still the marijuana research capital of the world, mostly due to the renowned works of the grandfather of the marijuana research Prof. Raphael Mechoulam (Hebrew University, Jerusalem) and large group of his fellows and collaborators.

Since 1995 Israel has issued approximately 25,000 licenses (~20,000 valid today) for MC (the actual demand of licensure is estimating ~ 100.000 potential permanent patients); all under the Dangerous Drugs Ordinance Act permitting the use of a "dangerous" drug for medical purposes and all distributed under the auspices of the Ministry of Health (MoH) or any person authorized by the MoH". Since 2009 increased regulations have accompanied rapid growth (approximately 150 new licenses a month). To address the growth, three years ago the MoH created a supervisory administrative unit (The MC Authority) that regulates all MC issues.

The MC Authority issues licenses to certain patients after approving a specialist physician's recommendation. Thus, physicians in Israel cannot directly prescribe MC to patients but can sign a medical recommendation that is then processed by the MC Authority. Nevertheless, the number of licensed patients in Israeli has risen dramatically in the last few years, despite of the very limited list of the formal indication for MC approvals and very strict criteria for proposed patient's selections, which has been made by the MC Authority. The rapid growth of licensed patients accompanied by the increasing resistance of formal medical establishment (incl. Israel Medical Associations and its professional Societies, such as Pain Specialists, Family Physicians, Addiction Medicine etc.). In this atmosphere, the small group of clinicians, has became a responsible for vast majority of the cannabis-treated patients.

The medical community in generally reluctant to larger extension of the MC usage. Some hope has arrived from the recent study (Ebert et al., IMAJ 2015;17:437-41), that found most of the Israeli physicians participated in this study were in favor of cannabis use for medical purposes, acknowledging its legitimacy as a therapeutic agent for some medical indications. According to this study, the majority physicians are standing against legalization, but agreed be involved in the process of authorizing MC use but decline to prescribe it to patients directly, delegating this function to the MoH. Physicians agree unanimously that more education and professional training on MC is needed and should be readily available.

Most recently, MoH announced that medical cannabis will be available in pharmacies in Israel, and that more doctors would be allowed to prescribe it. Thus, nowadays the situation with MC in Israel could be described as transitional, as soon as the new regulations are being made by the authorities and soon will be presented to the public. We have a lot to do: to improve relationships with patients organizations and general public, and with suppliers/growers; to organize systematic learning and training for health care practitioners etc. I hope that the recent clinical and scientific advances and our success with the cannabis-treated patients will attract more physicians and other health care practitioners to be involved in MC, which allow to promote National MC program in Israel to the new achievements.

PERSPECTIVES FROM A DOCTOR'S OFFICE IN GERMANY

Franjo Grotenhermen[1,2]

[1]Medical Practice, Ruethen, Germany; [2]Nova-Institute Huerth/Rhineland, Germany;

Legal situation: In Germany cannabis-based medicinal drugs can be prescribed on a special prescription (THC/dronabinol, nabilone, Sativex). In addition, patients may apply to a body of the health ministry („Bundesopiumstelle") for an exemption from the narcotics law to buy cannabis flowers from the pharmacy in the frame of a „doctor's accompanied self-therapy". This approval is only granted if a doctor writes a medical certificate, where he demonstrates, that no other treatment is efficient and that therefore a treatment with cannabis is necessary.

Background for an approval for a self-therapy with cannabis flowers: After a long court case of patients, in 2005 the Federal Administrative Court of Germany ruled that the German Government has to allow the medical use of cannabis in certain cases, where the patient has no other legal alternatives. The ruling says: "The right of physical integrity cannot only be violated in that bodies of the state themselves produce an assault or inflict pain through their actions. The extent of protection of this fundamental right is also affected if the government takes measures to prevent a medical condition to be cured or at least be mitigated and thereby physical suffering is continued and maintained needlessly."

Costs of a treatment: Health insurances usually do not cover the costs. They only have to pay for a treatment with Sativex in spasticity due to MS. The monthly costs for a treatment with dronabinol, which is produced by two German companies with a mean daily dose of 10-15 mg is about 250 to 400 euros, for a treatment with Sativex with the same dose (4-6 sprays with 10.8-16.2 mg dronabinol) about 120-160 euros. Three bottles of Sativex cost 314 euros in Germany (810 mg THC and 750 mg CBD). After approval for self-therapy with cannabis flowers patients can buy the cannabis produced by the Dutch company Bedrocan in a pharmacy, which has the approval to supply this patient with the medication. The cannabis flowers have to be paid by the patient. Depending on pharmacy costs are 14 to 25 euros a gram.

Numbers of patients treated with cannabis-based medicines: Currently about 10,000 patients receive a treatment with dronabinol or Sativex. About 500 patients receive a treatment with cannabis flowers. Last official number of July 2015: 463. There is a severe undersupply of the German population with cannabis-based medicines compared to Canada, the Netherlands and Israel.

Diagnoses for which approvals for self-therapy with cannabis have been granted: Acne inversa, Anorexia and cachexia, Anxiety disorder, Asthma, Attention deficit / hyperactivity disorder (ADHD), Autism, Bladder spasms, Blepharospasm, Borderline personality disorder, Cervical and lumbar spine syndrome, Cervikobrachialgy, Chronic Fatigue Syndrome (CFS), Chronic pain syndrome after polytrauma, Cluster headaches, Crohn's disease, Depression, Epilepsy, Fibromyalgia, Headache, HIV, Cervikobrachialgy, Hyperhidrosis, Irritable bowel syndrome, Lupus erythematosus, Lyme disease, Migraine, Migraine accompagnée, Mitochondropathy, Multiple sclerosis, Neurodermatitis, Obsessive-compulsive disorder, Osteoarthritis, Painful spasticity in syringomyelia, Paraplegia, Paresis of the brachial plexus, Paroxysmal dyskinesia nonkinesiogene, Polyneuropathy, Posner Castle Man Syndrome, Post-traumatic stress disorder, Psoriasis, Restless legs syndrome, Rheumatoid arthritis, Sarcoidosis, Scheuermann's disease, Sleep disorder, Spasticity in cerebral palsy, Spondylitis ankylosans, Still's disease, Systemic scleroderma, Thrombangitis obliterans, Tics, Tinnitus, Tourette's syndrome, Trichotillomania, Ulcerative colitis, Urticaria of unknown origin.

Poster Presentations

INVOLVEMENT OF CB1 AND CB2 DURING OOCYTE MATURATION IN MOUSE MODEL: IMPORTANCE ON FERTILIZATION, EMBRYO DEVELOPMENT AND TRANSPORT OF EMBRYOS

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The oocyte is a complex cell and even today the whole biochemical and physiological processes necessary for successful oocyte maturation are not totally understood. There are some evidences indicating that endocannabinoids play an important role in cellular communication during the transport of embryos through the oviduct and also during implantation and placentation processes. However nothing is known about the role played in the maturation of the oocyte, a key process in achieving potentially fertilizable cells. Our aim was to analyze the involvement of cannabinoids in in vitro maturation (IVM) of mice oocytes. **Methods:** Oocytes in germinal vesicle (GV), metaphase I (MI) and metaphase II (MII) were analyzed by immunofluorescence and real time PCR to search the protein and mRNA from both receptors. During maturation wild type oocytes in GV stage were incubated with HU-210 and antagonist SR141716 and, JHW-015 and antagonist SR144528. After that, the oocytes were fertilized and cultured in vitro until blastocyst stage. Finally, knockout CB1-/- and CB2-/- oocytes matured in vivo or in vitro, were fertilized and culture in vitro to evaluate embryo development to blastocyst stage. Finally we used CB1-/- and CB2-/- females that were mated with wild type male and the embryos were recovered at 3.5 dpc (days post coitum) to evaluate the embryo development in vivo..
Results: 1) cannabinoid receptors CB1 and CB2 were present in mice oocytes during the different stages of both in vitro and in vivo oocyte maturation**. 2)** The activation of CB1 receptor during IVM using HU-210 synthetic cannabinoid was involved in a more efficient IVF and in a better in vitro development to blastocyst stage. In addition, this effect was reverted by the antagonist SR141716. **3)** The kinases PKB/Akt and ERK1/2, involved in different signalling pathways of meiosis, were regulated differently after cannabinoid treatment through CB1 receptor. **4)** The activation of CB2 with the agonist JHW-015 did not show differences in both the embryo development and the modulation of the studied kinases. **5)** When in vivo matured oocytes from knockout models (CB1-/- and CB2-/-) were used, it was observed that the absence of CB1 decreased the blastocyst rate, whereas the lack of CB2 did not have any effect compared with wild type mice. **6)** The absence of CB1 receptor at 3.5 dpc caused a delay in transport of the embryos into the oviduct and decreased the blastocyst rate, like the in vitro model. Interestingly a high percentage of embryos were arrested in early stages of development even at the oocyte stage. However the absence of CB2 receptor did not show a delay in the transport and blastocyst rate.
Conclusions: The identification of pathway(s) by which cannabinoids mediate the IVM of oocytes and/or the in vitro development of the fertilized egg will provide an interesting target to test if the poor quality of human oocytes obtained by IVM could be improved using new culture media supplemented with cannabinoids.

CANNABINOID RECEPTOR TYPE 1 (CB1) IN THE BRAIN AND THE EMOTIONAL PROCESSING OF VISCERAL PAIN

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Increasing evidence points to a major role of endocannabinoids and CB1 in mediating somatic and visceral nociception at the level of primary sensory neurons and the spinal cord. However, there is little knowledge about CB1 in the brain and pain processing.

Methods: Here, we used different visceral pain models in mice (acetic acid-induced abdominal constrictions and cerulein-mediated acute pancreatitis) and assessed the effects of CB1 conditional genetic deletion in different brain areas and neuron subtypes; CaMK-CB1 mice for conditional knock-out in forebrain neurons, Nex-CB1 mice for deletion in cortical glutamatergic neurons and Dlx-CB1 animals for knock-out in cortical GABAergic interneurons.

Results: So far, we observed that pancreatitis-related abdominal allodynia and hyperalgesia and acetic acid-induces writhing behaviour were not changed in these mutants. However, the emotional expression of pain was increased in CaMK-CB1 knock-out mice as assessed by the mouse facial grimacing score.

Conclusion: Our results suggest for the first time that CB1 located in limbic and forebrain neurons is crucially involved in the affective processing of visceral pain.

DYNAMICS OF EXPRESSION AND LOCALIZATION OF THE CANNABINOID SYSTEM IN HUMAN OOCYTES AND GRANULOSA CELLS DURING OOCYTE NUCLEAR MATURATION

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Introduction: The cannabinoid system has been implicated in the control of some reproductive events. With regard to the oocyte maturation, in female follicles have been detected the presence of components of the cannabinoid system and it is known that CB1 and CB2 receptors are present in human oocytes. Even so, little is known about the dynamics of expression and localization of cannabinoid receptors and degrading-enzymes during the maturation of oocytes and in their surrounding granulose cells.

Aim: The interest of the present research focuses in the detection of cannabinoid receptors (CB1 and CB2) and cannabinoids degrading enzymes (FAAH and MGLL) in human oocytes and their granulosa cells. Moreover, we wanted to describe the spatial and temporal location of these proteins during different stages of oocyte maturation: germinal vesicle (GV), metaphase I (MI) and metaphase II (MII).

Material and methods: In the study were used oocytes and granulosa cells of patients in the program of assisted reproduction (IVF/ICSI) of the Human Reproduction Unit of the Cruces University Hospital. These patients underwent a follicular controlled hyperstimulation protocol. The presence of the components of the cannabinoid system was analyzed by qRT-PCR, immunoblot and immunocytochemistry.

Results: The results showed the presence of some components of the cannabinoid system in human oocytes and granulosa cells. The location of each receptor and each degrading-enzyme varied during oocyte maturation but that location did not vary in granulosa cells from these oocytes. Notably, the CB1 receptor colocalized with FAAH in GV and MI phases but in MII phase, where CB1 is maintained in the periphery, the FAAH is internalized. The MGLL maintained a cytosolic location throughout the maturation process. In granulosa cells CB1 is also the most important receptor since, according to our results, we could not confirm the presence of CB2. In this regard, CB1 is located in the cell membrane while the enzymes also are present in the cytosol.

Conclusions: The presence of cannabinoid system components in human oocyte and granulosa cells during oocyte maturation, suggests a possible action of the cannabinoids in female gamete maturation. On the other hand, the coordinated redistribution of cannabinoid system proteins suggests a possible role for this system in the communication between oocyte, granulose cells and sperm that it must be a dynamic and coordinated process.

CIRCADIAN OSCILLATIONS OF ENDOCANNABINOID 2-ARACHIDONOLGLYCEROL LEVELS MODULATE CARDIAC NEUTROPHIL RECRUITMENT AND HEALING AFTER MYOCARDIAL INFARCTION

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Myocardial infarction (MI) is the leading cause of death worldwide. Epidemiological studies show acute myocardial infarction to be more prevalent in the morning, which is associated with a poorer outcome. The mechanisms behind this association are unclear. Under homeostatic conditions, circadian oscillations of immune cell functions and hormones (e.g. glucocorticoid plasma levels) have been reported. Circulating leukocytes oscillate between blood and peripheral tissue, peaking at Zeitgeber time (ZT)5 in the blood (where ZT0 refers to lights on [= inactive phase in rodents] and ZT 12 to lights off [= active phase]) and at ZT13 in muscle tissue and bone marrow. We hypothesized that the time-of day dependency of MI outcome could be due to circadian oscillations in endocannabinoid levels which might affect inflammatory cell recruitment, infarct size and healing after MI.

We measured circadian kinetics of murine (wildtype, C57BL6/J) plasma endocannabinoid levels and tissue mRNA expression of endocannabinoid-related enzymes and cannabinoid receptors. Myocardial infarction was induced by permanent ligation of the left anterior descending coronary artery (LAD). The ligation was performed at two different time points, ZT5 and ZT13. Baseline plasma 2-AG endocannabinoid levels were markedly increased at ZT13 compared to ZT5. 24 hours after MI, plasma levels of 2-AG levels as well as neutrophil mobilization factors (G-CSF, CXCL1 and CXCL2) were significantly higher in ZT13 MI mice than in ZT5 MI mice. At baseline, we found lower levels of CXCL12 and higher numbers of neutrophil progenitors in the bone marrow at ZT13, suggesting facilitated neutrophil mobilization in response to MI at this time point. Concomitantly, LAD occlusion at ZT13 resulted in a 2-fold higher neutrophil count in the infarcted heart 24 hours post-MI $(2.95 \times 10^6$ vs. 1.6×10^6; n=3; p<0.01). This was associated with larger infarcts (46.22±1.74% vs. 27.28±3.83%; n=4; p<0,005), enhanced fibrosis and adverse remodelling, resulting in a worsened cardiac function (ejection fraction 3d post-MI: 22.75±1.18% vs. 32.3±1.8%; n=3-4; p<0.01) and a significantly higher mortality. In an attempt to limit exaggerated neutrophil-mediated inflammation after MI at ZT13, we injected a neutrophil-depleting antibody (Ly6G, 50µg/mouse/day), which significantly reduced neutrophil counts, infarct size and scar formation and improved cardiac function. Conversely, increasing endocannabinoid plasma levels by systemic administration of 2-AG metabolizing enzyme MAGL inhibitor JZL184 increased infarct size, cardiac neutrophil infiltration and worsened cardiac function after MI at ZT5.

Our data reveal that the time-of-day of ischemia onset determines the outcome after MI. Acute MI during the sleep-to-wake transition period leads to larger infarcts and a worsened cardiac function, which can be explained, at least in part, by circadian oscillations of 2-AG levels and thus neutrophil recruitment to the myocardium.

EFFECT OF PREGNENOLONE ON SYNAPTIC DEPRESSION ELICITED BY CANNABINOIDS

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The CB_1 cannabinoid receptor is widely distributed in the nervous system. It is typically localized in presynaptic axon terminals. Activation of these CB_1 receptors by exogenous and endogenous cannabinoids (endocannabinoids) inhibits synaptic transmission. Such presynaptic inhibition of synaptic transmission is the basis of most behavioural effects of cannabinoids, like analgesia, sedation and extrapyramidal motor effects (B Szabo [2014] Effects of phytocannabinoids on neurotransmission in the central and peripheral nervous systems. Handbook of Cannabis, ed. RG Pertwee, Oxford University Press, pp 157-172). Pregnenolone is an intermediate product of steroid hormone synthesis, but as a "neurosteroid" it can also directly affect neuronal functions. Recently it was reported that pregnenolone attenuates behavioural effects of cannabinoids (M Vallee et al., Science 343: 94-98, 2014). Presynaptic inhibition elicited by Δ^9-tetrahydrocannabinol (Δ^9-THC) in nucleus accumbens brain slices was also attenuated. The aim of our study was to analyze the interaction between pregnenolone and cannabinoids on synaptic transmission.

Methods: Slices containing the cerebellum and the nucleus accumbens were prepared from brains of mice and rats. Synaptic transmission was analyzed in superfused brain slices with patch-clamp electrophysiological techniques.

Results: **1)** In mouse cerebellar slices, spontaneous GABAergic synaptic input to Purkinje cells was inhibited by the synthetic cannabinoids JWH-210 (5×10^{-6} M) and JWH-018 (5×10^{-6} M). This inhibition was not (or only minimally) affected by pregnenolone (10^{-7} M). **2)** Depolarization-induced suppression of GABAergic inhibition (DSI) was elicited in Purkinje cells. Pregnenolone (10^{-7} M) did not interfere with this endocannabinoid-mediated form of synaptic inhibition. **3)** In rat nucleus accumbens slices, GABAergic and glutamatergic synaptic input to medium spiny neurons was activated by electrical stimulation of axons. Δ^9-THC (2×10^{-5} M) suppressed the GABAergic and glutamatergic synaptic transmission. These effects of Δ^9-THC were not modified by pregnenolone (10^{-7} M).

Conclusions: The results show that inhibition of GABAergic synaptic transmission elicited by synthetic exogenous and endogenous cannabinoids in the cerebellum was not influenced by pregnenolone. Inhibition of GABAergic and glutamatergic synaptic transmission by the phytocannabinoid Δ^9-THC in the nucleus accumbens was also not changed by pregnenolone. Thus, further research is necessary to verify or reject the concept that the neurosteroid pregnenolone interferes with cannabinoid-elicited inhibition of synaptic transmission.

GENE EXPRESSION AND EPIGENETIC REGULATION BY CANNABINOIDS IN NEUROGENESIS

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INTRODUCTION: Endocannabinoids (eCB) in the central nervous system (CNS) have recently emerged as instructive cues in the development of the CNS as they are able to attenuate detrimental effects on neurogenesis and neuroinflammation that are associated with ageing. New evidence suggests that eCB signalling regulates gene expression by inducing epigenetic modification such as DNA methylation or histone modification in the regulation of a range of neurobiological processes in the brain, including CNS development, learning, memory and neurodegeneration associated with ageing.

AIMS: The aim of this study is two-fold: firstly to determine if pharmacological targeting of the CB1 or CB2 subtype of cannabinoid (CB) receptors can regulate epigenetic activity in neural stem cells (NSC) from C57BL6/J mice, and secondly to determine the potential involvement of key genes in the regulation of this epigenetic activity.

METHODS: We analysed if CB signals are able to regulate DNA methylation in NSC by modifying the DNA methyltransferases (DNMT) activity. This study was performed with a mini library of 5 highly selective CBs (CB1 agonist: ACEA; CB1 antagonist: AM251; CB2 agonist: JWH133; CB2 antagonist: AM630; DAGL inhibitor: RHC-80267). Experiments were performed in nuclear extracts from NSC (t= 48 and 72h). To evaluate the role of CB on NSC proliferation, the expression of the mitotic marker phospho-Histone H3 (PH3) and BrdU incorporation were assessed. DNA cell cycle analysis of NSC was performed using flow cytometry (FACS). Gene expression levels in NSC were determined by microarray analysis (Affymetrix). Furthermore we investigate the epigenetic control of neurogenesis by the CB1/CB2 cannabinoid signalling pathway (Epigentek).

RESULTS: Cells expressing PH3 or BrdU incorporation significantly increased in NSC after exposure to CB agonists ($P<0.001$ vs. control). The incubation of the NSC with IL-1ra blocking antibody (R&D Systems), abolished the above proliferative effects. Interestingly, NSC proliferation rate was significantly increased ($P<0.001$ vs. control) after exposure to recombinant murine (rm)IL-1ra. The above results strongly suggest that IL-1ra is a critical mediator for the protective actions of CB in the CNS. Microarray-based gene expression analysis of NSC identified a set of novel candidate genes being up regulated or down regulated in NSC after CB exposure. These genes are specifically involved in the cell cycle regulation. Finally, we determined that basal DNA methyltransferase (DNMT) activity in the NSC was downregulated after CB agonists exposure (by approx. 50%). Interestingly, rmIL-1ra, caused a significant decrease in DNMT activity (by approx. 61%).

CONCLUSION: Our results suggest that CBs are potent signals that induce NSC proliferation and migration via IL-1ra. These data reveal an unexpected role for this signalling pathway in neurogenesis, which might have important implications for brain repair.

EVALUATION OF THE FUNCTIONAL SELECTIVITY OF CANNABINOID RECEPTORS SIGNALING IN MOUSE BRAIN CORTEX

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Evidence shows that, for most of G protein-coupled receptors (GPCRs), distinct agonists can differentially regulate several signaling pathways through the same receptor by a selective activation of different intracellular effectors, a mechanism known as functional selectivity. In the case of cannabinoid receptors, both the CB_1 and CB_2 receptors have been shown to preferentially couple to the $G_{i/o}$ family of heterotrimeric G-proteins. Furthermore, CB_1 receptor has been demonstrated to be capable of coupling to different families of G-proteins when activated by an agonist drug suggesting that different intracellular responses may be activated by the CB_1 receptor depending on the ligand.

The aim of the present study was to evaluate the functional coupling of both CB_1 and CB_2 receptors to different subtypes of G proteins ($G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, $G_{\alpha z}$, $G_{\alpha 12/13}$ and $G_{\alpha q/11}$) in mouse brain cortex membrane homogenates.

Methods: Stimulation of the $[^{35}S]GTP\gamma S$ binding by the CB_1/CB_2 cannabinoid agonists WIN55,212-2 and $\Delta^9 THC$ (10^{-5} M) was determined by Scintillation Proximity Assay (SPA) technique.

Results: WIN55,212-2 stimulated $[^{35}S]GTP\gamma S$ binding to all the G_α proteins evaluated (E_{max} range $117\pm2\%$ to $131\pm3\%$). On the other hand, $\Delta^9 THC$ induced a stimulation of the $[^{35}S]GTP\gamma S$ binding to $G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, and $G_{\alpha q/11}$ (E_{max} range $111\pm2\%$ to $134\pm3\%$) but not to $G_{\alpha z}$ and $G_{\alpha 12/13}$ proteins. In all cases, activation of the G_α proteins by WIN55,212-2 and $\Delta^9 THC$ was blocked by the antagonist O-2050 (10^{-5} M). To elucidate the role of each cannabinoid receptor in this G protein activation, the same experiments were carried out with brain membranes from CB_1ko, CB_2ko and CB_1/CB_2 double ko mice. Results suggest that the stimulation of $G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, $G_{\alpha z}$, and $G_{\alpha q/11}$ G protein subtypes by WIN55,212-2 is CB_1 receptor-mediated. However, WIN55,212-2 seems to stimulate the coupling to $G_{\alpha 12/13}$ protein through a CB_2 receptor-dependant mechanism. In the same manner, $\Delta^9 THC$ appears to activate $G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, $G_{\alpha z}$ and $G_{\alpha 12/13}$ through CB_1 receptors but stimulates $[^{35}S]GTP\gamma S$ binding to $G_{\alpha q/11}$ by activating CB_2 receptors.

Conclusions: Our results demonstrate that, in mice brain tissue, different exogenous cannabinoid ligands are able to selectively activate different G_α protein subtypes, inhibitory and non-inhibitory G_α protein subtypes, through the activation of both CB_1 and CB_2 receptors.

CANNABIDIOL (CBD) DECREASES RESTING BLOOD PRESSURE AND THE BLOOD PRESSURE RESPONSE TO STRESS IN HEALTHY VOLUNTEERS

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Cannabidiol (CBD) is a non-psychoactive phytocannabinoid that reduces the cardiovascular response to stress in rodents. The aim of this study was to investigate if CBD similarly reduces cardiovascular stress in humans.

Methods: This was a randomised, placebo-controlled, double blind, cross over study in 9 healthy volunteers. A single dose (600mg) of CBD or placebo was followed, 2 h later, by mental arithmetic, isometric exercise and cold pressor test. Cardiovascular parameters were monitored, from ingestion of CBD/placebo until the end of study, using a finometer and laser doppler.

Results: At rest, CBD reduced systolic blood pressure (-6 mmHg; $P<0.05$) and stroke volume (-8ml; $P<0.05$), with raised heart rate and maintained cardiac output. In response to isometric exercise, volunteers who had taken CBD had a blunted mean arterial pressure (-5 mmHg, $P<0.05$), increased heart rate (10 bpm, $P<0.01$), decreased stroke volume (-13 ml; $P<0.01$) and blunted forearm blood flow. In response to cold stress, volunteers who had taken CBD had a blunted mean arterial pressure response (-6 mmHg, $P<0.01$) and increased heart rate (7 bpm; $P<0.05$), with no associated increase in total peripheral resistance. There was no difference in diastolic blood pressure, between CBD and placebo, during exercise and cold stress. None of the parameters were different between two treatments during mental stress.

Conclusion: Acute administration of CBD reduces resting blood pressure, and reduces blood pressure response to stress in humans, associated with increased heart rate. These changes in haemodynamics should be considered for people taking CBD. Further research is required to establish the underlying mechanisms and whether CBD has a potential therapeutic use in cardiovascular disorders.

DIFFERENT PHYSIOLOGICAL EFFECTS OF CHRONIC URB597 TREATMENT IN PRIMARY AND SECONDARY HYPERTENSION IN RATS

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Introduction: The endocannabinoid system has been suggested to be up-regulated in hypertension. The fatty acid amide hydrolase (FAAH) is an enzyme responsible for the degradation of the endocannabinoid anandamide. Acute injection of the FAAH inhibitors reduced blood pressure (BP) in hypertensive rats (e.g. *Bátkai et al. Circulation 2004;110:1996-2002; Malinowska et al. Br J Pharmacol 2012;165:2073-88*). The aim of our study was to examine the effects of chronic administration of the FAAH inhibitor URB597 in hypertensive animals.

Methods: Experiments were performed on 8-11 weeks old spontaneously hypertensive rats (SHR) and two age groups (4 weeks old and 6-7 weeks old) of deoxycorticosterone acetate (DOCA)-salt rats, which represent primary and secondary hypertension, respectively. Appropriate normotensive control animals [Wistar Kyoto (WKY) and uninephrectomised (UNX) rats, respectively] were also used. Secondary hypertension was induced through 6 weeks by administration of DOCA (25 mg/kg twice weekly) and high salt diet to UNX rats. URB597 1 mg/kg or its vehicle were injected twice daily for two weeks (in case of secondary hypertension it was administered starting from 5th week of DOCA or its vehicle treatment). BP and heart rate (HR) were measured in conscious animals using the tail-cuff method.

Results: **1)** Systolic BP was higher than 200 mmHg in DOCA-salt rats and it was about 180 mmHg in SHR and 120 mmHg in all normotensive controls. The cardiac hypertrophy was detected in DOCA-salt and SHR animals and renal hypertrophy in DOCA-salt only. Hypertensive rats had lower weight and comparable rectal temperature to normotensive animals. **2)** URB597 decreased BP by about 20% in older DOCA-salt rats only, and it did not change BP in other groups. It also did not influence HR except of WKY rats in which it tended to increase HR. **3)** URB597 slightly by about 10-15% lowered cardiac and renal hypertrophy in younger DOCA-salt rats, and did not significantly affects heart and kidney weight to body mass ratio in other animals. However in SHR it showed a mild tendency to increase this ratio. **4)** URB597 treatment failed to affect the body weights, however it tended to improve the inhibition of increase in body mass observed in younger DOCA-salt rats. **5)** Except of younger UNX rats, chronic administration of URB597 increased or tended to increase rectal temperature in all experimental groups.

Conclusions: Two weeks of URB597 administration to hypertensive rats caused different effects on some physiological parameters which were dependant on age and type of hypertension. Thus, caution should be taken during studies of FAAH inhibitors because of their potential age- and model-specific activities.

EFFECTS OF THE CHRONIC ADMINISTRATION OF FAAH INHIBITOR, URB597, ON THE CANNABINOID-INDUCED VASORELAXATION IN DOCA-SALT HYPERTENSIVE RATS

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Endocannabinoids system is involved in the regulation of blood pressure (BP), especially under pathological condition, such as hypertension. It has been shown that fatty acid amide hydrolase (FAAH) inhibitors, including URB597, normalized elevated blood pressure, suggesting their role as potential antihypertensive agents (*Bátkai et al., Circulation 2004;110:1996-2002*). Up to date, nobody has examined the effect of chronic administration of URB597 on vascular function in DOCA-salt hypertensive rats.

Methods: Experiments were performed on uninephrectomized rats divided into normotensive (unilateral nephrectomy only) (UNX) and hypertensive (rats on a high salt diet and injectioned by deoxycorticosterone acetate; DOCA-salt animals). URB597 1 mg/kg or its vehicle were injected twice daily for 14 days for both group of animals, UNX+URB, and DOCA-salt+URB, respectively. Functional studies were performed in the isolated endothelium-intact aorta and small mesenteric arteries (G3) pre-constricted with phenylephrine. CB_1 receptor and/or FAAH expression was assessed by Western blots and/or by immunohistochemistry.

Results: URB597 treatment **1)** decreased elevated systolic BP, **2)** tended to increase body weight and **3)** reduced medial hypertrophy in aorta but not G3 in DOCA-salt rats with no changes in UNX animals. **4)** Methanandamide (0.01-30 μmol/L) evoked enhanced or diminished concentration-dependent relaxation in G3 and aorta, respectively, in hypertensive DOCA-salt rats. Methanandamide-induced vasorelaxation in G3 was sensitive to antagonist of cannabinoid CB_1 receptor, AM6545 (1 μmol/L) only in DOCA-salt rats. The incubation with antagonist of vanilloid receptor TRPV1, capsazepine (1 μmol/L) impaired relaxant effect in normo- and hypertensive animals. The cannabinoid CB_1 receptors were up-expressed in G3 but not in aorta in DOCA-salt rats. **5)** Chronic URB597 treatment decreased the FAAH expression in G3 in DOCA-salt rats and failed to affect vascular effects of cannabinoids and CB_1 receptor expression.

Conclusions: These results for the first time showed, that **a)** enhanced methanandamide-mediated vasorelaxation in the endothelium-intact G3 in DOCA-salt hypertensive rats is CB_1 and TRPV1-dependent. **b)** CB_1 receptors are over-expressed in resistance vessels in DOCA-salt rats. **c)** The vascular responses to cannabinoid under normal and hypertensive conditions are region specific. **d)** Chronic administration of FAAH inhibitor URB597 lowered systolic BP, possible due to its potency to decreased medial hypertrophy in aorta and reduced FAAH expression in hypertensive DOCA-salt animals, however it has no impact on vasorelaxant potency of cannabinoids. **e)** Finally, in terms of FAAH inhibitors as potent drug, including antihypertensives, more studies is required to explain its hypotensive mechanism.

VCE-004.8, A NOVEL CANNABIDIOL DERIVATIVE ALLEVIATES BLEOMYCIN-INDUCED SCLERODERMA AND EXERTS POTENT ANTIFIBROTIC EFFECTS THROUGH PPAR-γ AND CB2 PATHWAYS.

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Scleroderma is a group of rare diseases that is associated with early and transient inflammation and vascular injury, followed by fibrosis affecting the skin and multiple internal organs. Fibroblast activation is the hallmark of scleroderma and disrupting the intracellular TGFβ/Smad signaling may provide a novel approach to controlling fibrosis. Because of its potential role in modulating inflammatory and fibrotic responses both PPARγ and CB2 represent attractive targets for the development of cannabinoid-based therapies. We have previously found that CBD oxidation increases its PPARγ agonistic activity and we have generated a non-electrophilic CBD quinol derivative, VCE-004.8, that is a dual agonist of PPARγ and CB2 receptors.

Methods: We study the *in vitro* effect of VCE-004.8 on collagen deposition, gene transcriptional activation and myofibroblast differentiation induced by TGFβ. To study the anti-fibrotic efficacy *in vivo*, dermal fibrosis was induced by daily subcutaneous injections of bleomycin into mice upper back for 6 weeks. During the last 3 weeks mice were treated in parallel by daily intraperitoneal injections.
Results: VCE-004.8 inhibits Col1A2 and Smad-dependent gene transcription induced by TGFβ as well as reduces collagen synthesis through a PPARγ-dependent pathway. The compound also prevents myofibroblast differentiation induced by TGFβ. In vivo results show that VCE-004.8 reduces dermal thickness, blood vessels collagen accumulation and prevents mast cell degranulation and macrophage infiltration in the skin. These effects were abolished in the presence of either T0070907 (PPARγ antagonist) or AM630 (CB2 antagonist). Using a fibrosis specific RT² Profiler PCR Array we found that VCE-004.8 downregulates the expression of several genes associated with fibrosis.
Conclusion: VCE-004.8 is a novel CBD derivative with agonistic activity on PPARγ and CB2 that has great therapeutic potential for the treatment of fibrotic diseases such as SSc.

THE EFFECTS OF CANNABINOID 2 RECEPTOR MODULATION IN EXPERIMENTAL PROLIFERATIVE VITREORETINOPATHY

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Proliferative vitreoretinopathy (PVR) can develop after ocular trauma or inflammation and is a complication of surgery to correct retinal detachment. The inflammation and fibrosis that occurs in PVR is characterized by the proliferation and migration of retina pigmented epithelial (RPE) cells to form contractile membranes comprised of cellular components and extracellular matrix. Currently, there are no pharmacological treatments for PVR. Cannabinoids acting at the cannabinoid 2 receptor (CB2R) can decrease inflammation and fibrosis. Therefore, the objective of this study was to examine the anti-inflammatory actions of CB2R as a candidate novel therapeutic target in experimental PVR.

Method: A model of experimental PVR was induced for 24, 72 hr or 1 week in CB2R knock-out (CB2RKO), wild-type (WT; C57BL6) and CD1 mice by intravitreal injection of dispase/collagenase. Ocular internal tissue morphology was evaluated by histopathological scoring using a modified previously published scoring table. Microglia, astrocytes and neutrophils were identified using immunohistochemistry. Real time intravital videomicroscopy (IVM) was used to visualize and quantify leukocyte-endothelial interactions in the iridial mcirovasculature, as a measure of ocular inflammation.

Results: Intravitreal injection of dispase significantly increased histopathological scoring compared to saline in CB2RKO ($p < 0.01$), but not in WT animals ($p > 0.05$). WT animals treated with CB2R antagonist, AM630 had significant increases in histopathological scores compared to WT dispase control ($p < 0.05$). In dispase-injected CB2RKO and WT + AM630 groups, retinal damage was correlated with increases in microglia and activated astrocytes ($p < 0.001$). Consistent with inflammation, leukocyte adhesion was significantly increased in the iris microvasculature after dispase-injection in CD1 mice at 24 ($p < 0.01$) and 72 hr ($p < 0.001$). Treatment with the CB2R agonist, HU308, significantly decreased leukocyte-endothelial adhesion at 24 hr compared to dispase + vehicle ($p < 0.05$).

Conclusion: Absence of CB2R or treatment with CB2R antagonists was associated with increased inflammation and exacerbated tissue damage in experimental PVR. Consistent with an immunosuppressive role for CB2R, activation of CB2R reduced inflammation and ocular pathology. Therefore, intervention at early stage PVR with drugs targeting the endocannabinoid system, specifically CB2R, reduces inflammation and disease severity and may prevent PVR progression and vision loss.

TRPV1 IN THE DORSOLATERAL PERIAQUEDUCTAL GREY DIFFERENTIALLY MODULATES FORMALIN-EVOKED NOCICEPTIVE BEHAVIOUR IN SPRAGUE-DAWLEY AND WISTAR-KYOTO RATS

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Introduction: The Wistar-Kyoto (WKY) rat is a stress-hyperresponsive strain that exhibits a hyperalgesic phenotype, compared with the Sprague-Dawley (SD) strain (Burke et al., Neuroscience 2010; 171:1300-13). Transient receptor potential vanilloid receptor 1 (TRPV1) within the midbrain periaqueductal grey (PAG) plays a key role in regulating nociceptive behaviour via modulation of neuronal activity in the rostral ventromedial medulla (Palazzo et al., Mol. Pain, 2010;6: 66).The present study tested the hypothesis that pharmacological modulation of TRPV1 in the dorsolateral (DL) PAG would differentially regulate formalin-evoked nociceptive behaviour in SD versus WKY rats.

Methods: Adult male WKY and SD rats (n=5-7 per group; 260-290g) received intra-DLPAG injections of either vehicle (100% DMSO), the TRPV1 agonist capsaicin (6nmoles/0.2μL), the TRPV1 antagonist 5'-IRTX (0.5nmoles/0.2μL) or co-administration of capsaicin and 5'-IRTX via bilaterally implanted stainless steel guide cannulae, 10 minutes prior to intra-plantar formalin injection (2.5%, 50μl). Nociceptive behaviour was assessed for 60 minutes using EthoVision XT. In a separate experiment, we used qRT-PCR to compare levels of TRPV1 mRNA in the DLPAG of SD and WKY rats (with and without formalin administration). Data were analysed by two-way ANOVA (with or without repeated measures) followed by Fisher's LSD post-hoc test. $P<0.05$ was considered statistically significant.

Results: In SD rats, intra-DLPAG administration of 5'-IRTX or capsaicin significantly increased formalin-evoked nociceptive behaviour, in the later phase of the formalin trial compared with vehicle-treated rats. These effects of 5'-IRTX or capsaicin were not observed in WKY rats. WKY rats receiving either intra-DLPAG vehicle or capsaicin injection, but not 5'-IRTX, exhibited higher nociceptive behaviour over the entire formalin trial compared with SD counterparts. Co-administration of capsaicin with 5'-IRTX had no effect on formalin-evoked nociceptive behaviour when compared with vehicle treatment in either SD or WKY rats. TRPV1 mRNA levels were significantly higher in the DLPAG of non-formalin treated SD rats compared with WKY rats. Formalin administration decreased the TRPV1 mRNA levels in SD rats but not in WKY rats.

Conclusion: Pharmacological blockade/desensitisation of TRPV1 in the DLPAG results in elevation of formalin-evoked nociceptive behaviour in SD but not WKY rats. These data, together with evidence for higher expression of TRPV1 mRNA in the DLPAG of SD rats, suggest a differential role of TRPV1 in the DLPAG in nociceptive responding between the two rat strains.

ASSESSING THE NEED FOR MEDICAL CANNABIS AND CANNABINOID EDUCATION AMONG HEALTH PROFESSIONALS

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As cannabis research progresses and with the formation of a growing cannabis industry it is important to prepare medical professionals and patients for new challenges that will arise. At this time in Europe there are no established educational programs for medical professionals. More countries are changing their policies on medical cannabis and cannabinoids; some have even formed medical cannabis programs. However, without educated medical personnel the practical application of medical cannabis will be greatly diminished. This is a cross-sectional survey assessing how informed medical professionals are about medical cannabis and their opinions on the subject.

Methods: A cross-sectional study was conducted from January to April 2015 among medical professionals from different countries by way of an online questionnaire. The data was analysed using descriptive statistics.

Results: There where 124 questionnaire responses from different health professionals (medical students, GP/family medicine trainees, GP/family medicine practitioners, hospital specialists, pharmacists). 41% where approached by patients about cannabis, but only 8% had prescribed cannabis to a patient. They believe that the media has a strong influence on patients (83%), and 54% believe that patients are or may be better informed then them on the subject of medical cannabis. 85% say they would attend a seminar on the subject, while 86% think it should be included in official medical training. 82% believe that medical cannabis should be regulated inside the health system.

Conclusion: This study points towards a need for educating medical personal on the subject of medical cannabis and cannabinoids as well as the endocannabinoid system. The current level of knowledge among medical personnel does not make them feel comfortable implementing this treatment. It also shows that there is an interest in the medical community for good quality information on the subject.

REGION- AND FUNCTION-DEPENDENT SUFFICIENCY OF CANNABINOID CB1 RECEPTOR RESCUE: CONTRIBUTIONS OF GABAERGIC AND GLUTAMATERGIC COMPONENTS OF THE ENDOCANNABINOID SYSTEM

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Introduction: Endocannabinoids modulate synaptic transmission through retrograde depolarization-induced suppression of inhibition (DSI, on GABAergic neurons) and excitation (DSE, on glutamatergic neurons). The endocannabinoid system is involved in the regulation of a large variety of functions, including feeding behavior, seizure susceptibility, anxiety, and extinction of aversive memories, behaviors in which cannabinoid type-1 (CB1) receptor null mutant mice show alterations. Necessity of cell-type specific populations of the CB1 receptor has been assessed using conditional knockout models of the receptor. Using a conditional rescue approach, we recently reported sufficiency of CB1 receptor signaling in glutamatergic neurons for some hippocampal functions, but not for amygdalar functions.

Methods: To address the sufficient role of CB1 receptor signaling in distinct cell populations, we applied the Cre/loxP system in mouse lines for conditional rescue (Stop-CB1 which phenotypically equals a complete CB1-KO) of this receptor. The CB1 receptor was rescued cell-type specifically to endogenous levels in forebrain GABAergic neurons (GABA-CB1-RS) or in both dorsal telencephalic glutamatergic neurons and forebrain GABAergic neurons (Glu/GABA-CB1-RS). These animals with cell-type-specific rescue of the CB1 receptor were tested in paradigms for innate anxiety, fear learning, feeding behavior and seizure susceptibility.

Results: Functional rescue of the CB1 receptor was confirmed by immunohistochemistry, radioligand binding, and electrophysiological measurement of DSE and DSI. GABAergic rescue of CB1 receptor signaling did not improve the exaggerated susceptibility of Stop-CB1 mice to kainic acid-induced seizures, whereas the anxiogenic phenotype found in Stop-CB1 mice was largely restored to normal levels. Neither GABA-CB1-RS nor the double rescue Glu/GABA-CB1-RS restored the reduced food intake after fasting as present in Stop-CB1 mice. Fear extinction seemed slightly recovered in the GABAergic rescue, whereas a combined Glu/GABA rescue resulted in considerable improvement towards normal levels of extinction.

Conclusion: Using a conditional rescue approach to restore CB1 receptor signaling in a cell-type-specific manner, we showed distinctive sufficiency of the forebrain GABAergic subpopulation of this receptor for different brain functions. Whereas GABAergic CB1 receptor signaling was largely sufficient for normal anxiety-like behavior and some involvement in fear extinction was seen, no sufficiency was detected for either fasting-induced feeding or protection against chemically induced seizures. Furthermore, experiments with a combined glutamatergic and GABAergic rescue of the receptor showed that the behavioral effects of endocannabinoid-dependent modulation of neurotransmission in these neuronal populations are not merely additive. Rather, in some brain processes a more complete endocannabinoid system seems to be required for a normal output behavior.

THE MITOCHONDRIAL CANNABINOID RECEPTOR 1 (MTCB1) REGULATES MITOCHONDRIAL AXONAL MOTILITY

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Neurons strongly rely on mitochondrial energy metabolism with particular high energy demands at locations distant from the cell body and, for this reason, mitochondria have to be placed correctly through mechanisms involving fusion, fission and transport. Emerging evidence indicates a strong link between mitochondrial trafficking and neurodegenerative diseases. The cannabinoid receptor CB1 regulates neuronal activity and deregulations of its signaling are centrally involved in brain diseases. This function is exerted at least at two sites: (i) at the plasma membrane, where CB1 (pmCB1) acts as a classical G-protein coupled receptor (GPCR) and (ii) at the mitochondria, where it was recently described as the first GPCR associated to these organelles (mtCB1), regulating the mitochondrial activity in the brain. Here we show that in the central nervous system mtCB1 is implicated in the trafficking of mitochondria.

Methods: Hippocampal primary cultures from WT or KO-CB1 (-/-) PO-P1 pups were transfected with a mitochondrial fluorescent protein (mtDsred) and kept for 7-9DIV. Then, transfected neurons were recorded with a confocal spinning-disk microscope and mitochondrial trafficking parameters were analyzed (percentage of mobile mitochondria, velocity, distance travelled by mitochondria and dwelling time). Agonists of CB1, when used, were applied for 15min and neurons recorded before and after the treatment.

Results: The percentage of transported mitochondria both, anterograde and retrograde, is increased in CB1 KO axons compared to control WT neurons. Also, the total mitochondrial transport is reduced in WT neurons after an acute treatment of a specific CB1-agonist (HU). Moreover, KO axons overexpressing CB1 show a reduced percentage of mobile mitochondria when CB1 is activated, but not when a mutant lacking the predicted signal to mitochondria (DN22CB1) is expressed. Confirming these results, when a non-permeable version of HU (HU-Biotin that can only act in the pmCB1) is used, the reduction in mitochondrial transport is not observed. Finally, when neurons are pretreated with KH7, an inhibitor of soluble Adenylyl cyclase and downstream effector of mtCB1 is used, HU cannot decrease mitochondrial movement.

Conclusions: Our data show that mitochondrial CB1, in hippocampal axons, cause a reduction in the percentage mobility of this organelle in both senses, anterograde and retrograde. Moreover this regulation occurs through sAC signaling pathways. Together, these results indicate an additional mode of cannabinoid regulation of brain bioenergetics processes.

A RETROSPECTIVE DESCRIPTION OF THE USE OF NABILONE IN UK CLINICAL PRACTICE

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Background: Nabilone, a synthetic cannabinoid analogue, is licensed in the UK for chemotherapy induced nausea and vomiting but is known to be frequently used off-label for intractable chronic pain and spasticity in conditions such as multiple sclerosis (MS).

Objective: To describe nabilone use in the UK for licensed and non-licensed indications.

Methods: This multicentre observational study was undertaken in two phases; a 'pilot study' (3 NHS Trusts) and then an 'extension study' (5 Trusts). Sites were selected following identification by the manufacturer as high-prescribing centres. Data were collected retrospectively from medical records of 250 patients prescribed nabilone between 1st January 2005-March 2013, mean observation period 31.1 (standard deviation (SD) 22.8) months.

Results: MS was the most common distinct condition for which nabilone was prescribed (19%, 48/247 patients with data recorded). Results are presented here for these 48 patients. Where n<48, data were missing from medical records. 67% (32/48) patients were female. At nabilone initiation mean age was 51.9 (SD 9.0) years and prior symptom duration 6.9 (6.7) years (n=33); 76% (28/37) patients had been prescribed ≥3 other classes of medication for the same symptoms. Nabilone was most commonly prescribed for pain (81%, 29/36) and spasticity (61%, 22/36). 50% (21/42) patients started on 1mg and 38% (16/42) on <1mg daily. 52% (25/48) patients were recorded as benefitting from nabilone; most common benefits were pain relief (n=19), improved sleep (n=15) and spasticity relief (n=7). 31% (15/48) patients were recorded as experiencing adverse effects, most commonly drowsiness (n=8), fatigue (n=5) and dizziness (n=2). The estimated mean cost of nabilone was £3,296 (SD £3,300)/patient/year (n=42).

Conclusions: Almost all nabilone use was for unlicensed indications. Although most patients prescribed nabilone for MS had experienced symptoms for many years and had been prescribed a number of other medications, over half derived benefit and adverse effects were no different to other CNS-active medications. The cost of nabilone should be considered in light of numbers of prior treatments and the comparatively high cost of other MS medications. Incomplete recording was a problem for many data fields collected in the study. Careful and complete documentation is important, particularly when a drug is used off-licence.

DOSE-DEPENDENT EFFECTS OF CANNABIS ON THE NEURAL CORRELATES OF ERROR MONITORING IN FREQUENT CANNABIS USERS

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Introduction: Cannabis has been suggested to impair the capacity to recognize discrepancies between expected and executed actions. However, there is a lack of conclusive evidence regarding the acute impact of cannabis on error monitoring.

Methods: In order to contribute to the available knowledge, we used a randomized, double-blind, between-groups design to investigate the impact of administration of a low (5.5 mg THC) or high (22 mg THC) dose of vaporized cannabis vs. placebo on the amplitudes of the error-related negativity (ERN) and error positivity (Pe) in the context of the Flanker task, in a group of frequent cannabis users (required to use cannabis minimally 4 times a week, for at least 2 years).

Results: Subjects in the high dose group ($n = 18$) demonstrated a significantly diminished ERN in comparison to the placebo condition ($n = 19$), whereas a reduced Pe amplitude was observed in both the high and low dose ($n = 18$) conditions, as compared to placebo.

Conclusion: The results suggest that a high dose of cannabis impairs both the conscious (late), as well as the initial automatic processes involved in error monitoring, while a low dose of cannabis deteriorates only the conscious (late) processing of errors.

mTORC1 ACTS THROUGH HYPOTHALAMIC MELANOCORTIN AND ENDOCANNABINOID SIGNALING TO MODULATE FOOD INTAKE

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The mammalian target of rapamycin complex 1 (mTORC1) pathway is an intracellular fuel sensing pathway whose hypothalamic activity is known to affect food intake and body weight. Previous data from our laboratory have shown that the central administration of the mTORC1 inhibitor, rapamycin, induced hyperphagia. During refeeding, rapamycin rapidly decreases the hypothalamic content in α-MSH, an anorexigenic neuropeptide released by POMC neurons of the arcuate nucleus (ARC) and that acts through the melanocortin receptors (MCRs) on parvocellular neurons of the paraventricular nucleus (PVN). Furthermore, mTORC1 blockade increases hypothalamic levels of the endocannabinoid anandamide, which, by engaging cannabinoid 1 receptor (CB1R), could modulate synaptic inputs onto the PVN, thereby determining hyperphagia. Despite these observations, the detailed mechanisms linking mTORC1 and endocannabinoid system in the modulation of the hypothalamic melanocortin system remain unclear.

Methods: *In vitro* electrophysiology in acute coronal hypothalamic slices: (i) Ability of mTORC1 pathway in controlling POMC neuron excitability was assessed by monitoring action potential firing in POMC-YFP mice; (ii) Implication of mTORC1, melanocortin and endocannabinoid signaling in the control of neurotransmitter release was evaluated by recording miniatures excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) in parvocellular neurons of the PVN from WT and POMC-CB1–KO mice. *In vivo*, a pharmaco-genetic approach (hM3D DREADD/CNO system in POMC-Cre mice) allowed the specific and reversible activation POMC neurons, while measuring food intake at refeeding in response to intracerebroventricular (icv) rapamycin administration. *Ex vivo*, assessment of hypothalamic endocannabinoid levels by liquid-mass spectrometry following pharmaco-genetic approach.

Results: 1) Rapamycin rapidly induced the firing of POMC, GABAergic-like, neurons while inhibiting POMC, glutamatergic-like, neurons. **2)** In parvocellular neurons from WT mice, rapamycin or CB1R agonist (WIN55-212) reduced mEPSC frequency. No effect was observed in POMC-CB1–KO. Activation of MCRs (MTII) or CB1R blockade (AM251) fully reversed the rapamycin-induced decreased frequency. **3)** Activation of CB1R reduced mIPSC frequency in WT but not in POMC-CB1 mice. **4)** *In vivo* acute pharmaco-activation of POMC neurons induced mTORC1 signaling in POMC neurons, prevented the orexigenic action of rapamycin and inhibited rapamycin-induced increase in hypothalamic anandamide content.

Conclusions: Here we demonstrate that the mTORC1 pathway controls food intake by modulating POMC neurons activity (excitability and synaptic release) and endocannabinoid signaling. Altogether, these findings pinpoint the exact relationship between mTORC1 and endocannabinoids in the regulation of POMC neuronal function and feeding.

HEMP MERISTEM MACERATES AS PLANT-BASED RAW MATERIAL

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Vital processes in buds and shoots ensure the mobilisation of lipids as fatty acids as well as the digestion of storage starch into diverse sugars. There are high amounts of oligosaccharides, phytohormones (e.g. auxines, gibberellines), aminoacids or proteins and enzymes. All these building materials are needed for the metabolism in undifferentiated cells that build up the division-active tissues called meristems. Moreover primary substances are available in meristems and in differentiated cells they are used for the synthesis of species-typical secondary plant compounds.

In phytotherapy the whole plant, roots, leaves, stalks, flowers, seeds or even barks are used as starting material. In gemmotherapy, as a specialised form of phytotherapy, specific macerates are used from meristems of buds and shoots. The total information, the vital and growth force of the bud is utilized as regeneration and healing power for humans.

Therapy-specific or as a preventive measure gemmotherapeutics take an increasingly important role in natural medicines. With its vasodilatative and catabolic effect they help release the body tissue from endotoxins and ensure a better regeneration and healing. With the direct protein communication in the cells gemmotherapeutics stimulate the immune system and arrange for the re-establishment of the protein balance in the blood.

In this context the Terra Energetika GmbH, as first company worldwide, is focussing on the manufacture and application of meristem-macerates from *Cannabis sativa* L. Such plant-based raw materials are of great value to the food and cosmetics industry as well as to phytopharmacy.

Method: Cell tissue from buds of diverse, originally continental landraces of *Cannabis sativa* L. (Tattwas certified bio-dynamic hemp cultivation) was used as starting material. This material was further processed according to recipes of the Terra Energetika GmbH. From the single buds and shoots, carefully picked by hand, a macerate of ethanol, glycerine and water was manufactured under good manufacturing practice. After the maturing time the extract was filtered and scientifically investigated for dissolved ingredients, purity and legal conformity. The scientifical and analytical examination in compliance with GMP and ISO 17025 has been carried out by an international renowned analytic laboratory and by our partner laboratory in Switzerland.

Result: Among the principal ingredients a number of important cannabinoids were found for example cannabichromen (CBC) or cannabigerol (CBG) as well as other ingredients such as α-bisabolol and vitamin E acetate.

Conclusion: These ingredients show a broad application area and are proved in case of infections, inflammations, pain, psychical discomforts (e.g. depression, stress, fear etc.), loss of appetite, psychosomatic disorders, skin diseases and asthma. For example α-bisabolol that was originally isolated from chamomile oil is not toxic for healthy cells but induces apoptosis (natural cell death) in cancer cells.

In comparison, the gemmotherapeutic from summer lime (*Tilia platyphyllos* Scop.) is known for its anxiety reducing, relaxing and nervine quality and is successfully applied in states of fear as well as for gastritis and bulimia. For depressive states fig tree (*Ficus carica* L.) as well as weeping birch (*Betula pendula* Roth) strengthens nerves and with both combined it could be achieved an even better success.

Initial studies have shown that concerning the ingredients of hemp meristem macerates there is a wide range of possible applications that have considerable importance for medicinal and therapeutical approaches.

PHARMACOLOGICAL TARGETING OF THE CANNABINOID TYPE-2 (CB$_2$) RECEPTOR PREVENTS INFLAMMATION-DRIVEN NEURODEGENERATION IN THE LIPOPOLYSACCHARIDE RAT MODEL OF PARKINSON'S DISEASE

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Introduction: The endocannabinoid system has recently emerged as a potential anti-inflammatory target to break the self-sustaining cycle of neuroinflammation and neurodegeneration that is associated with neurodegenerative diseases. Indeed, we have recently shown that there is significant CB$_2$ receptor upregulation, concomitant with microglial activation, in the inflammation-driven lipopolysaccharide (LPS) rat model of Parkinson's disease (Concannon et al., Exp Neurol 2015;269:133-141). Therefore, the aim of this study was to determine if pharmacological activation of CB$_2$ receptors can prevent inflammation-driven Parkinsonism in this model, which would further highlight the potential of the CB$_2$ receptor as an anti-inflammatory and neuroprotective target for Parkinson's disease.

Methods: Male Sprague Dawley rats were assigned to four groups: [1] LPS lesion & CB$_2$ agonist (n=10), [2] LPS lesion & Vehicle (n=10), [3] Naive & CB$_2$ agonist (n=4), [4] Naive & Vehicle (n=4). Animals in the lesion groups received a unilateral, intra-nigral LPS lesion. Animals were injected with a CB$_2$ agonist, JWH-133, or vehicle, 2 hr prior to surgery and daily for a further 13 days. Animals underwent twice daily behavioural testing for motor sfunction over the dosing period and were sacrificed 14 days post-surgery. Quantitative immunohistochemical analysis was performed to assess neuroinflammation and neurodegeneration in the nigrostriatal pathway.

Results: Injection of LPS into the rat substantia nigra caused significant motor dysfunction in the Stepping and Whisker tests of forelimb kinesis and sensorimotor integration respectively, and this was underpinned by significant nigral microgliosis and nigrostriatal neurodegeneration. Chronic JWH-133 attenuated LPS-induced dopaminergic neurodegeneration but this did not ameliorate motor dysfunction. Interestingly, the CB$_2$ agonist did not reduce microglial infiltration/proliferation at the lesion site, indicating that the protective effects of JWH-133 treatment may involve other anti-inflammatory mechanisms such as reduction in release of pro-inflammatory mediators from these cells.

Conclusions: Overall, this study has shown that pharmacological targeting of the CB$_2$ receptor protects the nigrostriatal pathway from inflammation-driven dopaminergic neurodegeneration via a mechanism that does not involve a reduction in microgliosis. This study indicates that targeting the CB$_2$ receptor may represent a viable target for anti-inflammatory disease modification in Parkinson's disease.

COMT-CANNABIS INTERACTION DURING ADOLESCENCE AS A RISK FACTOR IN DEVELOPING SCHISOPHRENIA

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Introduction: Schizophrenia is a complex disabling disorder possible caused by a combination of genetic and environmental factors. Previous studies have found an interaction between genetic mutations in the COMT gene (i.e. the Val/Met polymorphism) and cannabis use in the risk of developing schizophrenia. In particular, adolescents carrying the COMT Val allele (that produce a relative increased COMT activity) appeared to be the most sensitive to the early effects of cannabis on the development of psychotic symptoms later in life. Moreover, epidemiological studies have shown that during adolescence, a developmental period marked by considerable neuronal rearrangement especially in PFC, exposure to cannabis is a key factor that determines increased vulnerability to the development of schizophrenia. Thus in this study we wanted to investigate how a gene-environment interaction might influence brain development and lead to schizophrenia-relevant behavioral phenotypes, using genetically modified mice that carry the COMT-Val human variant (i.e. COMT-Val transgenic mice). **Methods:** COMT Val tg mice and their control littermates were injected with increasing doses of (1.25-5.0 mg/kg, i.p) Δ^9-THC (or vehicle for 15 days during adolescence (pnd 29-44) and then tested during adulthood (pnd>90-122). To control for the developmental effects of Δ^9-THC treatment interacting with the COMT Val genotype another cohort of COMT Val-tg and control littermates were instead treated with the same increasing doses of Δ^9-THC or vehicle only during adulthood (pdn75-90) and then tested after a 20 days of washout. Both groups of adolescence THC e adult THC were assessed during adulthood (pnd>90-122) for behavioral phenotypes relevant to schizophrenia, using different behavioral task such as Prepulse Inhibition(PPI), Locomotor Activity, Temporal Order Object Recognition, Social Interaction and Locomotor Activity after Amphetamine challenge. **Results:** THC exposure during adolescence leads to certain schizophrenia-like behavioral phenotypes in an exclusive COMT-dependent manner. Indeed, COMT Val tg mice exposed to THC during adolescence exhibit sensorimotor-gating deficits, reduced locomotor activity and also deficits in object recognition compared to their control littermates. Moreover, the same group of mice do not displayed behavioral sensitization after amphetamine challenge. On the contrary**,** adult COMT Val tg mice treated with THC during adulthood displayed decreased social interaction compared to their control littermates, but no significant differences between groups were evident in all other tasks. Furthermore, only WT mice treated with Δ^9-THC during adolescence displayed a significant behavioral sensitization after amphetamine challenge, no difference have been found in all other tasks. **Conclusions:** Our findings strength previous clinical evidence supporting a unique COMT Val x cannabis interaction in the development of schizophrenia-relevant phenotypes with a crucial timing component of adolescence as a vulnerability risk period. This novel and unique mouse model will then be suitable to elucidate the mechanisms underlying this gene-environment interaction in the development of schizophrenia related symptoms.

EFFECT OF CB1 RECEPTOR (CB1R) ACTIVITY ON IN VITRO SPINAL NETWORKS IN CONTROL CONDITIONS OR AFTER EXCITOTOXICITY

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The major endocannabinoid, anandamide (AEA) is shown to exert neuroprotection in various regions of brain including hippocampus and brain stem by lowering the excitotoxicity through CB1R-mediated signaling. Previous studies have also indicated that, immediately after spinal cord injury, an increase in AEA is an early response that may perhaps help recovery of locomotor function. However, sustained application of AEA has never been tested in an *in vitro* model of mammalian spinal cord for its effect on the standard locomotor rhythm and also after an excitotoxic insult. Hence in the present study, the outcome of sustained application of AEA levels or block of its uptake by the fatty acid amide hydrolase inhibitor (FAAH) LY 2183240 was investigated. We studied locomotor patterns in control conditions and after kainate (KA)-induced excitotoxicity. Since activation of CB1R has shown to inhibit adenylyl cyclase, cAMP levels were analyzed in both basal and forskolin stimulated conditions.

Methods: Isolated spinal cords of Wistar (P0-2) rats were maintained in vitro for up to 24 h for electrophysiological recording and subsequent histochemical processing. Locomotor-like rhythms were recorded from ventral lumbar roots by using suction electrodes. Spinal cords were treated with AEA (5-10 μM) or with LY 2183240 (1-5 μM) for 24 h to investigate any locomotor changes. To induce excitotoxicity, spinal cords were treated with kainate (50 μM; KA) for 1 h before the application of cannabinoid ligands for 24 h. Histology was done to analyze cellular damage, changes in neuronal and motoneuronal numbers, and to quantify CB1R expression changes. At the end of the experimental protocol of drug application, spinal cords were immediately processed to test the cAMP levels.

Results: Continuous (24 h) application of exogenous AEA or increasing the endogenous AEA levels (through FAAH inhibition with LY 2183240) significantly reduced the dorsal root afferent-elicited locomotor rhythms without changing mono or polysynaptic reflexes.
After 24 h AEA application, both basal and forskolin induced cAMP levels were decreased. Nevertheless, KA-evoked depression of electrophysiological activity was not significantly contrasted. A moderate protection at cellular level was observed after exogenous AEA application in comparison to only KA treated preparations. LY 2183240 did not produce any significant histological protection.

Conclusion: Sustained activation of CB1R reduced the number of bursts in electrically-evoked locomotion without changing the parameters of reflex activity or chemically evoked fictive locomotion. These results validate the notion that long-lasting application of cannabinoid agonists partially depresses spinal rhythmicity likely due to decrease in cAMP levels.
After KA-induced damage, lack of good recovery of functional activity associated with modest protection at cellular level despite prolonged cannabinoid ligand application suggests that any role of CB1R should perhaps be restricted to late stages after spinal cord injury when control of spasticity and pain is paramount.

EFFECTS OF PRENATAL EXPOSURE TO Δ^9-TETRAHYDROCANNABINOL ON EXCITATORY SYNAPTIC PROPERTIES OF VENTRAL TEGMENTAL AREA DOPAMINE NEURONS

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Early life adverse events can persistently affect brain functions, and contribute to the development of psychiatric trajectories. Hence, exposure to drugs of abuse early in life produces long-lasting cognitive deficits in humans. Particularly, prenatal Cannabis exposure has negative impact in humans on cognitive processing, such as reduced attention processing and impairments in motor and language skills as well as associative and discrimination learning. Despite the high prevalence of Cannabis use among pregnant women, its impact on the developing brain is still not accurate.

The present study was aimed at investigating on the effects of prenatal cannabinoid exposure on i) the postnatal maturation of glutamatergic transmission onto ventral tegmental area (VTA) dopamine (DA) neurons in the offspring; ii) endocannabinoid functional regulation of excitatory synapses onto VTA DA cells.

Methods: Sprague Dawley dams were administered the psychoactive ingredient of Cannabis, Δ^9-tethrayhydrocannabinol (THC, 2 mg/kg s.c.), once per day from GD 5 to GD 20. *Ex vivo* whole cell patch-clamp recordings were performed from VTA DA cells of off-spring of THC- and vehicle- exposed dams, during their third postnatal week. Excitatory postsynaptic currents (EPSCs) were stimulus-evoked and pharmacologically isolated. To examine whether or not prenatal THC treatment affected endocannabinoid system function, a form of short term synaptic plasticity at the same synapses was induced, CB1 receptor function studied, and endocannabinoid levels measured.

Results: We found that THC-offspring display **1)** AMPA receptor-mediated EPSCs larger at negative potentials when compared to positive potentials, **2)** a paired-pulse facilitation of AMPA receptor-mediated EPSCs, **3)** an augmented AMPA to NMDA ratio, **4)** a shorter decay time of NMDA receptor-mediated EPSCs, and **5)** an enhanced sensitivity to NR2A antagonist. We also found that excitatory synapses exhibit **6)** a larger depolarization-induced suppression of excitation (DSE), which could not be ascribed to either **7)** modifications of CB1 receptor agonist effects on AMPA receptor-mediated amplitude or **8)** endocannabinoid levels.

Conclusions: We conclude from these findings, that maternal Cannabis use alters **a)** development of both AMPA and NMDA receptors on VTA DA cells, and **b)** endocannabinoid system function at multiple levels. This changes might alter mesolimbic DA transmission, which might result in enduring changes in brain function and abnormal behavior. Whether or not these changes are long lasting and/or might contribute to affective dysregulation and addiction vulnerability later in life has to be examined yet.

ROLE OF N-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE IN (NEURO)INFLAMMATION

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Multiple sclerosis (MS) is a chronic progressive disease of the central nervous system (CNS) characterized by autoimmune and aberrant inflammatory responses. Histologic examination of bioptic samples reveals foci of severe demyelination, decreased axonal and oligodendrocyte numbers, and glial scars. Preclinical and clinical studies showed that N-palmitoylethanolamide (PEA), a naturally occurring lipid amide, exerts anti-inflammatory, analgesic and neuroprotective effects. PEA inhibits mast cells degranulation and glia activation; its levels change during CNS pathological conditions affecting the progression of the neuroinflammatory process. Plasma and cerebrospinal fluid levels of NAEs are altered in MS patients. PEA is preferentially degraded by the N-acylethanolamine acid amidase (NAAA), we have previously demonstrated that NAAA inhibition normalizes PEA levels in several inflammatory models. In order to investigate the role of NAAA in MS we induced the EAE model of MS in C57BL6/J mice and examined the expression of NAAA and inflammatory markers. Analysis of qPCR data showed that NAAA and iNOS levels are significantly up regulated in mice showing clinical signs of EAE. Immunofluorescence analysis demonstrated that NAAA is upregulated in activated microglial cells. To determine whether NAAA upregulation in microglia cells affects the onset and progression of EAE we generated transgenic mice overexpressing NAAA (NAAA ki) in CD11b-positive cells. NAAA ki were obtained by crossing NAAA conditional knock-in heterozygous mice carrying a NAAA isoform-1 coding sequence within the Rosa26 locus with CD11b-Cre transgenic mice. EAE was induced in NAAA ki mice and wild type littermates, clinical signs became evident 10 days post immunization in both groups, however NAAA ki mice showed significantly higher clinical sign scores than WT littermates. Moreover the abrupt weight loss that accompanies EAE onset was greater in NAAA ki mice than in WT mice. Our preliminary data suggest that the modulation of NAAA activity might be beneficial for the treatment of MS.

THE TWO NON-PSYCHOTROPIC CANNABINOIDS CANNABIDIVARIN (CBDV) AND TETRAHYDROCANNABIVARIN (THCV) PROMOTE PRIMARY HUMAN SATELLITE CELL DIFFERENTIATION: A NOVEL THERAPEUTIC OPPORTUNITY TO REINFORCE MUSCLE FUNCTION IN DUCHENNE'S MUSCULAR DYSTROPHY(DMD)

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Duchenne's muscular dystrophy (DMD) is one of the most common forms of hereditary myopathy resulting in the progressive degeneration of skeletal muscle tissue. It mainly affects young males since it is caused by alterations of the X-linked gene encoding for the structural protein dystrophin, resulting in irreversible muscle damage. In particular, the loss of the regenerative capacity of skeletal muscle precursor cells (satellite) and their decreased ability to differentiate into mature and functional myotubes leads to progressive muscle weakness with chronic degeneration (Shieh Neurol Clin. 2013,1009-29). Therefore, as ongoing therapies show poor efficacy (Bushby and Connor, Clin Investig 2011;1217-1235, we aimed at investigating the potential use of phytocannabinoids including cannabidiol (CBD), cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV) to promote satellite cell differentiation.

METHODS: a) Murine C2C12 myoblasts or primary human satellite cells were cultured *in vitro* and induced to differentiate with or without the aforementioned phytocannabinoids. After 4 days, total mRNA and proteins were collected to evaluate the expression level of skeletal muscle differentiation markers, such as myogenin (Myog), troponin-T (Tnnt-1) and myosin heavy chain (MyHC) which are normally up-regulated during muscle precursor cell commitment towards fused myotubes b) The ability of CBD, CBDV and THCV to influence intracellular calcium concentrations ($[Ca^{2+}]_i$) in skeletal muscle cells has been assessed in the present study.

RESULTS: **a)** In C2C12 cells, both CBD and CBDV 1-3 μM promoted the myotube formation. Whereas, 1-3 μM THCV did not produce significant effects. **b)** In primary human satellite cells CBD 1 μM, CBDV 3 μM and THCV 3 μM increase satellite cell transcript levels of MyHC, Myog and Tnnt-1. Western blot analyses confirmed the pro-myogenic effect of all the phytocannabinoids tested. **c)** Morphological analysis supported our molecular observations, showing that satellite cells induced to differentiate in the presence of THCV 3 μM or CBDV 3 μM exhibit a significant increase in myotube number, length and size compared to control. **d)** Moreover, in agreement with the pro-differentiating effects of CBDV and/or THCV, we found that these two phytocannabinoids in a dose-dependent manner increased $[Ca^{2+}]_i$ in both C2C12 and primary human satellite cells. In order to search for the molecular mechanism responsible for all these effects, we hypothesized the potential involvement of TRP channels as potential molecular targets for these phytocannabinoids.

CONCLUSIONS: We report that TRPA1, TRPV2 and TRPV4 showed the highest degree of expression in primary human satellite cells. A weak expression of TRPV1 was also found. Interestingly, the non-selective TRP channel antagonist Ruthenium Red (10μM) reversed the THCV-induced effects, whereas the effects of CBDV were only partially counteracted.

CBDV effect on Ca^{2+} seems to be specifically mediated by the TRPA1 channel, as revealed by the use the selective TRPA1 antagonist AP18, whereas THCV effect seems to be only partly mediated by this channel, suggesting that this compound exerts its effect through multiple TRP channel activation. TRPV2 antagonists did not counteract the effects of either CBDV or THCV.

In summary, these findings point to a novel opportunity for the use of certain phytocannabinoids to treat degenerative muscle diseases associated with defects in muscle differentiation and repair processes. Moreover, our study suggest that this beneficial effect of phytocannabinoids is dependent on their activation of TRP channels, and in particular of TRPA1.

CANNABIDIOL METABOLITES: FORMATION AND BIOLOGICAL ACTIVITY. AN OVERVIEW OF THE LITERATURE

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Of the phenolic terpene constituents of the *Cannabis sativa* plant, delta-9 tetrahydro-cannabinol (THC) has been well characterized pharmacologically over the past 50 years. In principle, acting as an agonist of cannabinoid CB1 and CB2 receptors, THC induces a multitude of central and peripheral effects. By contrast, the pharmacology of cannabidiol (CBD), the other prominent phytocannabinoid, is just beginning to be unfolded. CBD is not psychoactive and is a modest ligand of cannabinoid receptors. However, CBD has been shown to possess an astonishingly broad array of biological activities both *in vitro* and *in vivo*. Several preclinical studies in animal disease models and a limited number of human clinical studies have reported antipsychotic, anxiolytic, anti-inflammatory, analgesic, anti-emetic, muscle relaxant, neuroprotective, anticonvulsant (anti-epileptic) as well as anticancer, sebostatic and bone healing effects. Yet, the biochemical mechanisms responsible for the therapeutically useful effects are not fully understood.

The single-dose pharmacokinetics of CBD in humans has been studied after oral, smoking and intravenous administrations. The metabolism of CBD in mammals, including humans, involves extensive oxidation and conjugation. Since in typical therapeutic settings one hundred to several hundred milligram daily oral doses of CBD are administered, it is conceivable that metabolites formed from the natural substance may contribute to the overall pharmacological / therapeutic effects of the parent phytocannabinoid. While there is some information on the pharmacology of the main metabolite of THC, *i.e.*, 11-nor-9-carboxy-THC (Ujváry and Grotenhermen, *Cannabinoids* 2014;9:1-8), much less is known about the biological activity *in vitro* or *in vivo* of CBD metabolites. Exploring this area will help our understanding of the multitude of biological activity of CBD and could suggest novel structures related to the metabolites.

This presentation outlines the pharmacokinetics and metabolism of CBD in humans and reviews our currently limited knowledge on the biological activity (essentially studies *in vitro*) of CBD metabolites.

Methods: Chemical structure- and substructure based searches were conducted in SciFinder® and Reaxys® while text-based searches were done in PubMed and Google. Additional publications were located using snowballing techniques. Scholarly books on cannabinoids, including symposium proceedings, were also scanned for relevant information.

FASTING STIMULATES LIVER OEA MOBILIZATION THROUGH A HISTAMINE-DEPENDENT MECHANISM

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The fatty acid ethanolamide, oleoylethanolamide (OEA), is a lipid mediator that regulates feeding [1-3] and stimulates lipolysis and fatty acid oxidation in adipocytes and hepatocytes [4] through activation of nuclear peroxisome proliferator-activated receptor-alpha (PPAR-α) [5]. Fasting is accompanied by a substantial elevation in liver OEA levels [6], but the biochemical mechanism and physiological implications of this effect are unknown. The neurotransmitter histamine plays an important role in the control of feeding behavior and mediates, in part, the anorexic effects of OEA [7]. In the present report, we show that fasting promotes OEA mobilization in the mouse liver through a mechanism that requires activation of H_1-type histamine receptors by peripheral histamine.

Methods: Male HDC-null mice (mice deficient in the histamine-synthesizing enzyme histidine decarboxylase), wild-type littermates (129/Sv background) and male C57BL/6J mice (8-10 weeks-old) were fed a standard chow diet and were subjected to three different conditions: **a**) Free-feeding (FF); **b**) 12h food-deprivation (FD) and **c**) 1h refeeding after 12h food-deprivation (RF). Alpha-fluoromethylhistidine (α-FMH), a suicide inhibitor of HDC, was administered by intracerebroventricular infusion (5μg/5μl), while histamine receptor antagonists (fexofenadine and famotidine, 10 mg/kg) were administered by intraperitoneal injection during FD or RF. Endogenous OEA levels were quantified after lipid extraction by liquid chromatography/mass spectrometry.

Results: We found that: **1**) fasting stimulates liver OEA mobilization in wild-type mice, but fails to do so in mutant HDC-null mice; **2**) the H_1R antagonist, fexofenadine, prevents the effect of fasting on OEA accumulation in wild-type mice; **3**) the H_2R antagonist, famotidine, and intracerebroventricular infusion of α-FMH do not affect fasting-induced OEA accumulation in wild-type mice.

Conclusions: The results suggest that fasting stimulates peripheral histamine-dependent signaling, which promotes OEA mobilization in liver through activation of H_1, but not H_2 receptors. The mechanism underlying this novel function of histamine is under investigation.

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PEPTIDE ENDOCANNABINOIDS (PEPCANS) BEHAVE AS ENDOGENOUS PAMs FOR THE CB$_2$ RECEPTOR

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Peptide endocannabinoids (pepcans) are a family of hemoglobin-derived peptides recently identified [Heimann et al. PNAS 2007, Gomes et al. FASEB J 2009]. The length of pepcans ranges from 12 to 23 aminoacids and are N-terminal extended versions of the smallest and most abundant peptide, pepcan-12 (RVD-Hemopressin). Pepcan-12 was reported to interact with CB$_1$ receptors as agonist/inverse agonist. We recently identified that the entire family of pepcans acts as negative allosteric modulator (NAM) of CB$_1$ and pepcan-12 is the most potent and efficacious NAM [Bauer et al. JBC 2012].

Method: All the pepcans (-12,-14,-17,-20,-23) were tested in binding assay and [^{35}S]GTPγS assay using CHO- hCB$_2$ membranes. For β-arrestin recruitment and the modulation of cAMP levels, CHO- hCB$_2$ overexpressed cells were used. Competitive ELISA was developed using our in-house 1A12 antibody which recognized the entire family of pepcans. In the I/R (ischemia/reperfusion) model the hepatic artery and the portal vein were clamped with micro-aneurysm clamps for 60 minutes and reperfusion allowed for 2/6/24 hours. Liver was collected and pepcans levels measured by ELISA.

Results: Here we show that pepcans behave as positive allosteric modulators (PAMs) of CB$_2$ receptors. In binding assays pepcan-12 increased the affinity of orthosteric ligands ([^{3}H]-CP55,940 and [^{3}H]-WIN55,212-2) for hCB$_2$ receptors. When pepcan-12 was co-incubated with synthetic (CP55,940) or endogenous (2-AG) ligands it induced an increased efficacy and potency of the orthosteric ligand in functional assays ([^{35}S]GTPγS and cAMP). Pepcan-12 *per se* did not elicit any effect either in the binding or functional assays, thus behaving as pure PAM for CB$_2$.We quantified the levels of pepcans in different animal tissues by competitive ELISA exploiting our in-house antibody.The results showed that pepcans are present in different tissues with amounts ranging from 0.07 ng/mg tissue in the brain to 2.1 ng/mg tissue in the spleen.In LPS-challenged mice, the level of pepcans significantly raised suggesting a modulation of pepcan upon inflammation. In a mouse model of liver ischemia reperfusion, pepcans levels showed an increase after 2h and 6h of reperfusion returning to basal levels after 24h, following the kinetics of eCB as previously shown [Bàtkai et al. FASEB J 2007].

Conclusion: The different allosteric modulation properties of pepcans (negative at CB$_1$ and positive at CB$_2$) make them unique and versatile endogenous modulators of cannabinoid receptors.

EMOTIONAL AROUSAL STATE INFLUENCES ANANDAMIDE SIGNALING IN THE AMYGDALA TO MODULATE ANXIETY

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Introduction: Cannabinoid type 1 (CB1) receptors are abundantly expressed within the amygdala, where they regulate emotional responses. Emerging evidence indicates that cannabinoid drugs can induce distinct and often opposite effects on anxiety, cognition, and several other behaviors, depending on the stress level and the aversiveness of the environmental context. Although it is well established that cannabinoid drugs can induce opposing effects on emotionality, studies have not yet investigated whether the endocannabinoid anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are differentially released in the amygdala, in response to changes in the level of emotional arousal associated to the experimental conditions and whether this endocannabinoid release might also be a primary factor in determining the outcome of cannabinoid effects on anxiety behavior.
Methods: Male adult Sprague-Dawley rats were divided into two main groups and tested for anxiety behavior in the Elevated Plus Maze task (EPM). One group of rats was not handled, did not receive any prior habituation to the experimental room and was tested under high light condition (High Arousal condition; HA); the second group, instead, was extensively handled and habituated to the experimental room prior to the EPM and tested under red light condition (Low Arousal condition; LA) in order to decrease its novelty-induced stress response during the EPM testing. We measured amygdalar endocannabinoid AEA and 2-AG levels immediately after a 5-min EPM test in HA and LA rats and in a third control group that was not handled or tested (Home Cage group; HC). We also evaluated the effects on anxiety behaviour of intra-basolateral amygdala (BLA) administration of the AEA hydrolysis inhibitor URB597 (10 ng/0.2 ul), or its vehicle, either alone or together with the CB1 antagonist AM251, at a dose not-altering behavioural performance by itself (1 ng/0.2 ul).
Results: We found that the LA group displayed a significant low anxiety behavioral profile as compared to the HA group (e.g. higher % time spent in open arms and % number of open arm entries). We also found increased AEA levels only in the LA group as compared to both HA and HC groups. No changes were found in amygdalar 2-AG levels, thus, indicating that the increase in amygdalar AEA levels might be linked to the low anxiety profile shown by the LA group. URB597 was able to further decrease the anxiety response shown by LA rats, compared to the correspondent vehicle group, through the activation of CB1 receptors, without affecting emotional behavior in the HA group.
Conclusions: Taken together, these findings show how the endocannabinoid system is differentially activated to regulate anxiety response, depending on the level of the environment-associated emotional arousal and help to shed light on the neurobiological mechanism involved in the differential impact of stress on emotionality.

CANNABINOID SIGNALING IN STRESS AND DEPRESSION: THE ROLE OF DAGLα

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Depression is a highly prevalent psychiatric disorder associated with a high incidence of morbidity and mortality. Disruption of the endocannabinoid system (ECS) through pharmacological or genetic invalidation of cannabinoid CB1 receptors has been linked to depression in humans and depression-like behaviors in mice. Two main endogenous ligands of CB1 receptors, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), are produced on demand from phospholipids. The enzymes involved in the endocannabinoid biosynthesis thus play a major role in regulating the activity of this system. This study investigates the role of the main 2-AG producing enzyme diacylglycerol lipase α (DAGLα).

Methods
Here we generate and utilize knockout mice lacking DAGLα (Dagla$^{-/-}$), to assess the behavioral consequences of reduced endocannabinoid levels in the brain. We performed different behavior tests to determine anxiety- and depression-related behavioral changes in Dagla$^{-/-}$ mice. We also used the mitotic marker 5-bromo-2´-deoxyuridine to analyze adult neurogenesis.

Results
Dagla$^{-/-}$ animals show an 80% reduction of brain 2-AG levels, but also a reduction in cortical and amygdalar AEA. The behavioral changes induced by Dagla deletion include a maternal neglect behavior, a fear extinction deficit, increased behavioral despair, anhedonia, increased anxiety-related behaviors, and reduced hippocampal neurogenesis. Some of these behavioral changes resemble those observed in animals lacking the CB1 receptor.

Conclusion
Our findings demonstrate that the deletion of Dagla adversely affects the emotional state of animals and results in enhanced anxiety-, stress- and fear-responses.
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THE NAAA ACTIVITY INHIBITION AMELIORATES ADJUVANT-INDUCED ARTHRITIS IN RATS

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Fatty acid ethanolamides (FAEs) are a family of lipid messengers that participate in the control of multiple physiological functions, as well as pain and inflammation. They include agonists of cannabinoid receptors, such as anandamide (AEA), and agonists of type-α peroxisome proliferator-activated receptors (PPAR-α), such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). N-acylethanolamine acid amidase (NAAA), along with fatty acid amide hydrolase (FAAH), degrades these molecules and regulates their levels. NAAA, primarily localized in the lysosomal compartment of macrophages, is an N-terminal nucleophile cysteine amidase and displays a strong preference for saturated and monounsaturated FAEs, such as PEA and OEA. NAAA has attracted attention as a potential target for inflammation. In the present study we tested the effect of the first systemically active NAAA inhibitor, ARN726, in a model of rheumatoid arthritis (RA). Arthritis was developed in Sprague-Dawley rats by hind-paw injection of Complete Freund's Adjuvant (CFA): the disease developed within 24 hours and caused paw edema and thermal (heat) hyperalgesia. Robust increases in NAAA activity and immunoreactivity were observed in paws obtained from CFA-treated but not naïve rats. Additional immunohistochemical investigation confirmed an increment in NAAA signal in rheumatic paw tissues. Furthermore, histological investigation showed marked damages in terms of loss of the structure and organization of tissues, caused by edema formation, and the thinning of the epidermis, compared with naïve samples. Administration of ARN726 or dexamethasone, a steroidal anti-inflammatory drug, 7 and 14 days after CFA injection led to a reduction in both paw edema and heat hyperalgesia. Furthermore, the NAAA inhibitor was able to specifically reduce NAAA activity and lower PEA and OEA levels, pus production and MPO activity in paws. These results suggest that pharmacological blockade of intracellular NAAA activity causes significant analgesic and anti-inflammatory effects in the CFA model of arthritis by interfering with the production of pro-inflammatory cytokines at the site of inflammation.

ROLE OF THE CB_1 CANNABINOID RECEPTOR IN BRAF^{V600E} MELANOMA CELLS AND STEM-LIKE CELLS

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Introduction: Human cutaneous melanoma is a skin cancer with high metastatic potential, enhanced heterogeneity, and resistance to chemotherapy. It has been recognized that one of the most important factors responsible for treatment failure is the presence of a small subpopulations of cancer cells, known as melanoma initiating cells, that have stem cell properties, tumorigenic potential and the ability to self-renew. Cannabinoid signaling regulates cell proliferation, differentiation and survival, with different outcomes depending on the molecular targets and cellular context involved. The aim of the current study is to investigate whether the endocannabinoid system have a role in determining the neoplastic phenotype of human melanoma cells.

Methods: Human malignant melanoma cells, melanoma stem-like cells and normal human epidermal melanocytes were used as in vitro model system. Gene and protein expression were assessed by real-time PCR and western blotting. Knock-down of cannabinoid receptor type 1 (CB_1) was performed in BRAF^{V600E} mutant A375 cells using two independent CB_1 short hairpin RNAs (shRNAs) (LV-shCB$_1$-1 and LV-shCB$_1$-2) and confirmed by real-time PCR and western blot. Analyses of cell growth, colony formation, cell cycle assay, migration capability and self-renewal ability were assessed in control (LV-c) and CB_1-silenced cells.

Results: Quantitative real-time PCR analyses showed that melanoma cells harboring BRAF V600E mutation expressed CB_1 receptors, while BRAF-wild type cells, their correspondent melanoma stem-like cells, and normal melanocytes did not express or expressed CB_1 at low levels. CB_2 receptor expression was not found in the tested cell lines. Silencing of CB_1 receptor in A375 cells induced a significant reduction in the proliferation potential, clonogenicity, migration and promotion G2/M phase arrest. Moreover, preliminary experiments revealed a decrease in self-renewal capacity of A375 stem-like cells.

Conclusions: Our findings suggest a possible role of the endocannabinoid system, via CB_1 receptors, in determining aggressive phenotype in melanoma cells. Experiments are ongoing in trying to elucidate the molecular mechanisms involved, particularly the relationship between CB_1 receptor function and the BRAF^{V600E}-mutated pathway.

PHARMACOLOGICAL INHIBITION OF FAAH ATTENUATES TLR4-INDUCED INCREASES IN NFκB-INDUCIBLE INFLAMMATORY GENES IN THE FRONTAL CORTEX; EFFECTS PARTIALLY MEDIATED BY CENTRAL TRPV1 RECEPTORS

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Introduction: Enhanced endocannabinoid tone modulates neuroinflammatory responses and thus may provide a novel therapeutic target for neurodegenerative and psychiatric disorders. Accordingly, enhanced anandamide (AEA) tone following inhibition of the hydrolytic enzyme fatty acid amide hydrolase (FAAH) attenuates neuroinflammatory responses following toll-like receptor TLR3 or TLR4 activation (*Henry RJ et al J Neuroimmunol 2014, Kerr DM et al., Neuroscience 2012*). However, the contribution of FAAH substrates within the brain, and the receptor mechanisms, mediating such immunomodulatory effects, are not fully understood. As such, this study examined the effects of inhibiting FAAH, both systemically and centrally, on neuroinflammatory mediators in the frontal cortex following systemic administration of the TLR4 agonist lipopolysaccharide (LPS). Furthermore, we examined if the attenuation of TLR4-induced neuroinflammation following FAAH inhibition was mediated by endocannabinoid receptor targets within the brain.
Methods: Male Sprague-Dawley rats received systemic (i.p.:10mg/kg, i.p.) or central (i.c.v.:500nmoles; flow rate of 5μL/min) administration of the FAAH inhibitor PF3845 or the corresponding vehicle (i.p.: ethanol: cremophor: saline; 1:1:18, i.c.v.:100% DMSO), 15 (i.c.v.) or 30 (i.p.) minutes prior to systemic administration of LPS (100μg/kg, i.p.). In a separate experiment, rats received i.c.v. administration of antagonist at the CB_1 receptor, CB_2 receptor, PPARγ, PPARα, TRPV1 or GPR55 (AM251, AM630, GW9662, MK886, IRTX or CID16020046 respectively) 15 minutes prior to i.p. administration of PF3845, followed 30 minutes later with systemic administration of LPS. Animals were sacrificed 2h later, frontal cortical tissue excised, and expression of NFκB-inducible inflammatory genes determined using qRT-PCR. Concentration of AEA and the related *N*-acylethanolamines, PEA and OEA were determined using LC-MS-MS. Data were analysed using a one-way ANOVA followed by Fisher's LSD *post-hoc* test or unpaired two-tailed t-test. $p < 0.05$ was deemed significant.
Results: Systemic and central administration of PF3845 significantly increased frontal cortical levels of AEA, PEA and OEA, and attenuated the LPS-induced increases in cortical expression of NFκB-inducible inflammatory mediators, when compared to vehicle-LPS-treated counterparts. Prior central (i.c.v.) administration of AM251 or AM630 failed to alter the PF3845-induced attenuation of cytokine expression following LPS. However, prior i.c.v. administration of the IRTX significantly attenuated the PF3845-induced decrease in cortical expression of IL-6.
Conclusion: Increasing FAAH substrate levels directly within the brain potently attenuates TLR4-induced neuroinflammatory responses independent of central CB_1 and CB_2 receptor activation but rather is partially mediated via central activation of TRPV1. Overall, these findings support an important role for FAAH substrates directly within the brain in the regulation of acute neuroinflammatory responses. *Acknowledgements: Funding provided by Science Foundation Ireland Research Frontiers Project (Grant no. 11/RFP/NES/3175).*

PHARMACOLOGICAL BLOCKADE OF CANNABINOID CB[1] RECEPTORS IN DIET-INDUCED OBESITY REGULATES DIHYDROLIPOAMIDE DEHYDROGENASE IN MUSCLE

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Introduction: Cannabinoid CB1 receptors peripherally modulate energy metabolism. However, it is still unknown whether CB[1] receptor antagonism regulates glucose pathways in the striate muscle in obesity.

Methods: We investigate the role of the CB1 receptor on the expression profile of key glycolytic enzymes in skeletal muscle of rats fed with a high fat diet (HFD) and a high carbohydrate diet (HCD). We analyzed the same enzymes in a model of *CB1^{-/-}* mice. Dihydrolipoamide dehydrogenase (DLD), a flavoprotein component (E3) of α-ketoacid dehydrogenase complexes with diaphorase activity in mitochondria, was specifically analyzed. Finally, mitocohondria from myotube C_2C_{12} cell line were incubated with ACEA, an agonist, and AM251, an antagonist of CB1 receptor, to study the effect on diaphorase/oxidative activity of mitochondrial enzymes, such as DLD.

Results: AM251 blocked the HCD-induced expression of seven key enzymes from either glycolytic pathway or tricarboxylic acid cycle. DLD overexpression observed with AM251 was confirmed in muscle of *CB1^{-/-}* mice. Interestingly, we identified the presence of CB[1] receptors at the membrane of skeletal muscle mitochondria, as was described in brain. We also found that AM251 increased the DLD and CB[1] expression in the muscle mitochondria of high carbohydrate diet-fed rats, and elevated the diaphorase/oxidative activity in C_2C_{12} myotube mitochondria in a dose-response manner.

Conclusions: We conclude from these findings that **a**) the CB1 receptor blockade regulates the expression of several key glycolytic enzymes in the skeletal muscle. **b**) CB1 receptor is expressed on the mitochondria of the skeletal muscle. **c**) CB1 blockade under HCD feeding and CB1 knock-out produced an increase in DLD expression on the skeletal muscle. **d**) CB1 blockade increased the diaphorase/oxidative activity in a dose-dependent manner in an *in vitro* myotube model.

A PORTABLE AND BATTERY POWERED MEDICAL CANNABIS INHALER

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Research and Development Team

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Intended Use: The MIGHTY MEDIC Vaporizer is intended for vaporizing and then inhaling cannabinoids from hemp flowers (cannabis flos). The goal is the application of the active ingredients in the human body via the alveoli for the appropriate medical indication.

It is suitable for the temporary inhalative application of cannabinoids prescribed by a physician for use at home, in a hospital, or doctor's office.

This Vaporizer is already approved as a medical device in Europe (available Jan. 2016). Canada and Israel shall follow soon.
The poster shows excerpts out of the instructions for use, concerning e.g. the use of hemp blossoms including a scheme of the availability of THC and CBD within the flower, the vapor and the bloodstream of the patient. In short: approx. 33% of the Hemp blossom's THC and/or CBD reach the bloodstream by using the MIGHTY MEDIC or the VOLCANO MEDIC, world's first and so far only medical Cannabinoid Vaporizer.

It also shows the technical solutions in regard of an improved usability for hygiene (by disposable Lippieces) and the new Dosage Capsules with magazine, which allow user or nursing staff prepared portions to be already dosed and prefilled with its much easier handling especially for patients with fine motor dysfunction.
While Storz & Bickel GmbH & Co. KG (www.storz-bickel.com) is the manufacturer, Vapormed GmbH & Co. KG (www.vapormed.com) is the distributor of Storz & Bickel's medical devices.

MEDICAL CANNABIS ACTIVIST 2.0: ADVOCACY AND ILLNESS NARRATIVES

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Internet technologies transform our social interactions: The rapid rise and proliferation of different types of social media platforms and the active adoption of user generated content technologies has transformed human social interactions. Social media platforms like Facebook, YouTube and Twitter have created a space to exchange ideas, news and opinions about the mundane and magnificent. The technologies of web 2.0 provided tools, to create and share text, images, sound and video within social networks and online communities. These technologies enabled the active, engaged citizen to disrupt traditional pathways of communicating and sharing information. For example, Facebook, Twitter and texting were key tools used by activists during the Arab spring protests in 2010.

Patient activist 2.0 and medical cannabis: The normalisation of social media facilitated the emergence of the patient /activist 2.0. They created websites, social networks, online communities and blogs providing public platforms for advocacy, knowledge sharing and communal support. This created a different kind of influence and reach compared to the first generation patient/ activist who operated in the 90's pre-social media age. Patient/activists 2.0 marshalled social media platforms as strategic branding tools to construct online identities, personal narratives and ideologies. My poster will use case studies to illustrate how the patient/activist 2.0 is re- framing the discourse of cannabis self -medication and illness narratives and considers how this might impact the relationship with the cannabinoid research community.

SUCCESSFUL THERAPY OF TREATMENT RESISTANT ADULT ADHD WITH CANNABIS: EXPERIENCE FROM A MEDICAL PRACTICE WITH 30 PATIENTS

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Background: Attention deficit hyperactivity disorder [ADHD] may persist into adulthood. It may be treatment resistant to standard medication, that is methylphenidate, amphetamine derivatives and atomoxetine. Currently, no clinical studies have been conducted on cannabis-based medicines in ADHD, but a few case reports (*Strohbeck-Kuehner P, et al. Cannabinoids 2008;3(1):1-3. Available online at http://cannabis-med.org/data/pdf/en_2008_01_1.pdf*)
and basic research (*Adriani W, et al. Neurosci Biobehav Rev 2003;27:639-651.*) suggest therapeutic benefits in this condition.
In Germany, patients independent of medical condition may apply for an approval to use cannabis flowers from the pharmacy if the standard therapy of a certain disease or symptom is not efficient or associated with severe side effects.

Method: The medical certificates of 30 patients with adult ADHD of a medical practice (practice of FG), who were granted approval by the German Health Ministry to use cannabis flowers between 2012 and 2014 were analysed with regard to course of disease, previous treatment efforts, and effects of self-medication with cannabis or therapy with cannabis-based medications were analysed.

Results: Mean age of patients [28 male, 2 female] at first visit was 30 years [range: 21 to 51]. In 63% of cases ADHD was diagnosed only during adulthood. In all patients diagnosed in childhood [between 6 and 13 years of age] had previously been treated with methylphenidate. Further pharmacological treatment with was atomoxetine, deexamphetamine, lisdexamphetamine and amphetamine juice. Medication was usually discontinued due to side effects and often due to ineffectiveness. Eight patients continued to take stimulants and combined them with cannabis, by 22 patients were allowed to use it only. All patients had experienced an improvement of a variety of symptoms by cannabis flowers, including improved concentration and sleep, and reduced impulsivity, by the use of cannabis. In five cases dronabinol [THC] was tried, which was also effective.
Many patients were diagnosed before with cannabis use disorders by psychiatrists in hospitals or medical practices due to misinterpretation of effective illegal self-medication. Patients reported that their therapeutic experiences were not taken seriously by most physicians and that they were not listening to them due to strong prejudices. In many cases parents and/or spouses wrote testimonies on their observations confirming their statements.

Conclusion: For adult patients with ADHD, who experience side effects or do not profit from standard medication, cannabis may be an effective and well-tolerated alternative.

ENDOCANNABINOID-DEPENDENT PLASTICITY IN EXCITATORY AND INHIBITORY SPINAL DORSAL HORN NEURONS

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Introduction: Plastic changes in synaptic transmission between primary nociceptors and second order dorsal horn neurons have been implicated in many pathological pain conditions. Such plastic changes include among others synaptic short- and long-term depression (STD or LTD), and long-term potentiation (LTP) of synaptic transmission. These forms of plasticity are often NMDA receptor-dependent, but in particular STD and LTD can also involve endocannabinoids (eCBs).

Methods: Using acute slices of the murine lumbar spinal cord and whole-cell patch-clamp recordings of AMPAR-EPSCs, we have previously demonstrated the presence of eCB-dependent primary nociceptor LTD in second order neurons of the superficial dorsal horn. The occurrence of this LTD was reduced by about half both in cannabinoid $(CB)_1$ receptor–deficient mice and in the presence of the NMDA receptor antagonist APV, suggesting the presence of different LTD mechanisms at these synapses. To gain further insights into this heterogeneity, we investigated STD and LTD separately in excitatory and inhibitory dorsal horn neurons by use of "glutamatergic" vGluT2::eGFP or "GABAergic" Gad67[eGFP] transgenic mice.

Results: STD and LTD occurred in both subpopulations, but the underlying mechanisms were different. In GABAergic neurons, both STD and LTD were resistant to deletion of CB_1 and to blockade of NMDA receptors. Accordingly, deletion of DGL-α, the major synthesizing enzyme of the eCB 2-arachidonoyl glycerol (2-AG), did not prevent LTD in inhibitory interneurons. We also found that synaptic transmission between primary nociceptors and GABAergic neurons was resistant to modulation with CP-55,940, a mixed CB_1/CB_2 receptor agonist. In glutamatergic neurons, LTD, but not STD, was reduced by pre-treatment with APV suggesting that it depended at least partially on NMDA receptor activation. Since LTD in non-classified cells was reduced in CB_1 receptor-deficient mice, we hypothesize that LTD in glutamatergic neurons is also dependent on CB_1 receptor activation. This would be consistent with the modulation by CP-55,940 of nociceptor input to glutamatergic neurons.

Conclusion: Our results thus suggest that the mechanisms of plasticity at synaptic primary nociceptor synapses differ between synapses and that this difference is at least partially determined by the neurotransmitter phenotype of the postsynaptic neuron.

ADOLESCENT THC EXPOSURE AND EPIGENETICS: GENE TRANSCRIPTION ALTERATIONS AND CONSEQUENCES OF SUV39H1 MODULATION ON THC-INDUCED DEPRESSIVE/PSYCHOTIC-LIKE PHENOTYPE

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Adolescent female rats treated with the psychoactive ingredient of marijuana delta-9-tetrahydrocannabinol (THC) develop a depressive/psychotic-like phenotype in adulthood (75 PND). Therefore, the precise molecular mechanisms that link adolescent THC exposure to the development of this phenotype is not yet fully understood. In line with the recent involvement of epigenetic mechanisms in the pathogenesis of psychiatric illnesses, in the PFC of THC-treated animals we observed increased tri-methylation of Lysin9 on the histone H3 (H3K9me3) 2 hours after the end of the treatment. This increase was still present 24 hours later, together with increased di-methylation of Lysin9 and acetylation of Lysin14 on histone H3 (H3K9me2 and H3K14Ac). These alterations returned to control 48 hours after the last THC. Moreover, in parallel to the increase in H3K9me3 levels, we also observed increased Suv39H1 levels, the histone methyl-transferase responsible of the methylation on K9. On these bases, the first aim of this work was to check the transcriptional consequences of histone modification changes induced by the adolescent THC exposure. Finally, to understand the possible role of H3K9me3 in the development of depressive/psychotic-like phenotype, our last aim was to block Suv39H1 activity during adolescent THC treatment, and then evaluate the effect on the behavior in adult animals pre-exposed to THC.

Methods: adolescent female rats were treated with increasing doses of THC twice a day from PND 35 to 45 and Real-Time analysis were performed in the PFC 2, 24, 48 hours and 75 PND after the last THC injection. Gene profiling study was done using RT custom arrays to check 39 genes encoding for the endocannabinoid system (ECS) or involved in synaptic plasticity processes. For the last aim, Chaetocin, a selective inhibitor of Suv39H1, was injected at the dose of 0.05 mg/kg (i.p., once a day), during all the adolescent THC treatment and behavioral tests (Novel Object Recognition, Social Interaction and Forced Swim test) were performed at 75 PND.

Results: **1)** Adolescent THC exposure induced a global decrease of mRNA analyzed 2 hours after the last THC injection, but this effect was less intense than 24 hours after. In fact, 2 hours after the last THC injection only 2 genes were significantly downregulated (Crebbp and Arc), whereas 24 hours later 17 out of 39 genes were significantly downregulated (including genes of the ECS, gabaergic and glutamatergic system and genes coding for protein involved in synaptic plasticity). On the contrary, all the mRNA analyzed returned to controls or even increased 48 hours after the end of the treatment. **2)** At PND 75, 5 genes related to the gabaergic or glutamatergic system (e.g. Gria1, Gria2, Grm1 and 3, Gabra1 and ABAT), Dgl4 and Ntrk12 were still up regulated, whereas only the mRNA of Reln gene was strongly down-regulated. **3)** Chaetocin administration significantly prevented the cognitive deficit induced by the adolescent THC exposure in the Novel Object Recognition test. On the contrary, Chaetocin administration did not prevent social deficit in the Social Interaction test and behavioral despair in the Forced Swim Test.

Conclusions: We conclude from these findings, that **a)** adolescent THC treatment induces transcriptional repression of a set of genes involved in brain plasticity **b)** The up-regulation observed 48 hours after the last THC injection might be an adaptative response to counterbalance the down-regulation induced by adolescent THC exposure **c)** gene transcription alterations present at 75 PND could be relevant for the depressive/psychotic-like phenotype **d)** pharmacological block of SUV39H1 prevents cognitive deficits, but not the alterations of emotional behaviors, suggesting that the increase of H3K9me3 might play a role in the development of cognitive deficits induced by adolescent THC exposure.

2-OXO-1,2-DIHYDROPYRIDINE-3-CARBOXAMIDE DERIVATIVES AS A SOURCE OF CB$_2$ RECEPTOR MODULATORS WITH POLYPHARMACOLOGICAL FEATURES

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Cannabinoid receptor (CB$_1$R and CB$_2$R) and their endogenous ligands (endocannabinoids) anandamide and 2-arachidonoylglycerol constitute, together with several degrading and biosynthetic enzymes (FAAH, MAGL, ABDHs, DAGLs, NAPE-PLD) and other proteins (intracellular carrier proteins and the putative endocannabinoid membrane transporter, EMT) the endocannabinoid system (ECS). The ECS is involved in several physiological and pathological processes including cancer, appetite, memory, neuropathic and inflammatory pain, obesity and neurodegenerative diseases. In a research project aimed at obtaining new CB$_2$R ligands, we developed a series of 6-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide derivatives. Interestingly, we found that the nature of the substituent in position C5 of the pyridine ring is crucially involved in the functional activity of these molecules at CB$_2$R. To further investigate the structure-activity relationships of this class of compounds, different approaches were exploited: **a)** insertion of all the different halogens at the C5 position of the pyridine ring **b)** switch of the methyl group from the C6 to the C4 position; **c)** insertion of bulky substituents at the C4 or C6 position of the pyridine ring. We also tested the 2-oxo-1,2-dihydropyridine-3-carboxamide derivatives on all the main targets of the ECS.

Methods: The receptor affinities (K_i values) were evaluated performing classic radiometric binding assays using membrane preparations obtained in-house from stable transfected CHO-hCB$_1$ and CHO-hCB$_2$ cells. The functional activity of the hit compounds was evaluated with the [^{35}S]GTPγS assay in the same biological matrices. The enzymatic activity was assessed using radiometric assays and appropriate biological matrices such as pig brain homogenate (MAGL) , U937 cell homogenate (FAAH) and cell homogenates from transfected cells (ABHD6 and ABHD12). The effect on AEA and 2-AG uptake was evaluated in U937 cells using [^{3}H]AEA and [^{3}H]2-AG as substrate, respectively.

Results: **1)** The difference in electronegativity and atom size of the halogens (F, Cl, Br and I) did not affect neither the affinity nor the functional behavior of the relative compounds at CB$_2$R. **2)** The switch of the methyl group from the C6 to the C4 position did not influence the binding and functional properties of these compounds on CB$_2$R. **3)** Compounds with bulkier substituents in position C4 or C6 were less active. **4)** Some of the compounds, which showed the best binding properties at CB$_2$R, showed also a potent inhibition of AEA and 2-AG uptake (EC$_{50}$ values in the nM range). Some of these inhibitors might exert part of their action by inhibiting FAAH, while others not. **5)** Similarly to the binding properties, the nature of the substituent in positions 1 and 5 seems to influence also the EMT inhibition.

Conclusions: We developed a series of compounds that, beyond the modulation of CB$_2$R activity, are able to inhibit additional targets (EMT and FAAH). This led us to the identification of novel polypharmacology within the ECS. The effects of these compounds in different cellular systems is under evaluation.

METAPLASTICITY OF CORTICOSTRIATAL ENDOCANNABINOID SIGNALING IN HABIT LEARNING

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Background: According to current theories in the field, actions are controlled by the balance between two distinct cognitive processes acquired during instrumental learning: 1) flexible control of behavior, defined as "*goal-directed*", which is dependent on the causal relationship between the action and the outcome (A-O); 2) *habitual* behavior, which is largely independent of A-O, and is mainly triggered by contextual stimuli that were present during training. Distinct anatomical systems mediate goal-directed and habitual control of behavior. In particular, neuronal projections from the limbic and associative cortical areas to the medial part of the dorsal striatum are involved in goal-directed actions and behavioral flexibility, while projections from sensory motor cortices to the dorsolateral striatum in habit formation. Synaptic neuromodulation of these neuronal circuits appears to be crucial to determine action control. However, the precise physiological mechanism is still unclear.

Methods: In C57BL6/J mice (males, 40-50 days old), we assessed the molecular and synaptic impact of different training regimes of instrumental conditioning of nose poke for food reward, which promote either goal-directed (short-training) or habitual behavior (over-training). After training, to test whether behavior was goal-directed or habitual, we performed an omission procedure, in which the A-O contingency was reversed. Western blot analysis was carried out on aliquots of homogenates obtained from short and overtrained mice brains. Metabotropic group I receptors (mGluR1/5) -dependent plasticity and spike timing-dependent plasticity (STDP) were tested for each mouse with patch-clamp whole cell recordings in horizontal brain slices at excitatory cortico-striatal synapses in the dorsolateral striatum (DLS).

Results: In the DLS of overtrained habitual mice, we found that endocannabinoid (eCB)-mediated LTD induced either by pharmacological activation of mGluR1/5 or STDP protocol was specifically lost at cortical connections to the striatal medium spiny neurons of the striatopallidal pathway. This associated with reduced efficacy of the intracellular cascades downstream from mGluR1/5 activation, raising the possibility that training-induced metaplasticity of mGlur1/5 signaling and adaptations of the eCB pathway may contribute to habit formation. At the behavioral level, to test whether mGluR1/5 receptors were actively recruited during instrumental learning, we used the mGluR5 antagonist MPEP, either administered systemically or selectively within the DLS during training. We found that *in-vivo* treatment with the antagonist restored aspects of goal-directed behavior in overtrained mice.

Conclusions: The results reveal that metaplasticity of the mGluR1/5 signaling and adaptations of the eCB pathway at segregated striatal circuits contribute to habit learning, which occurs naturally after repeated practice.

SYNERGISTIC INTERACTION BETWEEN THE SEROTONIN AND CANNABINOID SYSTEM IN THE PREVENTION OF PILOCARPINE-INDUCED STATUS EPILEPTICUS

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Introduction: Cannabinoid type 1 receptor (CB1R) regulates neuronal excitability and has been shown to mediate the anticonvulsant effects of cannabinoids in different animal models of epilepsy. Several studies support the existence of crosstalk mechanisms between endocannabinoid (EC) and serotonin (5-HT) systems. The 5-HT_{2C} receptor ($5\text{-HT}_{2C}R$) subtype has received attention in epilepsy as KO mice for this receptor displayed increased seizure susceptibility, whilst its activation showed anticonvulsant effects in different models of epilepsy. Interaction between $5\text{-HT}_{2C}R$ and CB1R has been shown. For instance CB1R KO mice exhibit altered expression and impaired functionality of the $5\text{-HT}_{2C}R$ in several brain areas. Here we tested the interaction between $5\text{-HT}_{2C}R$ and CB1R in the prevention of status epilepticus (SE) using the rat pilocarpine (PILO) model.

Methods: Adult male Sprague Dawley rats were injected with PILO (360 mg/kg) after 30 min scopolamine treatment (1 mg/kg) and monitored for 3 hours by cortical electroencephalographic (EEG) and hippocampal local field potential recording. Seizure behaviour was also observed and severity was measured by Racine scale. Pre-treatment with the cannabinoid agonist WIN 55,2122 (WIN), the $5\text{-HT}_{2C}/_{2B}R$ agonist RO60-0175 (RO) or their combination (RO+WIN) was performed 45 min before PILO administration. Antagonists were injected 15 min before the agonists.

Results: PILO induced SE in the 85% of rat tested (Racine scale 4-5). During SE, a dramatic increase of EEG total power in both cortex and hippocampus was observed respect to the baseline recording. WIN and RO, administered alone, had no effect in preventing SE, although they were able to delay the onset of SE. Furthermore, only WIN reduced the severity of behavioural SE (Racine scale 2-3). Co-administration of RO+WIN significantly reduced the occurrence of PILO-induced SE. Power spectrum analysis revealed that RO+WIN significantly reduced EEG total power during SE, in respect to the vehicle group. Administration of CB1R antagonist AM251 completely blocked behavioural and EEG antiepileptic effects of RO+WIN. Intriguingly, antiepileptic effects of RO+WIN were potentiated by the administration of $5\text{-HT}_{2C}R$ antagonist SB242084 while were prevented by the treatment of $5HT_{2B}R$ antagonist RS127445. The administration of the $5HT_{2A}R$ antagonist MDL11,939 had no effect on RO+WIN treatment.

Conclusions: Data so far obtained indicate a synergistic interaction between the 5-HT and EC system in the prevention of SE, mediated by CB1R, $5HT_{2B}R$ and $5HT_{2C}R$, although the molecular mechanism of this interaction remains to be full clarified, and suggest that EC and 5-HT systems might represent a suitable target for the identification of new antiepileptic treatment.

THE ROLE OF CB_1 IN INTESTINAL PERMEABILITY IN HYPOXIA AND INFLAMMATION

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We have previously shown that exogenous applied endocannabinoids (anandamide and 2-AG) increase Caco-2 permeability through the CB_1, suggesting a role for this receptor in modulating gut permeability. The aim of the present study was to further investigate the role of CB_1 on Caco-2 permeability during normoxia, hypoxia and inflammatory conditions.

Methods: Caco-2 cells were grown until fully confluent on 0.4 μM pore size transparent polyethylene terephthalate (PET) membrane inserts. Transepithelial electrical resistance (TEER) measurements were made as a measure of permeability. URB597 (FAAH inhibitor) and JZL184 (MGL inhibitor)(to increase local levels of endocannabinoids) were added to the apical (luminal) or basolateral (systemic) membrane of cells. Hypoxia was induced using the GasPak™ EZ Anaerobe Pouch System. Inflammatory conditions were established by adding pro-inflammatory cytokines, interferon gamma (IFN γ 10ng/ml) and tumour necrosis factor alpha (TNF α 10ng/ml). AEA and 2-AG were measured by liquid chromatography tandem mass spectrometry. ELISAs were performed for the assessment of interleukin 8 (IL-8) and interleukin 6 (IL-6) levels in cell culture media.

Results: When applied to the apical or basolateral membrane of Caco-2 cells, URB597 (3μM and 10μM) decreased TEER levels (i.e. increased monolayer permeability) (p<0.001). JZL184 lowered TEER values when applied apically (p<0.01 for 3μM and p<0.001 for 10μM). Basolateral application of JZL184 (10μM) increased TEER (p<0.001), which mirrored the effect of 2-AG when applied again to the basolateral membrane. There effect of URB597 and JZL184 was abolished in cells where CB_1 was knocked down. In models of hypoxia or inflammation, URB597 and JZL184 further increased permeability. Furthermore, the permeability response to inflammation (but not hypoxia) was reduced in CB_1 knock down cells and both hypoxia and inflammation significantly increased cellular 2-AG levels (P<0.001). Despite this, IL-8 and IL-6 levels were reduced in media of cells treated for 24 h with AEA (p<0.05), URB 597 (P<0.001) and JZL 184 (p<0.001). Similarly IL-6 levels were reduced for AEA (p<0.01), 2-AG (P<0.0001), URB 597 (p<0.001) and JZL 184 (p<0.0001).

Conclusions: Increasing endogenous levels of endocannabinoids increases intestinal permeability in control conditions, in hypoxia and inflammation, through CB_1, and intestinal cells produce 2-AG in response to hypoxia and inflammation. The role of CB_1 in inflammation is demonstrated by the blunted permeability response to cytokines in CB_1 knock down cells. Despite this, endocannabinoids reduce inflammatory cytokines, suggesting this effect on intestinal permeability is distinct from their anti-inflammatory role.

RAW CANNABIS AND OTHER COMPLEMENTARY AND ALTERNATIVE MEDICINE IN RELAPSING-REMITTING MULTIPLE SCLEROSIS. A PILOT, RANDOMIZED, DOUBLE-BLIND, CROSSOVER TRIAL.

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Complementary and Alternative Medicine (CAM) is commonly used by Multiple Sclerosis (MS) patients to find symptomatic relief, in conjunction with or in lieu of, the prescribed standard therapy due a significant need of improving quality of life (QOL). (Schwarz,S et al. *Mult Scler.* 2008;14:1113-1119, Yadav,V et al. Neurology. 2014;82:1083-1092) Cannabis has been proposed as an Unique Dietary Essential, providing a large array of "nutraceuticals" that could help in the management of MS, such as lipid-soluble Vitamin E, Essential Fatty Acids (PUFA, Omega 3: Omega 6), Essential Amino Acids, antioxidants and Cannabinoid Acids. (Courtney,W.(2010).Treating Yourself. 24 (1), 52-54) Cannabis and cannabinoid extracts have been found beneficial to MS patients suffering neuropathic pain, spasticity or bladder incontinence, (Wade D et al. (2010) Mult Scler 16:707–714, Corey-Bloom et al.(2012).CMAJ.184 (10),1143-1150), but it has not yet been systematically explored the potential benefit of a daily supplementation of immuno-regulatory cannabinoids in their acid form. The main aim of this study is to compare changes in MS progression and side effect profiles of current therapies versus dietary supplementation with a low-fat, plant- based diet containing daily Cannabis S. in its raw/unprocessed form.

Methods: RRMS patients with EDSS <= 6, aged 20-50, without relevant co- morbidity nor previous history of substance abuse are assessed with biochemical, behavioural, neurological, neuropsychological and instrumental tests, at week 0 and week 9. The intervention comprises 8 weeks of dietary supplementation of vitamins (low-fat vegan diet) and Essential Fatty Acids, occupational therapy, mind-body therapy and natural setting.

Raw cannabis extracts are achieved by blending fresh flowers and juicing stems and leaves. This preparation is thought to be devoid of the psychotropic effects due lack of decarboxylation of naturally occurring THC Acid into THC. (Verhoeckx, K et al. (2006). International Immunopharmacology. 6 (1), 656-665). In the trial, active juices (2.5 g fresh Cannabis flowers + 300 g fresh hemp leafs + 7.5 g hemp oil) are randomized with placebo (300 g fresh spinach + 7.5 g hemp oil) and delivered in 5 supplementation daily. Support for this project is through a unique crowdfunding initiative combined with volunteer work, and represents a novel approach for progressing clinical research in this area.

Results: We will establish whether there is a decrease of chemical index of the inflammatory cascade, a general improvement in symptom management as well as a enhanced QOL related to lower side effects when compared with current treatments.

Conclusions: This project will explore the therapeutic potential of dietary supplementation with raw cannabis extracts, which builds on an unmet clinical need for cannabinoid-based therapies for the treatment of MS.

MODULATION OF LATE STAGE ATHEROSCLEROSIS BY THE TYPE-2 CANNABINOID RECEPTOR

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Introduction: Atherosclerosis is a chronic inflammatory disease of the vascular system that is characterized by the build up of plaques, composed of lipids and other cellular debris, within arterial walls. Instability of advanced plaques can lead to rupture, manifesting serious health consequences such as myocardial infarction or stroke. As atherosclerosis advances, plaques can calcify; a factor which increases their vulnerability to rupture. The mechanism of plaque calcification is unclear but is thought to be a cell-mediated process similar to bone remodeling. The type-2 cannabinoid receptor (CB2) modulates processes in immune cells involved in atherogenesis as well as in osteogenic precursor cells involved in bone remodeling. Prior studies showed that CB2 alters the composition of early stage plaques in Ldlr$^{-/-}$ mice, a murine model of atherosclerosis; however, the function of CB2 in more advanced plaques has not yet been elucidated. We hypothesized that CB2 modulates the composition of advanced plaques and tested this by evaluating the effects of systemic CB2 gene deletion on advanced plaque formation and calcification in Ldlr$^{-/-}$ mice.

Methods and Results: Groups (n≥8) of 8-week old CB2$^{+/+}$Ldlr$^{-/-}$ (WT) and CB2$^{-/-}$ Ldlr$^{-/-}$ (CB2$^{-/-}$) mice were fed a high fat diet (HFD) for up to 24 weeks to induce advanced atherosclerosis. Hyperlipidemia induced by the HFD did not differ between WT and CB2$^{-/-}$ mice, as determined using standard blood plasma analysis methods. LC-MS/MS analysis of aortic endocannabinoid levels showed increased anandamide (AEA) and 2-archidonylglycerol (2-AG) after 12 weeks of HFD for both groups compared to chow fed controls. After 24 weeks, aortic 2-AG levels were significantly elevated while the AEA levels decreased significantly in both groups. En face analysis revealed the extent of atherosclerosis in the aortic arch and thoracic aorta did not differ between WT and CB2$^{-/-}$ mice, but was ~1.9-fold greater in the abdominal aortas of CB2$^{-/-}$ mice (17.0±1.3% vs 9.0±1.3%, p=0.002). Morphometric analysis of von kossa stained serial cross sections showed calcium deposition in advanced aortic root plaque to be ~2.3 fold greater in CB2$^{-/-}$ mice compared to WT mice (12.9±1.1% vs 5.6±1.2%, p=0.002).

Conclusion: These results are consistent with our hypothesis that CB2 modulates plaque calcification in advanced atherosclerotic lesions. Information from this and future investigations could translate into CB2-targeted therapies for atherosclerosis.

ROLE OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 IN THE CONTROL OF BOTH NICOTINE-RELATED BEHAVIORS AND NICOTINE-INDUCED DOPAMINE NEURONAL ACTIVITY IN MICE

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Nicotine is the active substance in tobacco. It has been shown that nicotine-related behaviors are efficiently controlled by endocannabinoids, via different targets such as the cannabinoid type 1 (CB1) and peroxisome proliferator-activated receptors-α (PPARα) receptors. However, the brain transient receptor potential vanilloid 1 (TRPV1) is another important target of endocannabinoids. Interestingly, TRPV1 was recently implicated in addiction-related behaviors and TRPV1 can excite dopamine neuron activity in the ventral tegmental area (VTA). Thus we investigated the control of TRPV1 on nicotine-induced behavioral and cellular effects in mice.

Methods: TRPV1 knockout (KO) and wild type (WT) male mice were challenged with saline or nicotine 0.3 mg/kg i.p. using the protocol of repeated injections for 5 d. A group of mice was prepared for behavioral studies: they were challenged again with saline and nicotine in order to test nicotine-induced behavioral locomotor sensitization. Subsequently to the repeated injections, other mice were allocated to *ex vivo* electrophysiological studies: the firing activity of dopaminergic (DA) neurons within VTA was recorded before and after acute nicotine bath-application (1μM).

Results: Strikingly, TRPV1 KO mice did not show behavioral hyperactivity in response to the last challenge with nicotine when compared to WT mice. In brain slices, both acute and repeated injections of nicotine increased the spontaneous firing activity of VTA DA neurons in WT but not in KO mice. Similarly, while acute bath-application of nicotine (1 μM) induced a short but consistent increase in the spike frequency of WT VTA DA neurons, this amplifying effect was not observed in TRPV1 KO VTA neurons.

Conclusions: We conclude from these findings, that a) TRPV1 receptors control both the acute and chronic effects of nicotine. b) Part of the nicotine effects on VTA DA neurons are mediated by the TRPV1 receptors. c) TRPV1 receptors dependent regulation is involved in nicotine-induced behavioral sensitization. Thus our next step to further investigate the therapeutic opportunity with TRPV1 receptor pharmacology in nicotine addiction, is to test whether nicotine rewarding effects are lost in TRPV1 KO mice.

INVALIDATION OF THE CENTRAL CANNABINOID RECEPTOR ALTERS G PROTEIN SIGNALLING IN THE HIPPOCAMPUS

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The consequences of the invalidation of the central cannabinoid receptor to the intracellular signalling processes were studied in different regions of the mouse brain.

Methods: CB_1-KO mice and their wild type littermates were sacrificed and *hippocampi*, *cerebella* and *striata* isolated. Protein homogenates were used to measure stimulation of $[^{35}S]GTP\gamma S$ binding and stimulation/inhibition of adenylyl cyclase (AC) via the μ opioid (MOR), $GABA_B$, and β_1 adrenergic receptors. Furthermore, using the same samples we performed Western blots to assess the amount of Gi, Go and Gs proteins. Additional animals were used to collect RNA samples for quantitative PCR analysis of several regulators of G protein signalling (RGS) protein isoforms highly expressed in the hippocampus.

Results: In the *hippocampi* of CB_1-KO mice, Gi/Go-coupled GPCRs, MOR and $GABA_B$ but not Gs-coupled β_1 activated G proteins with a greater extent as measured by $[^{35}S]GTP\gamma S$ binding. However, the inhibition of AC activity, although reaching the same maximal value, was about 20 times weaker in mutant, than in wild type hippocampi by MOR-ligand morphine and $GABA_B$-ligand baclofen. Activators had the same effect on AC activity in both genotypes, indicating that the AC activity and subtype composition remained unchanged. We also assessed the expression level of $G\alpha i$, $G\alpha o$ and $G\alpha s$ proteins by Western blots. Quantitative analysis of the data revealed no significant differences between the $G\alpha$ protein levels in the *hippocampi* of CB_1-WT and CB_1-KO animals. RGS4 but not RGS2, RGS7, RGS14 or RGS19 expression levels showed a significant upregulation in the *hippocampi* of CB_1-KO mice.

Conclusions: We have found a region-specific change in the intracellular signalling in CB_1-KO mice, which was not present in *striata* or *cerebella*. In the *hippocampi* of mutant mice, however, we observed an altered G-protein signalling that was present in Gi/Go but not in Gs-coupled GPCR signalling. This particular alteration in G-protein signalling correlated with the upregulation of RGS4 in the *hippocampi* of CB_1-KO mice. From these findings, we hypothesise that the hippocampal upregulation of RGS4 caused the dysregulation of G protein signalling in CB_1-KO mice.

CANNABINOID RECEPTOR 2 DEFICIENCY RESULTS IN REDUCED NEUROINFLAMMATION IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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Background: Alzheimer´s disease is one of the most common forms of dementia worldwide. Pathological hallmarks include amyloid-β plaque (Aβ) depositions and intracellular tangles, comprised of hyperphosphorylated tau. Furthermore, AD is accompanied by an inflammatory response, which includes the activation and recruitment of microglia to sites of Aβ-deposition and the secretion of pro-inflammatory cytokines (Sastre et al., 2006; Int. J. Dev. Neurosci. 24, 167–176). The endocannabinoid system (ECS) is implicated in (patho-) physiological events in the CNS and changes in this system are related to many neurological diseases, including AD. CB2 is up-regulated on neuritic plaque-associated microglia (Benito et al., 2003; Neurology 23, 11136–11141). Furthermore, independent studies have shown that activation of the CB2 receptor reversed Aβ-induced memory impairments and neuroinflammation. However, the exact molecular mechanism of CB2 in AD remains elusive.

Methods: To examine the role of CB2 in microglia activation *in vitro*, CB2 deficient primary microglia were incubated with pro-inflammatory stimuli. Expression of cell surface markers and secreted cytokines were analyzed by flow cytometry and ELISA, respectively. To characterize the neuroinflammatory process in a transgenic mouse model of AD, APP/PS1 mice were crossed with CB2$^{-/-}$ mice. We quantified cytokine expression, Aβ plaque load, percentages of microglia and infiltrated macrophages, Aβ degrading enzymes as well as learning and memory performance in different age groups of WT, CB2$^{-/-}$, APP/PS1 and APP/PS1*CB2$^{-/-}$ mice.

Results: We demonstrate that **1)** microglia harvested from CB2$^{-/-}$ mice were less responsive to pro-inflammatory stimuli than CB2$^{+/+}$ microglia based on the cell surface expression of ICAM and CD40 and the release of chemokines and cytokines CCL2, IL-6, and TNFα.
2) Transgenic APP/PS1 mice lacking CB2 showed lower expression levels of pro-inflammatory chemokines and cytokines in the brain, as well as diminished concentrations of soluble Aβ$_{40/42}$. **3)** Furthermore, they showed increased levels of Aβ degrading enzymes neprilysin and MMP9 when compared to age-matched APP/PS1 mice. **4)** APP/PS1*CB2$^{-/-}$ showed reduced microgliosis seen as diminished Iba1 staining in IHC as well as reduced percentages of microglia and infiltrating macrophages. **5)** Cognitive performance in the Morris water maze paradigm showed increased escape latency to the hidden platform in APP/PS1*CB2$^{-/-}$ compared to APP/PS1 mice, indicating enhanced learning capacities.

Conclusions: We conclude that the reduction in neuroinflammation affected spatial learning and memory in aged APP/PS1*CB2$^{-/-}$ mice. Our data suggest an important role for CB2 in AD-associated neuroinflammation, most probably by changing the inflammatory profile of aging microglia. Consecutively, these changes have a prominent impact on Aβ induced inflammatory responses and learning abilities.

GENDER- AND REGION-DEPENDENT CONSEQUENCES OF ADOLESCENT THC EXPOSURE ON BEHAVIOR AND SYNAPTIC PLASTICITY

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In humans as in animals, gender differences have been frequently observed in the biological and behavioral effects of substances of abuse, including cannabis.

Accordingly, our previous works showed that exposure to delta-9-tetrahydrocannabinol (THC), the major psychotropic ingredient of Cannabis sativa, during adolescence results in long-term disturbances of cognitive performances and emotional reactivity in adult female rats, whereas preliminary findings suggested that this complex phenotype was not entirely present when the same treatment protocol was performed in adolescent male rats.

In this study, we fully investigated the long-term behavioral consequences of adolescent THC treatment in male rats, as compared to females.

To this aim, adolescent male Sprague-Dawley rats were treated with increasing doses of THC twice a day from PND 35 to 45 and, in adulthood, a series of behavioral tests were performed in order to check for the presence of (1) cognitive deficits (through the novel object recognition test, either classic and spatial), (2) social withdrawal (through the social interaction test), (3) depressive-like behaviors (through the forced swim test) and (4) psychotic-like signs (by monitoring locomotor activity, stereotyped behaviors and ataxia after acute phencyclidine administration).

Adolescent THC treatment induced a significant reduction of the discrimination index in the spatial but not in the classic version of the novel object recognition test, whereas it did not affect the other behaviors under investigation, suggesting that THC in male rats is associated with lasting cognitive impairment without alterations in the emotional sphere.

Based on these data, we investigated the possible molecular underpinnings of the cognitive impairment observed in adult THC-treated rats, by focusing our analyses in synaptosomal fractions from the hippocampus of adult THC- and vehicle-treated rats, a brain area particularly involved in the modulation of cognitive functions.

Interestingly, altered rearrangement of NMDA and AMPA receptor subunits were observed in THC-exposed rats. Indeed, in the post-synaptic fraction, the levels of the NMDA receptor subunit, GluN2B, were significantly increased in THC-treated animals, as well as the levels of the AMPA subunits, GluA1 and GluA2. Furthermore, changes in the levels of the pre-synaptic marker, synaptophysin, and the post-synaptic marker, PSD95, were also present.

In the same brain region, we also found significant alterations in astrocyte but not microglia markers, suggesting that adolescent THC might have promoted an aberrant astrocyte reactivity within the hippocampus.

Intriguingly, these changes appear to be specific for the hippocampus as, unlike what it has been observed in females, they were not detected in the prefrontal cortex.

In conclusion, these data demonstrate for the first time that the gender-dependent detrimental effects induced by adolescent THC exposure on behavior may rely on its ability to trigger different region-dependent changes in synaptic plasticity in male and female rats. Specifically, the prevalence of alterations in the emotional sphere observed in females is associated with profound changes in the prefrontal cortex, whereas here we demonstrated that the cognitive impairment induced in male rats is strongly associated with a marked dysregulation in the hippocampus.

ADAPTIVE CHANGES IN THE HABENULOMESENCEPHALIC CIRCUIT DURING CANNABINOID WITHDRAWAL

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The mesolimbic dopamine (DA) system arising from the ventral tegmental area (VTA) shows a profound reduction in its spontaneous activity after chronic cannabinoid exposure and withdrawal[1], the critical phases of the drug addiction cycle. These adaptive changes are thought to play a critical role into withdrawal-induced negative effects, eventually leading to relapse into drug taking. The lateral habenula (LHb) exerts a negative control over the VTA via the GABA rostromedial tegmental nucleus (RMTg), encoding aversion-related stimuli2[2-4]. In fact, both RMTg and LHb cells are activated by negative/unpleasant events, and inhibited by rewarding/positive stimuli. Therefore, these nuclei represent a potential convergence point for drug-evoked reward and aversive opponent processes. On these bases, we hypothesized that the LHb-RMTg pathway might be causally involved in the hypodopaminergic state which occurs during cannabinoid withdrawal.

Methods: We took advantage of standard single unit extracellular recordings from either VTA, RMTg and LHb neurons in anesthetized male Sprague–Dawley rats. To induce Δ^9-tetrahydrocannabinol (Δ^9-THC) dependence, rats were chronically treated with Δ^9-THC (15 mg/kg, i.p.), or its vehicle, twice daily for 6.5 days[4]. Rats were withdrawn spontaneously or pharmacologically with the cannabinoid antagonist rimonabant (5 mg/kg, i.p.).

Results: Administration of rimonabant precipitated an intense behavioral withdrawal syndrome (one-way ANOVA and Dunnett's test , $p < 0.0001$ versus control), whereas abrupt Δ^9-THC suspension caused only milder signs of abstinence (one-way ANOVA and Dunnett's test, $p < 0.01$ versus control). Electrophysiological experiments confirmed that Δ^9-THC withdrawal produced a marked decrease in the firing rate and burst firing of VTA DA neurons (one-way ANOVA and Dunnett's test, $p < 0.01$ versus control). As expected, RMTg electrical stimulation elicited a complete suppression of spontaneous activity in approximately half of the DA neurons examined. Remarkably, in Δ^9-THC withdrawn rats the duration of RMTg-evoked inhibition lasted longer (one-way ANOVA and Dunnett's test, $p < 0.05$ versus control), suggesting an augmented GABA inhibitory input onto DA cells. By contrast, the spontaneous activity of RMTg GABA neurons was reduced in cannabinoid-withdrawn rats (one-way ANOVA and Dunnett's test, $p < 0.0001$ versus control). Consistent with results, we also found that firing rate of RMTg-projecting LHb neurons was markedly suppressed during cannabinoid withdrawal (one-way ANOVA and Dunnett's test, $p < 0.0001$ versus control).

Conclusions: These findings support the hypothesis that enhanced GABA inputs from RMTg might contribute to the hypodopaminergia induced by cannabinoid withdrawal, and confirm that the LHb-RMTg pathway is implicated in the neurobiological mechanisms underlying drug dependence and addiction.

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THE PPARα AGONIST FENOFIBRATE PREVENTS DISRUPTION OF DOPAMINE NEURON ACTIVITY IN A NEURODEVELOPMENTAL RAT MODEL OF SCHIZOPHRENIA

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Peroxisome proliferator-activated receptors-alpha (PPARα) are members of a family of nuclear receptors widely expressed in the CNS by neurons and glial cells. PPARα are activated by endocannabinoid-like N-acylethanolamines, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), abundant in the brain and which share with the endocannabinoid anandamide anabolic and degradative pathways. In recent years, there is growing interest in the roles of CNS PPARs, which span from regulation of neuronal functions and metabolism, neurodegeneration and neuroinflammation. These properties might prove beneficial in the therapy of psychiatric disorders.

The aim of our experiments was to test the hypothesis that PPARα activation protects against the detrimental effects of maternal immune activation on fetal brain maturation. Hence, in utero exposure to maternal viral infections is associated with a higher incidence of psychiatric disorders with a supposed neurodevelopmental origin, including schizophrenia.

Methods: Here, we used an immune-mediated neurodevelopmental disruption model, based on prenatal administration of the polyriboinosinic-polyribocytidilic acid [poly(I:C)] in rats, which mimics a viral infection and recapitulates behavioral and cognitive abnormalities relevant to schizophrenia in the offspring. In these animals, we studied activity of ventral tegmental area (VTA) dopamine (DA) neurons, which are prominently involved in the pathophysiology of psychoses. Sustained PPARα activation was achieved by administration of a diet enriched with fenofibrate (0.2 % w/w in food pellets), a clinically approved PPARα agonist used as lipid-lowering medication. Pregnant dams were fed from gestational day 8 to gestational day 18 with either a standard or fenofibrate-containing diet. At gestational day 15 they were injected with poly(I:C) (4 mg/kg i.v) or vehicle. We carried out in vivo single cell extracellular recordings in urethane-anesthetized male offspring at PND 75-90, which were divided in four experimental groups, according to the prenatal treatments.

Results: **1)** Poly(I:C) prenatally-treated offspring had a lower number of spontaneously active VTA DA neurons (0.72±0.09) and a reduced mean firing rate (2.51±0.17 Hz), when compared to controls (cells per track, 1.15±0.13 and frequency, 3.13±0.13 Hz, respectively). In addition, burst parameters of DA cells were strongly altered by poly(I:C) administration. **2)** Notably, fenofibrate administration to poly(I:C)-exposed rats prevented this disruption as it restored the number of spontaneous active VTA DA cells (1.48±0.19) and normalized firing rate (3.73±0.22 Hz).

Conclusions: Our findings provide evidence that in utero exposure to maternal immune activation disrupts activity of DA neurons in adulthood and that PPARα activation prevents these detrimental effects. Further studies are needed to assess the mechanisms by which PPARα prevent neurodevelopmental disruption. One possibility is that they reduce (neuro)inflammation and/or damage produced by neuroinflammatory cytokines of maternal origin.

This study highlights the ability of PPARα agonists in the prevention of psychiatric conditions of neurodevelopmental origins and extend their potential therapeutic applications in neuropsychiatric disorders.

ENDOCANNABINOID-MEDIATED MODULATION OF HIPPOCAMPAL LONG TERM POTENTIATION DEVELOPS IN THE THIRD WEEK OF LIFE IN MICE

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The endocannabinoid system (ECS) regulates synaptic transmission by post-synaptically released endocannabinoids that bind to pre-synaptic cannabinoid receptor 1 (CB1) where they inhibit the presynaptic neuron and eventually attenuate transmitter release. This on-demand negative-feedback also influences various forms of synaptic plasticity with the ECS's involvement in long-term potentiation (LTP) being well established.

To clarify the exact onset of this phenomenon and assess the timeframe of this shift during post-natal development we set out to analyze the LTP formation during the postnatal ages P10, P14 and P18. We assessed CB1 mRNA- and protein levels through this important developmental phase.

Methods: To measure LTP facilitation, field EPSP's were recorded in stratum radiatum of CA1 in slices from C57BL/6 mice and CB1-KO mice at the ages P10, P14 and P18 after theta-burst-stimulation. Fluorescent in-situ hybridization was employed on hippocampal sections to detect and count cells expressing the CB1 mRNA in the dorsal hippocampus of wild-type mice in the aforementioned ages. Immuno-histochemistry was used to asses CB1-protein-levels.

Results: Theta-Burst-Stimulation in hippocampal slices of mice of the ages P10, P14 and P18 has shown differential capacities of LTP-induction when comparing CB1 deficient to WT animals. In the CB1-KO-animals, LTP was enhanced. This difference however is not yet present when comparing KO to WT at the age P10 and is getting more pronounced up to the age of P18. Data from in-situ and immuno-histo-chemistry suggested comparable amounts of CB1 mRNA and protein in the hippocampus across all ages.

Conclusions: Hippocampal LTP in CB1-KO mice is undistinguishable from that in wild types at the age of P10 but significantly different at the age of P18. CB1 mRNA and protein distribution in the hippocampus of early postnatal mice shows a pattern similar to adults. Changes in CB1's capability to signal through the downstream signaling pathways or differential generation of endocannabinoids between ages P10 and P18 may be responsible for an attenuated hippocampal LTP in wild type mice.

THE ROLE OF CB$_1$ IN INTESTINAL PERMEABILITY IN HYPOXIA AND INFLAMMATION

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We have previously shown that exogenous applied endocannabinoids (anandamide and 2-AG) increase Caco-2 permeability through the CB$_1$, suggesting a role for this receptor in modulating gut permeability. The aim of the present study was to further investigate the role of CB$_1$ on Caco-2 permeability during normoxia, hypoxia and inflammatory conditions.

Methods: Caco-2 cells were grown until fully confluent on 0.4 μM pore size transparent polyethylene terephthalate (PET) membrane inserts. Transepithelial electrical resistance (TEER) measurements were made as a measure of permeability. URB597 (FAAH inhibitor) and JZL184 (MGL inhibitor)(to increase local levels of endocannabinoids) were added to the apical (luminal) or basolateral (systemic) membrane of cells. Hypoxia was induced using the GasPak™ EZ Anaerobe Pouch System. Inflammatory conditions were established by adding pro-inflammatory cytokines, interferon gamma (IFN γ 10ng/ml) and tumour necrosis factor alpha (TNF α 10ng/ml). AEA and 2-AG were measured by liquid chromatography tandem mass spectrometry. ELISAs were performed for the assessment of interleukin 8 (IL-8) and interleukin 6 (IL-6) levels in cell culture media.

Results: When applied to the apical or basolateral membrane of Caco-2 cells, URB597 (3μM and 10μM) decreased TEER levels (i.e. increased monolayer permeability) ($p<0.001$). JZL184 lowered TEER values when applied apically ($p<0.01$ for 3μM and $p<0.001$ for 10μM). Basolateral application of JZL184 (10μM) increased TEER ($p<0.001$), which mirrored the effect of 2-AG when applied again to the basolateral membrane. There effect of URB597 and JZL184 was abolished in cells where CB$_1$ was knocked down. In models of hypoxia or inflammation, URB597 and JZL184 further increased permeability. Furthermore, the permeability response to inflammation (but not hypoxia) was reduced in CB$_1$ knock down cells and both hypoxia and inflammation significantly increased cellular 2-AG levels ($P<0.001$). Despite this, IL-8 and IL-6 levels were reduced in media of cells treated for 24 h with AEA ($p<0.05$), URB 597 ($P<0.001$) and JZL 184 ($p<0.001$). Similarly IL-6 levels were reduced for AEA ($p<0.01$), 2-AG ($P<0.0001$), URB 597 ($p<0.001$) and JZL 184 ($p<0.0001$).

Conclusions: Increasing endogenous levels of endocannabinoids increases intestinal permeability in control conditions, in hypoxia and inflammation, through CB$_1$, and intestinal cells produce 2-AG in response to hypoxia and inflammation. The role of CB$_1$ in inflammation is demonstrated by the blunted permeability response to cytokines in CB$_1$ knock down cells. Despite this, endocannabinoids reduce inflammatory cytokines, suggesting this effect on intestinal permeability is distinct from their anti-inflammatory role.

EXPRESSION ANALYSIS OF CB2-GFP BAC TRANSGENIC MICE

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Background: The endocannabinoid system (ECS) is a retrograde messenger system, consisting of lipid signaling molecules that bind to at least two G-protein-coupled receptors, cannabinoid receptor 1 and 2 (CB1 and 2). As CB2 is primarily expressed on immune cells such as B cells, T cells, macrophages, dendritic cells, and microglia, it is of great interest how CB2 contributes to immune cell development and function in health and disease. Understanding the mechanisms of how CB2 is involved in immune function, as well as trafficking and regulation of CB2 expressing cells are crucial issues. However, up to now, commercially available CB2 antibodies produce ambiguous results, especially those targeting the murine protein. Therefore, we have generated BAC transgenic GFP reporter mice (CB2-GFPTg) to trace CB2 expression *in vitro* and *in situ*.

Methods: CB2-GFPTg mice were generated via the BAC technology. A modified WT-BAC construct, in which the CB2 open reading frame (ORF) was replaced by the GFP ORF, was used for microinjection. Expression of GFP in CB2-GFPTg and WT littermates was analyzed in different tissues and cell types, using quantitative real-time PCR, Western blot, flow cytometry, and immunohistochemistry. Furthermore, isolated splenocytes were stimulated *in vitro* with LPS and CpG to analyze GFP expression under inflammatory conditions.

Results: 1) CB2-GFPTg mice express GFP under the CB2 promoter and display GFP expression paralleling CB2 expression on the transcript and protein level in spleen, thymus and brain tissue. Highest expression levels were measured in the spleen. **2)** Immunohistochemical analysis of the spleen showed that GFP is predominantly expressed by B cells, while T cells, macrophages, and dendritic cells show weaker expression. In the brain, GFP expression was observed in microglia, but not in neurons or astrocytes **3)** Flow cytometric analysis of isolated splenocytes showed that GFP expression is increased after pro-inflammatory stimulation with LPS or CpG, caused by increasing numbers of GFP expressing cells.

Conclusions: We provide a novel CB2-GFP transgenic reporter mouse line, representing a powerful resource to study CB2 expression in different tissues on protein level. This is, to our knowledge, the first model to study CB2 protein expression using GFP as a corresponding reporter. This will open new insights into the regulation of this molecule in different cell types and under different inflammatory conditions. Furthermore, it will be a valuable tool for studying CB2-mediated mobilization and trafficking of immune cells and for analyzing the fate of recruited immune cells in models of acute and chronic inflammation.

CIRCULATING FIBROCYTES AND LIVER DISEASE: IMPLICATIONS FOR CANNABINOIDS IN LIVER FIBROSIS

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Fibrocytes are a bone-marrow derived cell with dual characteristics of haematopoietic cells and fibroblasts ($CD45^+Col^+$). These cells migrate to tissue sites of injury and contribute to the remodelling process through differentiation into collagen type I producing myofibroblasts. They can also migrate to the spleen with a subsequent role in the immune response, differentiating into macrophages or dendritic cells. Little research is currently available with regard to circulating fibrocytes in human liver disease. Various cytokines and cannabinoids have been shown to mediate or attenuate some of the mechanisms involved in fibrosis. This study assessed levels of circulating fibrocytes in patients with cirrhotic and non-cirrhotic liver disease compared to healthy controls. Fibrocyte differentiation in culture following treatment with cytokines and cannabinoids was also investigated.

Methods: Peripheral blood mononuclear cells (PBMCs) extracted from whole blood samples from patients with liver disease and healthy volunteers were dual stained with fibrocyte markers CD45 and collagen-I antibodies for flow cytometric analysis. PBMCs obtained from healthy volunteers were cultured in the presence of transforming growth factor beta 1 (TGF-β1), with or without anandamide (AEA) or cannabidiol (CBD). Fibrocytes were stained with alpha smooth muscle actin (α-SMA) as a myofibroblast marker and observed under confocal microscopy.

Results: **1)** Circulating fibrocytes were significantly increased in patients with liver disease (7.1 ± 2.2) compared to healthy controls (0.3 ± 0.2, p=0.02). **2)** Subgroup analysis revealed greater circulating fibrocytes in patients with non-cirrhotic liver disease compared to those with liver cirrhosis. **3)** Fibrocytes treated with AEA showed a significant reduction in α-SMA expression following immunofluorescence microscopy (0.69 ± 0.04, p=0.04).

Conclusions: We conclude from these findings, that **a)** Circulating fibrocytes may have a role in inflammatory liver disease. **b)** AEA may have potential anti-fibrotic properties through attenuation of fibrocyte differentiation into a pro-fibrotic phenotype. **c)** The prognostic value of fibrocytes as a biomarker for disease severity has potential. **d)** Finally, the tissue fate of circulating fibrocytes requires further validation to determine the nature and extent of fibrocyte involvement in liver disease.

PRELIMINARY RESEARCH ACTIVITIES TO SUPPORT THE NATIONAL PROGRAM TO PRODUCE MEDICAL CANNABIS FOR ITALY

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Introduction: Ten years ago, CRA-CIN (now CREA-CIN) moved the first steps to breed Cannabis sativa which could be devoted to medical application. Origin of new varieties derives from a large germplasm collection that now counts about 300 genotypes. Traditional breeding take advantage of modern strategies like sex reversion and chemical analysis of plants using gas chromatography.
Minimum financial support has been derived from public Italian organizations, despite today we are supporting the first official program that is intended to produce medical Cannabis necessary to support the needs of 11 Regions that have recently introduced the law that provides medical Cannabis to the patients that are affected by diseases that need this medicine.

Methods: Female plants of many genotypes have been analyzed when plants were at least one month old using gas chromatography to identify the most interesting chemotypes. The THC, CBD, CBG, CBDV, THCV prevalent chemotypes are our priority tasks in association with the breeding program to produce the placebo strain (Cannabis without cannabinoids, $< 0,05\%$ of d.w.).
Stability of the lines have been reached using one or more steps of playing with selfing to increase the homozygosis condition of genes involved in cannabinoid and yield characters.

Results: Cannabinoid concentration in the more advanced Cannabis lines is close to 20% of dry weight of flower. Actually we have available some lines with THC prevalent that could produce 400-500 gr/m2 of dry flowers with 20% of THC. The CBD prevalent chemotype could produce about 18% of cannabinoid with purity close to 97%. CINRO is the first hybrid genotype (CBD:THC, at 6%:7% respectively) that has been cloned to produce the first batches of Italian medical Cannabis.

Conclusions: The CREA-CIN in collaboration with Military chemical pharmaceutical factory of Florence have produced the first medical Cannabis under the license of the Ministry of Health, with the AIFA (Italian Medicines Agency) validation. This is a trustable condition for all patients to receive and use Cannabis products (granulated flowers) that meet the most strict pharmaceutical quality that are required for a pharmaceutical grade plant product and in the future, extracts and others preparations could be produced by the military factory, offering alternative and more practical assumption methods of drugs, using chemotypes and varieties with variable concentration and combination (cannabinoids and terpenes at first) that will take in account the most frequent request of the patients groups that use Cannabis as medicine.

CANNABIDIOL (CBD) AND PALMITOYLETHANOLAMIDE (PEA) DO NOT MODULATE THE INFLAMMATORY RESPONSE IN CACO-2 INTESTINAL CELLS

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BACKGROUND: Cannabinoids CBD and PEA possess anti-inflammatory effects in the gut *in vivo*[1,2]. We have also shown that CBD increases monolayer resistance (i.e. decrease permeability) in cultured Caco-2 intestinal epithelial cells in a model of inflammation[3]. We sought to investigate if the effects of PEA and CBD on Caco-2 cell permeability were secondary to decreased local cytokine production. IL-8 is secreted by epithelial cells in response to inflammation, inducing chemotaxis of macrophages and other immune cells. This was used as a surrogate marker for production of other cytokines.

METHODS: Caco-2 cells were seeded at 5×10^4/ml per well in 12 well plates. Cells were grown for 15 days with media changed every other day. Media used was 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 to the media IFNγ (10μM) for 18 hours, followed by TNFα (10 μM) for 6 hours. PEA (10 μM) or CBD (10 μM) were added simultaneously with IFNγ. The effect of PEA (10 μM) or CBD (10 μM) on IL-8 secretion was also tested. A cannabinoid and cytokine free control was achieved with 0.01% ethanol. Media was snap frozen and stored at -80°C until analysed. IL-8 levels from culture media was measured after 24 hours of incubation by ELISA.

RESULTS: Secreted IL-8 increased significantly after the application of IFNγ and TNFα compared to control ($p < 0.001$). The increase in IL-8 secretion was not significantly affected by administration of PEA or CBD ($p < 0.01$ and < 0.05 respectively). Addition of PEA or CBD alone to Caco-2 cells did not affect IL-8 secretion compared to control.

CONCLUSIONS: We hypothesized that PEA and CBD acted through inhibition local cytokine production, preventing further inflammation through recruitment of macrophages. Although IL-8 levels rose under inflammatory conditions, this was not significantly affected by PEA and CBD administration. This suggests that the anti-inflammatory effects of PEA and CBD act through different cell types than epithelial cells *in vivo*. Inhibition of local cytokine production is not likely to be the mechanism of action of PEA and CBD in preventing falls in epithelial permeability.

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COORDINATED REGULATION OF SYNAPTIC PLASTICITY AT STRIATOPALLIDAL AND STRIATONIGRAL NEURONS ORCHESTRATES MOTOR CONTROL

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Background: Motor information originating from cortical inputs converges on the dorsal striatum and is conveyed by the medium spiny neurons (MSNs) of the striatonigral and striatopallidal pathways to the final basal ganglia (BG) outputs. Emerging evidence suggests that for appropriate function of BG circuits during movement, the coordinated activity of these two neuronal subpopulations is crucial. Despite their functional importance and potential implications for motor disorders, the cellular mechanisms shaping the coordinated activity of striatopallidal and striatonigral MSNs have yet to be established. We hypothesized that coordinated activity occurs through the concurrent, cell type-specific regulation of synaptic plasticity, and this directly relates to motor behavior and its pathophysiology. To begin to test these hypotheses, we investigated whether the functional interplay of presynaptic and postsynaptic molecular determinants of synaptic plasticity at the two MSN subpopulations is lost in the unilateral 6- hydroxydopamine (6-OHDA)-lesioned mouse model of PD, and the behavioral consequences of restoring it.

Methods: Parkinsonian mice were obtained upon unilateral stereotaxic injection of 6-hydroxydopamine (6-OHDA) in the medial forebrain bundle. High frequency stimulation (HFS)-induced corticostriatal synaptic plasticity was tested with patch-clamp whole cell recordings in horizontal brain slices at excitatory cortico-striatal synapses in the dorsolateral striatum (DLS). Immunohistochemical analysis of molecular markers of plasticity was performed on slices from mice receiving pharmacological treatment in the DLS through guide cannulae implanted at the time of the surgery. Behavioral analysis of different aspects of motor function (spontaneous and drug-induced rotational behavior, rotarod, spontaneous locomotion) was operated after intra-DLS and systemic infusion of drugs.

Results: We have found that in the PD mouse model, the concurrent and cell-type specific activation of the endocannabinoid and ERK signaling cascades in the DLS normalizes striatal circuitry and restores effective control of motor functions. This activation occurs by pharmacologically manipulating the activity of the Small Conductance Ca^{2+} activated K^+ channels (SK), and is associated with the modulation of eCB-LTD and of a novel form of adenosine-mediated LTD in striatopallidal and striatonigral neurons, respectively.

Conclusions: These results establish that coordinated regulation of synaptic mechanisms of plasticity at striatal MSN subpopulations plays a major role in the control of motor function and in

motor pathology.

WELLPAD: A NEW STANDARDIZED ASSESSMENT AND DATA VISUALIZATION TOOL FOR CLINICIANS AND RESEARCHERS IN THE FIELD OF MEDICAL CANNABINOID THERAPY

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Introduction: Reliable tools to guide clinicians and researchers in the emerging field of cannabis medicine remain lacking. Furthermore, in providing short- and long-term follow-up, it is challenging to authentically and reliably obtain clinical data in a standardized format. We have developed a digital global assessment tool for initial and recurrent assessment of patients engaged in cannabinoid therapy, and present proof of concept and emerging clinical research data for this tool. Ultimately intended be inclusively designed, i.e. highly usable by clinical participants coming from a wide variety of ages, cognitive abilities, cultures and digital literacy levels, Wellpad originates from successful employment in standardized assessment supervised therapeutics assessment of patient progress over the past two years for PRAXIS. (*Moller & Saynor, 2014,13th Intl Mobile HCI Health Conf., Toronto, ACM, Canada; Moller et al., 2014; Proc. 10th ICDVRAT, Gothenburg, Sweden*).

Method: Wellpad consists of five core lines of patient wellbeing enquiry, based on most common clinical presentation. Assessment clusters consist of (1) sleep quality (2) extrinsic stress perception (3) pain perception (4) internalized relaxation state (5) internalized mood state. Each symptom cluster is presented in digital format, (i.e. IPad) to patients in supervised fashion, using hand-swiped (1-5) Likert scales via gamification and responsive aesthetic digital design principles. As per standard clinic protocol, demographic data such as age, gender, and place of residence are obtained only at initial intake, while clinical data are obtained on each visit to provide quantitative symptom tracking and visual communication tool with patient or in research.

Results: Despite significant comorbidity, we were readily able to parse out four primary clinical indications that 15 fully informed consecutive sample patients (11M, 4F, mean age =38.8) sought therapy for as a primary therapeutic indication: 14.3% sleep disorder, 32.1% mood/anxiety disorder, 21% pain disorder, 32.1% other serious medical conditions. A wide range of strain variety use was reported, and Wellpad's geolocating feature showed 4 sample patients resided in the Toronto downtown core, 6 in suburban regions, and 5 in outlying towns or cities. The number of clinical data assessment data points ranged from 1-5, based on the number of assessments. Longitudinal clinical symptom patterns and fluctuations based on touchscreen Likert Scale inputs from initial baseline assessment and subsequent assessments are graphically displayed using the Wellpad data visualization panel, including fluctuation in our core assessment clusters of pain, sleep quality, stress perception, relaxation and mood.

Results: We report satisfactory usability for both clinicians and patients in entering demographic and clinical data using the Wellpad for patient assessment and follow-up in cannabinoid clinical research settings. Further results are graphically illustrated within the body of the presentation. The focus on inclusive digital design and aesthetic data visualization display allows for a standardization of clinical assessment data points over time, enhancing ease-of-use as well as providing a facile visual communication tool between clinician and patient as clinical progress is tracked based on illness course, change of medication variety or other clinically relevant factors. In the context of clinical research, the ability to readily extract and display data that has been shown to be clinically relevant is a major advance in studying short- and long-term follow-up of patients.

STUDY OF THE CANNABINOIDS RECEPTORS AND MITOCHONDRIAL ELECTRON TRANSPORT CHAIN IN A MODEL OF PARKINSON'S DISEASE

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Introduction

The Parkinson´s disease (PD) is a neurodegenerative disease that mainly affects the dopaminergic neurons of the *substantia nigra*. The MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treated non-human primate (Macaca fascicularis) is one of the best characterized models that clearly reproduces the main pathological and clinical features of PD. As this neurotoxin blocks the mitochondrial electron transport chain, which is also modulated by cannabinoid ligands, we decided to study the effects of the cannabinoid agonist, CP55940 on the mitochondrial complexes such as the cytochrome c oxidase (CCo), as well as the density and activity of the cannabinoid receptors.

Methods

Cell membrane microarrays were elaborated using membranes isolated from different brain areas of control and MPTP treated monkeys. These microarrays were used in the enzymatic activity assays, as well as in binding studies with radioligands or $[^{35}S]GTP\square S$. After the incubation, microarrays were exposed to a film (only in binding studies, as enzymatic assays are colorimetric), scanned and quantified using image analysis software.

Results

The *substantia nigra* of the MPTP treated animals showed an increase in the density of cannabinoid receptors and in the succinate dehydrogenase (SDH) activity, whilst the CCo activity was reduced. However, in other areas such as the cerebellum, the decrease in the cannabinoid binding sites was also accompanied by a reduction in both enzymatic activities.

Conclusion

The MPTP treatment induces alterations in the cannabinoid receptors and in the mitochondrial function in specific brain areas, although further studies must be carried out to understand this relationship in Parkinsonian monkeys.

ENDOCANNABINOID REGULATION OF TOLL-LIKE RECEPTORS 3 AND 4 ACTIVITY IN NEUROGENESIS

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Toll-like receptors (TLRs) play a key role in innate immunity by mediating neuronal and glial call responses to injury and infection. TLRs are also believed to play a functional role in developing the nervous system as they are present in early embryonic stages and neural progenitor cells. TLR3 and TLR4 in particular are linked to negative regulation of proliferation in the developing brain. The neuroimmune system is also known to be tightly linked with the endocannabinoid system in regulating neurogenesis. As well, IL-1β is a cytokine with pro-inflammatory involvement in neurogenesis. In this study, using wildtype and IL1-β KO neural stem cell cultures, we investigated the effects of TLR3 and TLR4 activation and their interactions with the endocannabinoid system, as well as their impact on cytokine activity for IP10, IL6 and TNF, three important neuroinflammatory mediators.

Methods: Neural stem cell cultures were prepared from C57BL/6J wild type mice or IL1-β knockouts. The effects of toll-like receptor agonists were tested in vitro, using MTT as a mitochondrial marker for metabolic activity, BrdU as a marker for neurogenesis and LDH as a marker for cell death. TLR3 was activated via polyinosinic:polycytidylic acid (polyI:C), a strong immune-stimulant. TLR4 was activated with lipopolysaccharide (LPS), a bacterial endotoxin. TLR activation was also tested in conjunction with JZL184, a blocker for the enzyme which degradates the endocannabinoid 2AG, and URB597, an inhibitor for the enzyme FAAH which degradates the endocannabinoid anandamide. We then proceeded to test cytokine expression under the same conditions for IP10-, IL6- and TNF-murines, both in wildtype and IL-1β KO NSC cultures.

Results: TLR3 activation with polyI:C decreased neural proliferation in wild type neural stem cell cultures and increased cell death. Both effects were enhanced with FAAH and MAGL inhibitors URB-597 and JZL-184, respectively. Opposite results were found in IL-1β knock-out neural stem cell cultures under the same treatment conditions. No significant cytotoxicity was found in IL-1β KO cultures following TLR3 activation. Further, co-treatment with MAGL inhibitor JZL-184 increased neural proliferation.

Conclusions: From this, we would suggest that the endocannabinoid system interacts with toll-like receptors of the innate immune system to mediate neurogenesis. In future studies, blocking these toll-like receptors could provide insights into potential avenues for brain repair. A better understanding of the physiological underpinnings of neural proliferation is crucial to the development of therapeutic treatment for neurological disorders such as traumatic brain injuries, neurodegenerative diseases, epilepsy and stroke.

CANNABINOID RECEPTOR 2 GENE POLYMORPHISMS ARE ASSOCIATED WITH ASTHMA ONSET, DISEASE SEVERITY AND TREATMENT OUTCOME

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The complex immune mechanisms that underlie airway inflammation are associated with, and probably causal to, acute and persistent asthma episodes, which indicate that an imbalanced immune system is the primary driving force underlying asthma. The interaction of several genes as well as environmental factors influences the asthma pathogenesis. The rs2229579 (1073C>T; Tyr316His) and rs2501432 (315A>G; Arg63Gln) are missense SNPs in the *CNR2* gene that codes for the cannabinoid receptor 2, while rs4237 (1029T>C) is located in the UTR-3 region of a neighboring gene, ~83kbp downstream of *CNR2*. We analyzed rs4237, rs2229579 and rs2501432 association with childhood asthma onset and severity, and the effect of these polymorphisms on the response to inhaled corticosteroids (ICS) or leukotriene receptor antagonist (LTRA) treatment and *CNR2* gene expression.

Methods: We studied a case-control cohort of 341 children mild/moderate persistent asthma (226 atopic, 100 non-atopic, 15 undetermined atopy), and 245 controls. Blood samples were collected before treatment, and 69 matching samples 4-6 weeks after treatment with ICS or and 115 matching samples after LTRA.

Results: *CNR2* mRNA levels were found to be significantly higher in asthmatic patients when compared with controls. *CNR2* mRNA levels of asthmatics with rs4237 TT genotype was higher compared to those with CT or CC and according to the recessive model of genetic association, the frequency of rs4237 CC genotype in atopic asthmatics was lower than in controls. No association was found between rs2229579 and rs2501432 genotype, and asthma or *CNR2* mRNA levels. Forced expiratory volume in 1 second (FEV1) was higher in atopic asthmatics with rs4237 CC genotype vs. CT or TT. FEV1 increased significantly after ICS treatment in atopic asthmatics with rs4237 TT genotype vs. CT or CC. Atopic asthmatic patients with rs2229579 CT genotype presented significantly higher FENO, total IgE and lower log PC_{20}. Non-atopic asthmatics with CT presented significantly higher eosinophil count. After ICS treatment atopic asthmatics with rs2229579 CT genotype had less improvement of condition as measured by dFEV1/FVC, while after LTRA treatment asthmatics with CT genotype had worsening condition measured by dFEV1/FVC. Atopic asthmatic patients with rs2501432 G allele presented significantly higher FENO. After ICS treatment atopic asthmatics with rs2501432 G allele had less improvement of condition as measured by dFEV1/FVC and by dFEV1%, while after LTRA treatment no significant difference in treatment outcome was found associated with rs2501432.

Conclusion: Our results suggest rs4237, rs2229579 and rs2501432 are associated with asthma severity and with ICS or LTRA treatment response in children with asthma. Furthermore, rs4237 is also associated with the onset of childhood asthma and with *CNR2 mRNA levels.*

INSECTICIDAL ACTIVITY AND CHEMICAL COMPOSITION OF THE VOLATILE OIL OF DIFFERENT CANNABIS VARIETIES

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The aim of this research is to compare the composition and the insecticidal activity of the volatile oils of three different varieties of *Cannabis sativa* grown at the University of Mississippi.

Methods:
The volatile oils of high THC/low CBD, high THC/high CBD and high CBD/low THC varieties of *Cannabis sativa* were obtained by hydro-distillation of both fresh and dried plant materials. The chemical profile of each oil was analyzed by both GC/FID using
Varian CP-3380 gas chromatograph equipped with a Varian CP-8400 automatic liquid sampler, a capillary injector and flame ionization detector and GC/MS using Thermo Finnegan Trace MS interfaced to a Trace 2000 GC equipped with an AS2000 auto-sampler and a single capillary injector and electron impact (EI+) source. A (30 m x 0.25 mm) DB-5 MS, 0.25 μ film (J&W Scientific, Inc.) column was used for GC and GC/MS analyses. The components identification was confirmed by comparing the retention times of the major components with those of authentic samples as well as using The NIST Mass Spectral Search Library. The essential oils were screened for their insecticidal and biting deterrent activity against mosquitoes.

Results: **1**) The number and the percentage of the compounds in each oil was determined by GC/FID analysis as 154 from high THC, 139 from high THC/high CBD and 157 from high CBD varieties. **2**) The composition and ratio of monoterpenes and sesquiterpenes were different among the three varieties. **3**) The drying process affected both the composition and ratio of the monoterpenoids. **4**) The oil obtained from the fresh and dried high THC/high CBD *Cannabis* showed good biting deterrent activity at 10 µg/cm^2 compared to DEET at 4.87 µ/cm^2, while the oil of both fresh and dried high CBD variety showed good larvicidal activity.

Conclusions: We conclude from these findings, that the volatile oil composition of the *Cannabis* plant material is variety dependent (at least quantitatively) and that may play a role in the insecticidal activity of the oil. Identification of the most active terpene in the oil(s) will be identified.

CLINICAL OBSERVATIONS OF 15 CASES
OF ENCEPHALOPATHY/EPILEPSY/CEREBRAL PALSY
USING STANDARDIZED NATURAL PRODUCT CANNABIS

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15 cases where caregivers requested cannabis therapy for treating refractory encephalopathies/epilepsy/cerebral palsy were observed from November 2014 while ingesting standardized quantities of cannabidiol as a natural product cannabis preparation. Positive effects were observed in 13 of the 15 patients, with reduced seizure frequency and spasm, with improved motor skills and mood. These results were achieved by administering between 1 and 6 mg/kg/day of natural product cannabidiol with no adverse side effects noted.

Methods: Caregivers requesting cannabis therapy for young persons with refractory epilepsy were provided standardized (* all/vsi: THC 2,5 mg/g (0,25%); CBD 34 mg/g (3,4%); CBN 0,6 mg/g (0,06%) natural product cannabis capsules and instructed to administer at dosages from 1 to 6 mg/kg/day, while continuing their routine medications. Various drugs such as Dilantin, Phenytoin and Topamax were co-administered throughout the study. Patients were seen for routine check ups and diagnostics, where caregivers were questioned as to observed effects and testimonials.

Results: The first patient, an 8-year-old male with epileptic seizures resulting from encephalopathy began in November 2014 and was immediately without seizures from the first dose of 1 mg/kg/day …that have not returned. In addition there was improvement in cognition and mood. Of the others that began later in 2014/2015, 7 of the ten now have no seizures, 100% reduction in all cases, two had 50% and 30% reduction in seizures, respectively and one there was no response and stopped. All of the patients in the study had underlying encephalopathies (one diagnosed as Lizen) and 3 were with comorbid cerebral palsies. In the 13 of 15 cases that responded favorably all showed better behavior, less absences, improved motor skills and less aggression. There were no adverse side effects in any cases. The antiepileptic mechanisms of CBD are not known, but may include effects on the equilibrative nucleoside transporter; the orphan G-protein-coupled receptor GPR55; the transient receptor potential of vanilloid type-1 channel; the 5-HT1a receptor; and the $\alpha3$ and $\alpha1$ glycine receptors, all being sites of cbd activity.

Conclusions: Admittedly this is mere observation of 15 patients in a non-controlled study, but it is the dramatic reduction in seizure frequency that calls direct attention to the phenomenon of a simple cannabis formulation. Definitely warranting further controlled studies of standardized cannabidiolpreparations that do not appear to present adverse side effects when used in conjunction with other anti-seizure drugs.

ASTROGLIAL TYPE-1 CANNABINOID RECEPTORS (CB$_1$) ARE NECESSARY FOR LONG-TERM OBJECT RECOGNITION MEMORY

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Astrocytes express a wide variety of G protein-coupled receptors (GPCR) that can influence cognitive functions such as learning and memory. Cannabinoids and endocannabinoids modulate memory processes through the GPCR CB$_1$ receptor. Interestingly, similarly to neurons, astrocytes express functional CB$_1$ receptors capable of modulating the effects of exogenously administered cannabinoid agonists on hippocampal synaptic plasticity and working memory. However, the endogenous roles of astroglial CB$_1$ receptors in long-term memory remain unknown.

Methods:To assess the role of astroglial CB$_1$ receptors in long-term memory, we used conditional mutant mice lacking CB$_1$ receptors specifically in astrocytes (GFAP-CB$_1$R-KO), drug applications (intra-peritoneal and intra-dorso hippocampal) and tested memory performances through an object recognition task performed in a L-maze.

Results: GFAP-CB$_1$R-KO mice have impaired long-term object recognition memory. Notably, administration of D-serine, a gliotransmitter that is a co-agonist at the N-methyl-D-Aspartate receptor (NMDAR), restores the long-term memory deficit when injected systemically. In line with this idea, raising endogenous levels of D-serine, through the inhibition of its degrading enzyme DAAO, also rescues the performances of GFAP-CB$_1$R-KO animals. Interestingly, local infusion of D-serine into to dorsal hippocampus also completely restores long-term memory deficits.

Conclusion:

Hippocampal astroglial CB$_1$ receptors are necessary to guarantee appropriate long-term memory through the modulation of the co-agonist site of the NMDAR. This study provides the first proof of a physiological role of astroglial CB$_1$R in behavior and reveals an unexpected role of astroglial CB$_1$ receptors in memory formation.

HABENULAR CB$_1$ RECEPTORS CONTROL THE EXPRESSION OF AVERSIVE MEMORIES

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SUMMARY

Expression of aversive memories is key for individual survival, but the underlying brain mechanisms are not fully understood. Medial habenular (MHb) axons co-release glutamate and acetylcholine onto target postsynaptic interpeduncular (IPN) neurons, but their involvement in aversive memories has not been addressed so far. We found that cannabinoid type-1 (CB$_1$) receptors, key regulators of aversive responses, are present at presynaptic terminals of MHb neurons in the IPN. Conditional deletion of CB$_1$ receptors from MHb neurons reduces freezing responses in fear conditioning paradigms and abolishes conditioned odor aversion in mice. Interestingly, local inhibition of nicotinic but not glutamatergic receptors in the target region IPN before retrieval rescues these phenotypes. Finally, optogenetic electrophysiological recordings of MHb-to-IPN circuitry revealed that blockade of CB$_1$ receptors specifically enhances cholinergic, but not glutamatergic, neurotransmission. Thus, CB$_1$ receptors control the expression of aversive memories by selectively modulating cholinergic transmission at MHb synapses in the IPN.

GENE EXPRESSION AND EPIGENETIC REGULATION BY CANNABINOIDS IN NEUROGENESIS

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INTRODUCTION: Endocannabinoids (eCB) in the central nervous system (CNS) have recently emerged as instructive cues in the development of the CNS as they are able to attenuate detrimental effects on neurogenesis and neuroinflammation that are associated with ageing. New evidence suggests that eCB signalling regulates gene expression by inducing epigenetic modification such as DNA methylation or histone modification in the regulation of a range of neurobiological processes in the brain, including CNS development, learning, memory and neurodegeneration associated with ageing.

AIMS: The aim of this study is two-fold: firstly to determine if pharmacological targeting of the CB1 or CB2 subtype of cannabinoid (CB) receptors can regulate epigenetic activity in neural stem cells (NSC) from C57BL6/J mice, and secondly to determine the potential involvement of key genes in the regulation of this epigenetic activity.

METHODS: We analysed if CB signals are able to regulate DNA methylation in NSC by modifying the DNA methyltransferases (DNMT) activity. This study was performed with a mini library of 5 highly selective CBs (CB1 agonist: ACEA; CB1 antagonist: AM251; CB2 agonist: JWH133; CB2 antagonist: AM630; DAGL inhibitor: RHC-80267). Experiments were performed in nuclear extracts from NSC (t= 48 and 72h). To evaluate the role of CB on NSC proliferation, the expression of the mitotic marker phospho-Histone H3 (PH3) and BrdU incorporation were assessed. DNA cell cycle analysis of NSC was performed using flow cytometry (FACS). Gene expression levels in NSC were determined by microarray analysis (Affymetrix). Furthermore we investigate the epigenetic control of neurogenesis by the CB1/CB2 cannabinoid signalling pathway (Epigentek).

RESULTS: Cells expressing PH3 or BrdU incorporation significantly increased in NSC after exposure to CB agonists ($P<0.001$ vs. control). The incubation of the NSC with IL-1ra blocking antibody (R&D Systems), abolished the above proliferative effects. Interestingly, NSC proliferation rate was significantly increased ($P<0.001$ vs. control) after exposure to recombinant murine (rm)IL-1ra. The above results strongly suggest that IL-1ra is a critical mediator for the protective actions of CB in the CNS. Microarray-based gene expression analysis of NSC identified a set of novel candidate genes being up regulated or down regulated in NSC after CB exposure. These genes are specifically involved in the cell cycle regulation. Finally, we determined that basal DNA methyltransferase (DNMT) activity in the NSC was downregulated after CB agonists exposure (by approx. 50%). Interestingly, rmIL-1ra, caused a significant decrease in DNMT activity (by approx. 61%).

CONCLUSION: Our results suggest that CBs are potent signals that induce NSC proliferation and migration via IL-1ra. These data reveal an unexpected role for this signalling pathway in neurogenesis, which might have important implications for brain repair.

OREXIN-INDUCED ENDOCANNABINOID BIOSYNTHESIS DISABLES SATIETY-INDUCING POMC NEURONS

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Anatomical, biochemical and pharmacological evidence supports a mutual involvement of cannabinoid and orexin-A (OX-A) in the regulation of appetite. Hypothalamic pro-opiomelanocortin (POMC) neurons and the derived peptide, α-MSH (melanocyte-stimulating hormone), promote satiety. POMC neurons receive OX-A fibers and express both OX-A receptor-1(OX-1R) and cannabinoid type 1 receptor (CB_1R). In this study, we interrogated whether OX-A–mediated feeding in a satiety state is associated with suppressed activity of POMC neurons, and if so, whether the altered activity of these neurons is important for CB_1R-induced feeding. We show that leptin signal deficiency during obesity increases OX-1R activity and promotes feeding via a CB_1R-mediated inhibition of *pomc* gene transcription, POMC expression and α-MSH release in the hypothalamus of sated mice. These processes involve OX-A-induced biosynthesis of the *endocannabinoid* 2-arachidonoyl glycerol (*2-AG*), CB_1R-induced ERK1/2 activation and concurrent STAT3-mediated inhibition of *pomc* gene expression. This loop participates in hyperphagia and weight gain induced by systemic injection of OX-A in lean mice, which are prevented by the selective OX-1R antagonist SB334867 at doses that are ineffective at regulating sleep and reduce body weight, fat liver content and serum transaminases in obese mice. In human obese subjects an inverse correlation was found between OX-A and α-MSH serum levels, together with corresponding alterations of markers of liver fatty disease. These results uncover a previously unsuspected role for OX-A in the promotion of feeding by down-regulation of hypothalamic POMC synthesis and α-MSH release via CB_1R.

A FLUORESCENT NOLADIN ETHER PROBE TO INVESTIGATE ENDOCANNABINOID CELLULAR UPTAKE AND TRAFFICKING

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The effects of endocannabinoids (ECs) are regulated by cellular biosynthesis, release, reuptake, trafficking and enzymatic metabolism. Unlike the clear knowledge about the biosynthetic and metabolic pathways, the mechanisms of cell membrane trafficking is not yet elucidated. Although the identification of the putative endocannabinoid membrane transporter (EMT) remains still elusive, the best experimentally supported theory relies on a passive membrane transporter-mediated mechanism. One of the main issues in elucidating the uptake process is the tight inter-play between ECs plasma membrane movement and their rapid and almost complete cleavage mainly dependent on FAAH and MAGL activity. Recently we have shown that all ECs compete for the same EMT independently of their intracellular fate (trafficking and enzymatic inactivation). Thus, we have synthesized and characterized a fluorescent-analogue of noladin ether (NBD-2AGE), the only EC hydrolysis-resistant.

Methods: The biological properties of NBD-2AGE were investigated testing CB receptor binding, bidirectional trafficking across plasma membranes and enzymatic cleavage. The stability of the probe was investigated using HPLC and FACS methods. The kinetics of cell uptake and release was investigated in different cell lines and in PBMCs. NBD-2AGE was also incubated with mouse brain slices and the distribution was analyzed by using confocal microscopy and high-resolution laser scanner (Typhoon FLA 9500). The distribution of NBD-2AGE into the brain and peripheral tissues was investigated after i.p. and intracisternal (i.c.) injection in mice. A LC-MS/MS method was established to detect noladin ether in the brain and peripheral tissues.

 Results: **1**) NBD-2AGE retains all main characteristics of ECs, including binding to CB receptors and trafficking across plasma membranes. **2**) NBD-2AGE showed a saturable and inhibitable kinetics of uptake and release in different cell lines and in PBMCs. **3**) NBD-2AGE confirmed to be a hydrolytic-resistant EC analogue and to selectively compete with the main ECs for cellular uptake. **4**) The fluorescent probe showed a specific distribution in different brain regions which was prevented by the pre-treatment with EMT inhibitors and incubation at low temperature. **5**) Upon i.p. and i.c. injection, NBD-2AGE showed a different pattern of distribution in the brain and peripheral tissues. **6**) Using a LC-MS/MS method, noladin ether was identified in human and rodent plasma but not in the brain.

Conclusions: Our data suggest that NBD-2AGE is a very useful probe to investigate the kinetics of EC trafficking across plasma membranes with a sensitive and radioactivity-free based method. Unlike the other ECs, NBD-2AGE is resistant to the fast and very efficient FAAH- and MAGL-mediated hydrolysis, which is a well known confounding factor for studying cellular uptake and trafficking of AEA and 2-AG. Finally, fluorescently-tagged noladin ether would allow monitoring the ECs distribution in different cell types and tissues when applied to complex matrices such as whole blood or brain slices or injected in animals.

EARLY LIFE INFLAMMATION WITH LPS AFFECTS ENDOCANNABINOID-MEDIATED SOCIAL BEHAVIOR IN ADOLESCENCE

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Introduction: Inflammation is a common physiological stressor, especially in childhood, and a normal mechanism to clear pathogens. We recently discovered a susceptible window, during which exposure to a bacterial compound (LPS) can cause long-lasting changes in physiology and behaviour. The full impact on behaviour and pathways is unknown at this point. Preliminary data identified behavioural changes in fear, anxiety, and sociability; those behaviours are substantially mediated by the amygdala. Here, I address the hypothesis that a single postnatal immune challenge causes alterations in the amygdaloid endocannabinoid (eCB) system, correlated with decreased social behaviour *(Trezza et al., J. Neurosci. 2012; 32, 14899–14908)* and reversed by increasing anandamide (AEA) concentrations.

Methods: Sprague Dawley (SD) rats were injected on P14 with either LPS (100μg/kg) or saline. To assess the effect of P14 LPS on social behaviour (P40), animals were separated over night and then encountered a novel rat, matched for treatment, age and sex. The experiment was scored offline for social non-play and play behaviours *(Vanderschuren et al., Neurosci. Biobehav. Rev. 1997; 21, 309–326)*.

To characterize the eCB system, amygdala tissue was harvested on P40. Samples were subjected to a lipid extraction process. AEA and 2-AG concentrations were determined using isotope-dilution liquid chromatography/mass spectrometry. CB1 binding was determined using a radioligand binding assay. FAAH activity (Vmax and Km) was measured as the conversion of $^{[3H]}$AEA to $^{[3H]}$ethanolamine.

To rescue the effects of P14 LPS on social behaviour, animals were habituated to the consumption of a highly palatable food on P35 for 4 consecutive days and then single-housed over night. They were given a low dose (1mg/kg) of a FAAH inhibitor (PF-3845) or vehicle (100mg peanut butter) 4-6h before testing *(Ahn et al., Chem. Biol. 2009; 16, 411–420).* Rats encountered a novel partner for 15 minutes and were returned to their cage mates for 48h. Testing was then repeated as for day 1, with opposite treatment.

Results: P14 LPS significantly decreases total social behaviour (t (46) = 3.696 p = .0006). This change is accompanied by decreases in CB1 binding (t (18) = 2.672, p = .016) and increases in AEA concentration (t (32) = 2.319, p = .027) and FAAH activity (t (15) = 2.975, p = .009). Social behaviour can be restored by inhibiting the AEA degrading enzyme FAAH, which significantly increases social interaction in P14 LPS, but not saline treated animals.

Conclusion: P14 LPS can exert a long-lasting effect on the amygdaloid eCB system and appears to change amygdala-mediated behaviour into adolescence. Decreased social behaviour is an important feature of many psychiatric disorders such as schizophrenia and anxiety-related disorders. Defining the effects of inflammation on amygdala functionality may facilitate the understanding of such disorders and potentially lead to novel and more effective treatments.

LEPTIN-CONTROLLED OREXIN/ENDOCANNABINOID INTERACTIONS IN THE MOUSE PERIAQUEDUCTAL GREY: ROLE IN THE REGULATION OF THE DESCENDING ANTINOCICEPTIVE PATHWAY

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Introduction: In the ventrolateral periaqueductal gray (vlPAG), activation of excitatory output neurons projecting monosynaptically to OFF cells in the rostral ventromedial medulla (RVM) causes antinociceptive responses via OFF cells stimulation and ON cell inhibition (Behbehani et al., Pain, 1990) . We demonstrated that this descending nociceptive pathway is under the control of cannabinoid receptor type-1 (CB1) (Maione et al., J Pharmacol Exp Ther, 2006). Orexins are hypothalamic peptides known to modulate arousal, feeding, reward and antinociception via orexin receptors (OX-R). Recently, Ho and collaborators demonstrated that orexin-A (OX-A), by activating OX-AR (OX-A receptor) in the vlPAG of rats, stimulates the synthesis of 2-AG and retrograde inhibition of the tonically active GABAergic circuit thus inducing activation of descending nociceptive pathway (Ho et al., J. Neurosci, 2011).

Aim and Methods: On this basis we hypothesized the existence of a leptin-controlled orexin/endocannabinoid interaction in the modulation of the pain network leading to nociception. In this study we have validated this hypothesis using a combination of in vivo electrophysiology, immunohistochemical, ultrastructural and behavioral approaches in wt and *ob/ob* mice.

Results: We observed that OFF and ON cells are more and less active, respectively, in *ob/ob* compared to wt. We found a significant increase of number and intensity of OX-A fibers in the PAG of *ob/ob* mice. OX-AR/DAGLalpha expression colocalized in a limited subset of PAG neurons through a electron microscopy approach. Moreover, CB1 receptors were expressed at symmetric synapses to OX-AR-expressing neurons thus suggesting an heterosynaptic pathway. The pharmacological blockade of the OXR1 into the PAG produced pro-nociceptive effect in WT mice detected by both pow withrawal and ON OFF cell activity. Interestingly, in the ob/ob mice the dose of the OX-AR antagonist able to generate the pronociceptive effect were double as compared to WT mice suggesting a change of this system in the absence of leptin. On the other hand, AM251, a selective CB1 antagonist also induced pro-nociceptive effect in wt mice and needed of lower dose in the ob/ob mice suggestin a tight cross-talk between leptin-orexin and cannabinoid systems. The endocannabinoid level measurements further confirmed the data.

Conclusions: Here we provide evidence supporting that the heterosynaptic endocannabinoid spread in the vlPAG after OX-AR activation is modulated by leptin. The leptin-related increase of OX-A signalling in PAG is accompained by incresed activation of OX-AR which are GqPCRs and could initiate the GqPCR-PLC-DAGL-2AG retrograde inhibition onto tonic GABAergic transmission in the vlPAG , leading to the potentiation of antinociception. Finally, we show that, beside the feeding and arousal, the orexin system could be highly involved in the pain modulation and is activity is possibly regulated by the leptin-cannabinoid system interaction.

ASTROGLIAL CB$_1$ RECEPTORS ARE REQUIRED FOR *IN VIVO* HIPPOCAMPAL LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION

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Cannabinoids and endocannabinoids modulate synaptic function through the type-1 cannabinoid receptors (CB$_1$). In the hippocampus, the activation of neuronal CB$_1$ is known to modulate synaptic transmission and plasticity, and behavior. Interestingly, astrocytes also express functional CB$_1$ (astroglial CB$_1$) responsible for both memory impairments and a long-term depression (CB-LTD) of synaptic function induced by exogenous cannabinoids. Yet, whether the endogenous activation of astroglial CB$_1$ receptors can also modulate synaptic functions *in vivo* is not known.

Methods: *In vivo* recordings of evoked field Excitatory Postsynaptic Potentials (fEPSP) in the ipsilateral Schaffer Collateral-CA1 pathway were performed in anesthetized mice lacking CB$_1$ in astrocytes (GFAP-CB$_1$-KO) and wild-type littermate controls (GFAP-CB1-WT). Long-term potentiation (LTP) was induced by a high frequency stimulation (HFS) protocol (3 trains of 100Hz during 1s, 20s between each train). Pharmacological compounds, when used, were administered intraperitoneally (i.p.) before HFS.

Results: **1)** The deletion of CB$_1$ in astrocytes impairs the induction of LTP in the Shaffer collateral-CA1 synapses. Furthermore**, 2)** the administration of the selective non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist MK-801 (3.0 mg/kg) blocks the induction of LTP in WT mice. **3)** The administration of the NMDAR co-agonist D-Serine, two hours before HFS, restores the capacity of GFAP-CB$_1$-KO mice to express LTP.

Conclusions: These results reveal that astroglial CB$_1$ receptors are required for LTP in the hippocampus. Moreover, the administration of MK-801 confirms that this form of long-term plasticity is dependent on NMDAR transmission. Finally, the rescue effect of D-Serine suggests that CB$_1$ in astrocytes regulates the availability of this molecule in the synapse hence controlling the induction of plasticity. Altogether these results demonstrate that the endocannabinoid system in astrocytes is important for normal synaptic function through the modulation of neuro-glial interactions.

PALMITOYLETHANOLAMIDE INCREASES CB2 RECEPTOR EXPRESSION VIA PPAR-α

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Introduction: The endogenous fatty acid amide palmitoylethanolamide (PEA) has been shown to exert anti-inflammatory and analgesic effects mainly through inhibition of pro-inflammatory compound release from mast cells, macrophages, and microglia. Although several mechanisms of action have been proposed, indirect activation of the cannabinoid (CB) system is thought to be responsible for the effects of PEA observed in several pain models.

Methods and results: Using cultured rat microglia and human macrophages, we evaluated whether PEA affects CB receptor expression through several approaches. We showed that PEA treatment increases CB2 mRNA and protein expression levels through peroxisome proliferator-activated receptor-α (PPAR-α) activation. The involvement of PPAR-α was demonstrated through i) pharmacological PPAR-α manipulation, ii) PPAR-α mRNA silencing, and iii) molecular docking. Incubation of microglia with PEA also induced morphological changes associated with an anti-inflammatory phenotype, compared to the phenotype of untreated microglia. Moreover, we observed that chronic treatment with PEA significantly increased CB2R expression in the spinal cord of healthy animals and prevented the behavioural dysfunctions induced by peripheral nerve injury.

Conclusions: These results provide evidence for a new mechanism of action for PEA, indirect regulation of CB2R expression.

CANNABIS USE IN PATIENTS WITH CHRONIC PAIN: EFFECT ON SYMPTOMS RELIEF AND QUALITY OF LIFE

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OBJECTIVE

Describe effects of cannabis use and the associated benefits reported by patients with different chronic pain diagnosis with moderate to severe symptomatology and resistant to other pharmacological treatments (radiculopathy, PHN, plexopathy, neurodegenerative diseases, fibromyalgia oncologic pain, cephalea, osteoarthritis)

MATERIALS AND METHODS

- Patients were psychologically screened prior to the administration and those with psychiatric or personality disorders, history of abuse or dependence for cannabis or others psychoactive substances were excluded
- Pain medications, pain intensity using Visual Analogue Scale (VAS: from 0=no pain, to 100=worst imaginable pain), quality of sleep (VAS: from 0=good sleep, to 100=worst imaginable sleep) and a self-administered questionnaire for anxiety and depression symptoms (HADS), were recorded at baseline (T0), after 3 (T1), 6 (T2) and 12 (T3) months
- Cannabis administration was a decoction. The starting dose was 5 mg/daily of THC corresponding to 28 mg Cannabis Flos 19%
- Cannabis dosage and adverse effects were recorded during each visit

RESULTS

- Recruitment began in November 2013
- 477 patients were treated (mean age 61±14): 45% were administered 5 mg of THC daily, 36% - 10 mg, 15% - 15 mg, 5% - 20 mg
- 18% of patients interrupted the therapy, but only 22% of these because of side effects. The remaining 9% didn't begin the therapy due to prejudice linked to substance. 78% interrupted because of therapy inefficacy and excessive cost. However had they continued therapy with higher dosage they probably would have been responders
- Pain intensity VAS: T0=8.6, T1=5.8, T2=5.1, T3=5.1
- Levels of anxiety, depression, quality of sleep improved in significant way (p.<.0001)

DISCUSSION

A large body of evidences from clinical studies support efficacy of cannabinoids in the amelioration of chronic pain symptoms from different origin.

In our sample the improvement of anxiety and quality of sleep levels is significant as well the pain intensity improvement. Most patients reduced or interrupted the benzodiazepine and anxiolytic use reporting a restorative sleep and greater alertness during the day.

CONCLUSIONS

In our experience cannabis has proven to be an effective pain treatment in chronic pain patients. However the remarkable advantages were reported in the improvement of moods and sleep quality and as a result QoL and disability.

A PHARMACOLOGICALLY POTENTIATED STRESS DURING LACTATION ALTERS THE HEPATIC ENDOCANNABINOID SYSTEM WHICH IS RELATED TO ACCUMULATION AND ASSEMBLAGE OF TRIGLYCERIDES, AND SYSTEMIC INSULIN RESISTANCE IN ADULT MICE

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Introduction: Type 1 Cannabinoid receptors (CB_1R), anandamide and fatty acid amide hydrolase (FAAH) are part of the endocannabinoid system (ECS). We have previously shown that a soft nociceptive stress during the whole lactation leads to overweight and alters metabolism of adult mice. These effects were normalized by treatment with SR 141716 a well-known CB_1R antagonist, indicating involvement of the ECS (Valenzuela et al., Obesity 2011; 19:29-35). Since CB_1R and endocannabinoids are involved in the negative feed-back mechanism implicating corticosterone action on CRH release, stress could be exacerbated by simultaneous treatment with a CB_1R antagonist during the first half of lactation.

Since hepatic CB_1R is required for development of insulin resistance in mice (Osei-Hyiaman et al., J Clin Inv 2008; 118:3160-3169), we evaluated if adult mice subjected to manipulations during lactation develop glucose intolerance (GI) and insulin resistance (IR) during adulthood, associated to a liver physiopathology involving: a perturbed ECS, triglycerides (TG) accumulation and an imbalance of proteins involved in trafficking/storage of lipids such as Adipophilin (ADPF), aP2, FSP27 and microsomal triglyceride transfer protein (MTTP).

Methods: During lactation (days 1-10), mouse pups were daily treated with an oral dose (3ug/g) of the CB_1R antagonist AM-251, and 1 h later subjected to stress with an injection of saline solution in the back. This was the main AMST group. Appropriate controls were also run as follow: stress + the antagonist vehicle (STV); no-stress + antagonist (CAM) and no stress + the antagonist vehicle (CV). Mice (70 and 140 days) were subjected to a glucose tolerance and insulin sensitivity tests. At 150 days, mice were euthanized, and liver extracted. Expression of CB_1R, FAAH, ADPF, aP2, FSP27 and MTTP were evaluated by RT-qPCR and Western Blots. Hepatic TG concentrations were also measured. Activity of FAAH was measured by its ability to hydrolase ^{3}[H]-labelled anandamide.

Results: Double treated mice (AMST; 140 days old) had GI and IR and higher mRNA expression of ADPF, aP2 and FSP27 in liver when compared to other 3 animal groups. Protein content of CB_1R, ADPF, aP2 and FSP27 were higher, but FAAH and MTTP were lowered in AMST. FAAH activity was significantly lowered while liver TG concentration was elevated by 2 fold.

Conclusions: Stress during early lactation together with a CB_1R antagonist treatment (AMST group) reprograms SEC in adult mice liver, by elevating the amounts of CB_1R and decreasing FAAH protein and activity. This concerted mechanism, should result in overactivity of CB_1R, with long term consequences in TG accumulation, facilitated by increased levels of aP2, ADPF and FSP27, and decreased MTTP. All these factors may contribute to development of liver steatosis and systemic GI and IR.

CANNABINOIDS INFLUENCE ANNEXIN A1 AND NITRIC OXIDE IN HYPOPHYSEAL CELLS

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There is strong evidence that folliculo-stellate (FS) cells in the hypophysis interact with hormone-producing (HP) adjacent cell populations (*Devnath and Inoue 2008; Morris and Christian 2011; Vankelecom 2012*). Annexin A1 (Anx A1) is a protein with anti-inflammatory properties and is involved in inhibiting the phospholipase A2 (PLA2), an enzyme essential in the generation of arachidonic acid, the precursor of the endocannabinoids 2-AG and AEA (*Blackwell et al. 1982; Perretti and Dalli 2009*). We demonstrate that the cannabinoids (CB) act on cannabinoid type 1 (CB_1) receptors on FS cells and evoke changes in the concentration of Anx A1 and nitric oxide (NO). We additionally detected Fpr-rs1, a receptor for Anx A1, on two HP cell models. Furthermore investigations were carried out to determine whether endocannabinoids influence FS cells that can interact with HP cells through Anx A1 and NO.

Methods: Cell lineages TtT/GF and Tpit/F1 were used as FS models. Adrenocorticotropin (ACTH)-producing AtT20/D16v cells and prolactin (PRL)-producing GH4C1 cells represented the HP models. Incubations of the cells involved serum-starvation for at least 4h, subsequent supplementation with a predefined serum-substitute B-27® (Sigma-Aldrich) and incubation with the cannabinoid substance for 30 min. Cell media and cell extracts were collected and further processed. The applied techniques comprised immunoblotting (IB), immunocytochemistry (ICC), *in situ* hybridization (ISH), ELISA, and photometrical detection. GraphPad Prism software was used for statistical analysis of the obtained data.

Results: **1)** All of the used cell models, FS and HP, expressed CB1 receptors. **2)** Incubations with 2-AG but not with AEA led to an increase in the concentration of Anx A1 in both TtT/GF and Tpit/F1 cell models. WIN 55,212-2 a synthetic CB1 agonist, raised the level of Anx A1 as well. **3)** The concentration of NO was influenced negatively by AEA and WIN 55,212-2 in TtT/GF cell media. **4)** Both HP cell models were positive in the ISH analysis for Fpr-rs1. **5)** Incubations with 2-AG did not alter the concentration of ACTH and PRL significantly, but NO decreased both ACTH and PRL in the ELISA analysis.

Conclusions: We conclude from these findings, that **a)** the effects of the 2-AG and WIN 55-212,2 are mediated by CB1 receptors **b)** leading to an increase in the levels of Anx A1 protein. **c)** Cannabinoids decrease the concentration of NO. The increase in Anx A1 and the decrease in NO levels highlights the anti-inflammatory properties of CB1 agonists. **d)** The Fpr-rs1 receptors are present on both HP cell lines. **e)** The missing effect of 2-AG on ACTH and PRL concentrations can be ascribed to the involvement of a complex stimulatory mechanism. Yasuo et al. (2014) found that while 2-AG alone did not alter the PRL secretion from hamster pituitary slices, the addition of adenosine or forskolin potentiated the concentration of PRL. **f)** Finally, NO inhibited hormonal secretion consistent with the expectations.

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CANNABINOID AND TERPENOID CONTENT/POTENCY: FLOWER VS. CO$_2$ CONCENTRATE

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Introduction: An International Cannabis Use Survey revealed that Cannabis is typically selected by smell by 60% users. While visual appearance and THC/CBD concentration also guide uses, the olfactory indicator is the terpenoid fraction. In addition to smoking cannabis flower, users also vaporize flower and concentrate using a variety of techniques. We compared the potency of flower and trim to that of solventless extract, rich in the cannabinoid and terpenoid fractions, and report crowd-sourced information on "effects" of the varietals we analyzed.

Methods: We developed a GCMS method for quantification of 42 terpenoids. The HPLC/DAD method for quantification of 7 cannabinoids, THCA, THC, CBA, CBD, CBN, CBG and THCV was from the American Herbal Pharmacopoeia Cannabis Monograph. Five cultivars of cannabis flower were harvested and cured to 15% moisture. The flower trim was collected and extracted using a standardized supercritical fluid CO$_2$ method. We used crowd-sourced information on user-reported effects of the varietals tested to determine whether there are correlations between subjective experience and objective terpenoid and cannabinoid profiles.

Results: We compared the quantity and volume of the terpenoid and cannabinoid component across samples. We report the extraction efficiency of the CO$_2$ method, microbial and aflatoxin results. We complied the data and compared the potency of flower vs. concentrate. We also sought to correlate objective end-user data to the terpenoid and cannabinoid profile using multi-dimensional analysis. Statistical analysis was preformed using GraphPad Prism® software.

Conclusions: It is largely unknown what the effects of vaporization of concentrated cannabis extracts are on human health. Our results demonstrate that the product of CO$_2$ extraction is significantly different from that of Cannabis flower, as potency is increased by approximately a factor of two to three across varietals and with twice as many terpene compounds detected by GCMS in the concentrate. Neural network analysis of crowd-sourced data and terpene analysis will reveal interesting profile characteristics by which users may differentiate varietals. While inhalation is an excellent delivery system, the difference in potency/dose is significant, and further studies are necessary to determine the health-related consequences of inhaling concentrated Cannabis extracts.

INVESTIGATING THE MUNCHIES: AN INTERVIEW AND ONLINE SURVEY OF CANNABIS-RELATED APPETITE CHANGES

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Introduction: It is understood, predominantly from anecdotal evidence, that cannabis intoxication leads to increased, often voracious, appetite and enhanced appreciation of food; colloquially referred to as the "munchies". This phenomenon appears to reflect actions of the drug on brain systems involved in the normal regulation of appetite. In lab animals, cannabis-derived 'phytocannabinoids' (eg, THC) stimulate feeding, apparently through their ability to mimic the actions of the endocannabinoid class of neurochemicals at brain cannabinoid receptors (Kirkham 2005). Recent developments suggest that endocannabinoid systems are crucial components of the biological mechanisms that modulate appetite, energy balance and body weight in mammals.

Despite centuries of cannabis use, most of our knowledge about the drug's actions on the psychological and behavioural aspects of appetite in people remains largely anecdotal. There is little empirical evidence to substantiate users' claims, and human laboratory studies have focused principally on food intake measures, rather than the psychological factors which affect eating. This study addresses that shortfall in the literature, by characterising the nature of "the munchies" in terms of alterations to motivation to eat, modulation of appetite, sensory aspects, food reward, food intake, and changes in food preference and choice.

Method: Combined quantitative and qualitative approaches provide a holistic framework for investigating this phenomenon: An online survey directly targeting the cannabis user population worldwide, assesses cannabinoid influences on various aspects of eating experiences, and in-depth interviews examined sensory alterations and shifts in food preferences. Interviewed participants were required to provide photos of foods they typically crave after cannabis use. A hybrid interview technique combined laddering questions (means-end chains) with discussion of the food photos. Content-analysis identified key concepts and ladders were used to create a hierarchical value map (HVM) in LadderUX[©]. Preferred 'munchies-food' were explored based on their sensory characteristics (taste, texture, appearance, etc).

Results: Preliminary data collection from the online study is ongoing. However, results from interview content analysis with experienced cannabis users confirm that they experience an almost insatiable appetite. Liking of highly rewarding foods that are easy to prepare are reported. Sensory drivers included preferences for sweet taste, as well as savoury and fatty/greasy foods and mouth-feel. A desire for textural and food temperature contrasts were also reported.

Conclusion: This study begins to elucidate the psychological components of cannabis modulation of appetite. Preferences for food types are studied as well as alterations to eating pleasure and eating motivation. It is clear that the munchies is more complex than previously understood. Hypotheses about the mechanisms of action of phytocannabinoids and the role of endocannabinoids in the control of appetite derived from these results will guide future, more focussed laboratory studies.

CLINICAL DEVELOPMENTS IN CANNABINOID TREATMENT OF CHRONIC PAIN

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Introduction: Chronic pain is the main symptom for which patients use cannabinoid-based medicines (CBMs). Clinical research on the treatment of chronic pain with the use of CBMs has significantly developed in the recent years. Despite promising results, the available data lacks conclusive evidence. Moreover, given the preference of many patients for herbal preparations, there is a significant need to develop a convenient, reliable and standardized method to administer herbal cannabis.

Methods and Results: This presentation will provide an overview of the recent developments in the clinical investigation of the efficacy of CBMs on chronic pain and discuss the current needs and opportunities for future research. Specifically, an inquiry into the optimal conditions and requirements for a clinical trial with vaporized herbal cannabis will be presented.

Conclusion: It will be proposed that vaporizing, as a safe and reliable method of cannabinoid administration, can be effectively applied to deliver a standardized, pharmaceutical-grade, herbal cannabis product for the treatment of chronic pain. Possible benefits for the research on the treatment of chronic pain with CBMs will be presented.

THE EFFECT OF 0.1%CANNABIDIOL OINTMENT FOR INTRACTABLE ALOPECIA AREATA A CLINICAL CASE REPORT

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Alopecia areata is known as one of the autoimmune disease. In many case spontaneous remission is seen but there are also seen repeating recurrence cases or very intractable cases to any treatments includes steroid. In such cases the breakthough of novel treatment is waited. On the other hand recent studies revealed that CBD regulates immune system by several signaling pathway.

In this report a 10years long-lasting intractable Alopecia areata male patient is treated with 0.1% cannabis derived CBD ointment as clinical trial.

The result was very sufficient and supports many preclinical evidences.

But in Japan it is still illegal to use cannabis as medical purpose even exclude THC.

So we are going to planning further clinical trial under governmental allowance in the near future.

Methods: 15% CBD raw hemp oil (PlusCBD^{TM} Cannavest) is mixed with white petrolatum and diluted to 0.1% concentrate.

0.1%CBD ointment is put on bold head once a day as a night cream.

Standard photograph is taken once a month.

Dropped hair root is observed under microscope and the severity of immune reaction is evaluated by morphologically.

Results:

The obvious hair growth is observed with in a month.

Microscopic hair root examination shows still severe immune reactions of hair root.

But clinical symptom improves day by day.

Conclusions:

Aropecia Areata is thought one of autoimmune disease caused by stress but it etiology is still unknown. Recent study reveal T helper 17 cells, characterized by interleukin-17 (IL-17) production, play a critical role in the pathogenesis of autoimmune disease, including alopecia areata. On the other hand Kozela et al reported both THC and CBD markedly reduce Th17 phenotype dose dependently. They also found decrease of m-RNA of IL-6 and increase of anti-inflammatory cytokine IL-10. These experimental studies and evidences suggest efficacy of CBD therapy for Alopecia areata.

 We tried 0.1% CBD ointment as additional therapy for intractable Alopecia areata male patient. The result supports CBD therapy may novel therapy for autoimmune disease including Alopecia Areata.

RESULTS FROM AUDITING MEDICAL CANNABIS OPERATIONS IN THE UNITED STATES

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Regulation is becoming mandatory in states that allow medical cannabis. The producers, manufacturers, dispensaries, and laboratories involved in this industry can operate legally in their states but function without much regulation or oversight. Due to increasing concerns over the need to standardized medicinal cannabis preparations, the American Herbal Product Association (AHPA) has created industry guidelines on manufacturing, producing, dispensing, and laboratory operation standards. Additionally, the American Herbal Pharmacopeia (AHP) completed the Cannabis monograph, a guide for the standardization of cannabis. The work of AHPA and AHP laid the foundation for a certification body called Patient Focused Certification (PFC) a project of Americans for Safe Access. AHPA and AHP guidelines are being incorporated into state level regulations as mandatory product safety standards in new state programs. PFC launched in early 2014 with facilities in several states having successfully completed the auditing process. Over a dozen operations have been certified in over 8 states. Results from over a year of auditing of medical cannabis facilities will be discussed, including data on corrective actions with research on the impact of such regulations on patients, facilities, government, universities, and neighborhoods.

EVOLUTION OF THE CONTENT OF CANNABINOIDS AND TERPENES DURING THE GROWTH OF DIFFERENT *CANNABIS SATIVA L.* PLANTS

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The increasing use of cannabis as medicine and the growing interest on the medicinal effects of non-psychotropic cannabinoids as CBD and CBG has brought the need to produce specific plants and pure compounds in a large scale. Terpenes are also very promising for many applications since they work synergistically with cannabinoids in addition to their characteristic smell which possess specific medical effects. In order to optimize the production for each compound and to have a deeper understanding of their development during the growth, the evolution of cannabinoids and terpenes content of 9 cannabis varieties of 3 different chemotypes (3 THC high varieties, 3 CBD enhanced varieties and 3 mix varieties) during the growth of the plants was studied.

Methods: Around 50 clones of each mother plant were grown indoor under controlled conditions. In the beginning, they were cultivated in 25 x 25 mm slabs until the roots grow and were transferred to 2 L pots. During these two steps, plants were grown under an indoor vegetative lights cycle (18 h of light). When their sizes were appropriate, plants were transferred to 10 L pots and were exposed to a flowering light cycle (12 h of light) until harvest. Each week some plants were cut, dried, and analyzed for cannabinoid and terpene content. The evolution of 8 cannabinoids content was monitored by means of High-performance liquid chromatography with photodiode array detection (HPLC-DAD) and more than 20 terpenes were quantified by Gas Chromatography with Flame Ionization Detection (GC-FID) and verified by Gas chromatography–mass spectrometry (GC-MS).

Results: **1)** The chemotype of the plant was clear since the beginning and stable during the growth. **2)** CBD/THC ratios and total cannabinoids/total terpenes ratios were stable during the growth. **3)** Cannabinoid and terpene content was clearly decreased during the vegetative phase while the plant was growing, achieving lower concentrations than in the mother plants. **4)** Characteristic terpenes for each chemotype were identified. **5)** The study is still ongoing in the flowering phase; therefore, more results as the highest concentration time for each compound and final conclusions will be presented later.

Conclusions: These results can help to optimize the production of specific plants facilitating to obtain pure compounds and to have a deeper understanding of the evolution of cannabinoids and terpenes during the plant growth. Moreover, the chemical profiles of the different chemotype plants can help studies who want to analyze potential interactions of the cannabinoids and terpenes or the therapeutic effectiveness of these plants.

IMPROVING QUALITY CONTROL METHODS FOR CANNABIS USING FLASH CHROMATOGRAPHY

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The American Herbal Pharmacopeia (AHP) recently published a *Cannabis* monograph, setting standards for identification, analysis, and quality control. Additionally, the American Herbal Products Association (AHPA) issued basic product safety guidelines this year for cultivation, manufacturing, dispensing, and laboratory operations for medical *Cannabis.* The recommendations from the AHP and AHPA are steadily being adopted and are implemented in US states through the 3rd party oversight program called Patient Focused Certification. However, a number of significant hurdles must be overcome in this industry to reach higher levels of product safety. Among the issues facing laboratories are access to reference standards, transportation of samples for pesticide or contaminant analysis, having flexibility to efficiently quantify several compounds from a variety of complex matrices in a high throughput manner, dependency on the use of high amounts of toxic solvents, and a shortened life span of expensive analytical equipment used in the routine analysis of viscous and particulate samples. Flash chromatography can help overcome some of the issues facing laboratories engaged in quality control of *Cannabis* medicines by offering a more efficient way to isolate cannabinoids and other compounds of interest from complex matrices, enhancing separation and identification techniques. Our data was obtained by analyzing plant and other complex matrices spiked with known amounts of cannabinoids.

*These authors contributed equally

THE VOICES OF WOMEN: NARRATIVES REVEALING SOCIAL STIGMA WITHIN THE MEDICAL PROFESSION

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This particular presentation will focus on the concerns of medical cannabis patients in discussing cannabis use with their personal physicians and stigmatization issues as voiced by female medical professionals. Using a phenomenological approach and qualitative methods to make problematic the standpoint of female medical cannabis patients, this project helps us understand why women choose to be recognized as medical cannabis patients (what are their reasons for obtaining a medical cannabis license/card) and the associated stigmatization (and fear thereof) that accompany this decision. The most prominent themes to emerge center on *Self*: personal health and necessary resources to use cannabis as medication; *Career*: fear of status or job loss (especially prominent among medical professionals); *Family*: familial acceptance and/or stigmatization; and *Society*: addressing the dominant perception that the use of cannabis is incompatible with the roles and responsibilities of female patients.

CANNABIS: A PAST ITALIAN HISTORY

Gastone Zanette & Enrico Facco

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We suppose that, within this context, most of you know the meaning of the words "*set and setting*". So here, the *setting* is represented by the city of Milan, on the edge of the 18° and 19° century, with its socio-economic and cultural expansion, whereas the *set* is represented by an empathic fellowship engaged in ethno-botany-pharmacology: Paolo Mantegazza, Carlo Erba, Andrea Verga, Cesare Lombroso, Raffaele Vallieri, Giovanni Polli, etc. These friends and scholars used to share knowledge regarding new healing plants, like Cannabis. Coming back from the Egyptian campaign, in 1802, the French Army brought back to Europe an old and forgotten plant, Cannabis Indica. Its influence on the European culture of those times is attested by some manuscripts: Moreau de Tours J.J. " *De l'hashisch et de l'aliénation mentale"*; Gautier T. "*Le Club des Hachichins*"; Baudelaire C. "*Paradis artificiels. Opium et haschisch* "; Cooke M.C. "*The seven sisters of sleep*"; Von Bibra B.E. "*Die narkotischen Genussmittel und der Mensch"*; Mantegazza P. *"Tales of human nature. Festivals and inebrations"*; Lewin L. "*Phantastika-die betaubenden und erregenden Genussmittel*". In those years of rapid medical progress, self-experimentation with vaccines, drugs and medical plants was a common practice among researchers. Even this Italian fellowship performed self-experimentation in order to compare Italian Cannabis Sativa versus Indica, imported mainly from North Africa, and better understand medical indications and uses. In Italy Cannabis Sativa was widely cultivated since the time of Roman empire (food, medicine, textiles); the Po Valley was the main production area and Italy was the second world producer after Russia. Carlo Erba is considered one of the pioneer of the chemical-pharmaceutical industry in Italy and, today, the Carlo Erba pharmaceutical company is part of Kabi-Pharmacia. Raffaele Valieri, Neapolitan physician, used to cure pain, epilepsy, depression, anorexia, insomnia, diarrhoea with Cannabis, as reported in the manuscript: "*On the native Italian Hemp and its extracts, as substitutes of Cannabis Indica*". Giovanni Polli published about Cannabis on "Annals of Chemistry applied to Medicine". Physician, anthropologist, ethnobotanist, writer and politician, Paolo Mantegazza is one eclectic figure of the late 19° century Italian culture. Mantegazza was a forerunner of what has come to be known as "*cultural studies*", on account of his interdisciplinary approach, his passionate blend of scientific and literary elements in his writings, and his ability to transcend the boundaries between '*high'* and '*low'* culture. Back from South America, he introduced the Erythroxilum Coca plant in the European pharmacopoeia and proposed a mind altering substances nosology 60 years before Lewis Lewin classification. He founded, the first Italian University Chair of Anthropology, the National Museum and the Italian Society of Anthropology and Ethnology, the Journal "Archives for Anthropology and Ethnology". In Mantegazza vision of the natural world, the mind altering plants are integral part of nature and, therefore, of Homo Sapiens.

USE OF MEDICAL CANNABIS IN THE TREATMENT OF VARIOUS DISEASES IN SLOVENIA

Milan Krek

National Institute of Public Health, Slovenia

Slovenia has a very active NGO movement, which permanently requires the right for self-medication by cannabis. They are connected with the pro legalize movements. In Parliament they have inserted a special law that defined the use of cannabis for self-medication and self-production of cannabis for self-medication of people. The law in the Parliament was not accepted. But activities are stronger than in the previous time, and we have to solve these problems on right way. Some parliamentary parties accepted and also support this approach.

In Slovenia it has expanded the use of homemade cannabis oil for treatment of various diseases. Oil is prepared by people who do not have medical and pharmacological knowledge and on illegal way. They produced oil from the illegally harvested cannabis grown in a very different environments. They use it for treatment for various illnesses, cancer, pain, multiple sclerosis, etc… Cannabis is not standardized and do not ensure safety for the patient. Also dosing of oil is problematic. We have a several overdosed patients accepted in hospital. Patients use treatments prescribed by doctors and in the same time they also used cannabis oil. Some clients refused the medical treatment and they use only cannabis oil. It is so bad for patients? Today the treatment in oncology has advanced and the treatment success rate is already relatively high.

The Government of the Republic of Slovenia is released using of THC for medical purposes. THC and its derivatives have been moved from the first group to the second group, which allows to doctors treatment of people with THC. Now doctors can prescribe dronabinonol(THC) for different diseases, but most often to treat pain and treat the consequences of cancer treatment and in palliative care.

The situation is particularly bad for patients who find it difficult to decide what to do and what form of treatment to choose illegal or legal. At the moment it will be the best as soon as possible to establish the treatment of various diseases with medical marijuana. The way to go is still long, because it will be necessary to establish a whole new system of treatment with cannabis in the state. Above all, it is necessary to ensure that the quality of cannabis for patients will be good and effective.

To end of this terrible situation, the Medical Chamber is preparing a position of Slovenian medical doctor about the treatment with medical cannabis and cannabinoids. The position will define what kind of system of treatment with medical marijuana will be introduced in Slovenia, and how patients will have access to treatment with medical marijuana. Without proper education of physicians it will not be possible to introduce new methods of treatment.

DEVELOPMENT OF A PORTABLE CERTIFIED VAPORIZER FOR THE INHALED USE OF MEDICINAL CANNABIS

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1: Bedrocan Beheer BV, The Netherlands - 2: Hermes Medical Engineering, Spain

Smoking is one of the most commonly used modes of administration for cannabis worldwide, by medicinal as well as recreational users [1]. It is considered a convenient method of administration, allowing easy self-titration of the desired effects. However, inhalation of toxic compounds during cannabis smoking poses a serious hazard. This risk is not thought to be due to cannabinoids, but rather to noxious pyrolytic byproducts. Consequently, the shortcomings of smoked cannabis have been widely viewed as a major obstacle for approval of herbal cannabis as a medicine by health authorities.

Cannabis ''vaporization'' or ''volatilization'' is a technique aimed at suppressing respiratory toxins by heating cannabis to a temperature where active cannabinoid vapors are formed, but below the point of combustion where pyrolytic toxic compounds are released. Vaporization offers patients who use medicinal cannabis the advantages of the pulmonary routes of administration (rapid delivery into the bloodstream, ease of self-titration, and concomitant minimizing the risk of over- and under-dosing) while avoiding the respiratory disadvantages of smoking. Over recent years, a wide variety of vaporizers have been developed for recreational cannabis use, but with the exception of the Volcano Medic [2], none of these devices have been tested or certified for medical use.

The aim of this poster is to show the different processes involved in the design and certification of a portable vaporizer as a medical device. The future vaporizer was inspired by the MiniVAP © vaporizer, and further optimized by the Industrial Design department of the University of Delft (The Netherlands). The device should be easy and comfortable to use for seriously ill patients, should deliver an accurate and reproducible dose, and must be hygienic and in compliance with pharmaceutical requirements for drug administration systems.

The following data are presented in the poster:

1.   Optimization of THC and CBD delivery. The vaporizer was systematically optimized for the delivery of THC and CBD in the form of herbal cannabis, while preventing the formation of degradation products. Parameters tested included: airflow, temperature setting, amount and variety of cannabis (THC/CBD), inhalation frequency and duration, distance between sample and heater, and size of the sample chamber (experiments performed by University of Leiden (The Netherlands) and Bedrocan Beheer BV).

2.   Toxicity testing of the internal airpath. Hot air that comes in contact with plastic or metal surfaces can release toxic chemicals that may be inhaled. Therefore, the internal airpath of the vaporizer was tested in order to detect potential hazardous chemicals released during standard conditions of use (experiments performed by University of Bask Country, Leioa-Spain).

3.   Optimization of the airflow. A computer model of the airflow through the mouthpiece was designed in order to optimize inhalation in terms of vapor temperature, THC/CBD condensation, and effort needed to inhale (experiments performed by TECNUN and Hermes Medical Enginnering SL, San Sebastián-Spain).

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Administration Forms. J. Psychoactive Drugs 45 (3), 199-210.

RAW CANNABIS AND OTHER COMPLEMENTARY AND ALTERNATIVE MEDICINE IN RELAPSING-REMITTING MULTIPLE SCLEROSIS. A PILOT, RANDOMIZED, DOUBLE-BLIND, CROSSOVER TRIAL.

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Complementary and Alternative Medicine (CAM) is commonly used by Multiple Sclerosis (MS) patients to find symptomatic relief, in conjunction with or in lieu of, the prescribed standard therapy due a significant need of improving quality of life (QOL). (Schwarz,S et al. *Mult Scler.* 2008;14:1113-1119, Yadav,V et al. Neurology. 2014;82:1083-1092) Cannabis has been proposed as an Unique Dietary Essential, providing a large array of "nutraceuticals" that could help in the management of MS, such as lipid-soluble Vitamin E, Essential Fatty Acids (PUFA, Omega 3: Omega 6), Essential Amino Acids, antioxidants and Cannabinoid Acids. (Courtney,W.(2010).Treating Yourself. 24 (1), 52-54) Cannabis and cannabinoid extracts have been found beneficial to MS patients suffering neuropathic pain, spasticity or bladder incontinence, (Wade D et al. (2010) Mult Scler 16:707–714, Corey-Bloom et al.(2012).CMAJ.184 (10),1143-1150), but it has not yet been systematically explored the potential benefit of a daily supplementation of immuno-regulatory cannabinoids in their acid form. The main aim of this study is to compare changes in MS progression and side effect profiles of current therapies versus dietary supplementation with a low-fat, plant- based diet containing daily Cannabis S. in its raw/unprocessed form.

Methods: RRMS patients with EDSS <= 6, aged 20-50, without relevant co- morbidity nor previous history of substance abuse are assessed with biochemical, behavioural, neurological, neuropsychological and instrumental tests, at week 0 and week 9. The intervention comprises 8 weeks of dietary supplementation of vitamins (low-fat vegan diet) and Essential Fatty Acids, occupational therapy, mind-body therapy and natural setting.

Raw cannabis extracts are achieved by blending fresh flowers and juicing stems and leaves. This preparation is thought to be devoid of the psychotropic effects due lack of decarboxylation of naturally occurring THC Acid into THC. (Verhoeckx, K et al. (2006). International Immunopharmacology. 6 (1), 656-665). In the trial, active juices (2.5 g fresh Cannabis flowers + 300 g fresh hemp leafs + 7.5 g hemp oil) are randomized with placebo (300 g fresh spinach + 7.5 g hemp oil) and delivered in 5 supplementation daily. Support for this project is through a unique crowdfunding initiative combined with volunteer work, and represents a novel approach for progressing clinical research in this area.

Results: We will establish whether there is a decrease of chemical index of the inflammatory cascade, a general improvement in symptom management as well as a enhanced QOL related to lower side effects when compared with current treatments.

Conclusions: This project will explore the therapeutic potential of dietary supplementation with raw cannabis extracts, which builds on an unmet clinical need for cannabinoid-based therapies for the treatment of MS.

CANNABINOID RECEPTOR CB2 EXPRESSION MODULATES Gβγ PROTEIN INTERACTION WITH THE ACTIVATOR OF G PROTEIN SIGNALLING 2/DYNEIN LIGHT CHAIN PROTEIN TCTEX-1

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Introduction: The activator of G-protein signalling AGS2 (Tctex-1) forms protein complexes with Gβγ proteins, and controls cell proliferation by regulating cell cycle progression. A direct interaction of Tctex-1 with various G protein-coupled receptors has been reported. Since, the carboxyl terminal portion of CB2 carries a putative Tctex-1 binding motif we investigated the potential interplay of CB2 and Tctex-1 in the absence and presence of G☐☐.

Methods: The interaction of CB2 with Tctex-1 and the influence of CB2 on the formation of Tctex-1-G☐☐ protein complexes were analysed by co-immunoprecipitation experiments in transiently transfected HEK293 cells. The analyses were performed in the absence and presence of the cannabinoid ligands JWH133 and AM630, the protein biosynthesis inhibitor cycloheximide or the protein degradation blockers MG132, NH_4Cl/leupeptin or bafilomycin.

Results: Our results show that CB2 neither directly nor indirectly *via* G☐☐ interacts with Tctex-1, but competes with Tctex-1 in binding to G☐☐. The Tctex-1-Gβγ protein interaction was disrupted by CB2 receptor expression resulting in a release of Tctex-1 from the complex, and its degradation by the proteasome. The decrease in Tctex-1 protein levels was induced by CB2 expression dose-dependently and was independent of CB2 receptor stimulation by agonist or blocking by inverse agonist treatment.

Conclusions: The results suggest that CB2 receptor expression independent of its activation by the specific agonist JWH133 or inactivation by the inverse agonist AM630 is sufficient to competitively disrupt Gβγ-Tctex-1 complexes, and to initiate Tctex-1 degradation. These findings implicate that CB2 receptor expression modifies stability of intracellular protein complexes by a non-canonical pathway.

GENETIC DELETION OF MONOACYLGLYCEROL LIPASE (MAGL) LEADS TO IMPAIRMENT OF CANNABINOID RECEPTOR CB1R SIGNALING AND ELICITS ANXIETY LIKE-BEHAVIOR

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Endocannabinoids (eCB) are key regulators of neuronal activity through modulation of excitatory/inhibitory neurotransmission at presynaptic sites expressing cannabinoid-1-receptors (CB1R). 2-arachidonoylglycerol (2-AG) is the most abundant eCB in the brain and is hydrolyzed by monoacylglycerol lipase (MAGL). Pharmacological and genetic studies indicate that lack of MAGL leads to dramatic elevations of 2-AG levels in the brain and loss of cannabimimetic behavioral effects through β-arrestin2-mediated CB1R desensitization via ERK inhibition. We exploited the MAGL knock-out mice (MAGL-/-), a genetic model for sustained 2-AG elevation in the brain, to provide morphological and biochemical evidence for β-arrestin2-mediated CB1R desensitization at presynaptic sites in prefrontal cortex (PFC), amygdala, hippocampus and cerebellar cortex which are brain regions involved in the control of emotional states.

Methods: Immunohistochemical studies and immunoprecipitation assay were used to reveal the presence of CB_1R/β-arrestin-2 complex the PFC, amygdala, hippocampus and cerebellar cortex of MAGL-/- . Furthermore, we provided morphological evidence of VGluT1/CB1R or VGAT/CB1R co-expression and alterations at presynaptic sites of the PFC, amygdala, hippocampus and cerebellar cortex of MAGL-/- and we applied an in vivo microdialysis method to assess amino acid contents in the mPFC of MAGL+/+ and MAGL-/- mice. Moreover, single-unit extracellular recordings were made from individual neurons in the mPFC to analyze the correct functioning of BLA-mPFC circuit in MAGL-/- . At the end, to assess if anxiety and depressive-like behaviors occur in these animals, we performed light/dark box test, tail suspension test and marble burying test.

Results: We found a widespread CB1R/β-arrestin2 co-expression in the mPFC, amygdala and hippocampus accompanied by impairment of ERK signaling and elevation of vesicular glutamate transporter (VGluT1), at CB1Rpositive excitatory terminals in the mPFC, or vesicular GABA transporter (VGAT), at CB1R-positive inhibitory terminals in the amygdala and hippocampus. The impairment of CB1R signaling in MAGL-/- mice enhanced the excitatory drive in the BLA-mPFC circuit which resulted in elevation of glutamate release in the mPFC interstitial fluid together with anxiety-like behavior assessed by the marble buring test.

Conclusions: Collectively, these data provide evidence for a β-arrestin2-mediated desensitization of CB1R, which likely impacts on the synaptic plasticity of brain circuits involved in emotional functions.

THE ENDOCANNABINOID SYSTEM IN THE ANTERIOR CINGULATE CORTEX PLAYS A ROLE IN THE TERMINATION OF FEAR-CONDITIONED ANALGESIA IN RATS

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Introduction: Fear-conditioned analgesia (FCA) is characterised by a profound suppression of pain upon reexposure to a conditioned aversive stimulus (Butler & Finn Prog Neurobiol. 2009; 88(3):184-202). The endocannabinoid system plays a key role in mediating FCA (Finn et al., Eur J Neurosci., 2004; 20, 848-852, Butler et al., Pain 2008; 140, 491–500). The medial prefrontal cortex (mPFC), of which the anterior cingulate cortex (ACC) is a subregion, has been implicated in FCA (Butler et al., Physiol Behav., 2011; 104, 1075-1081). The aim of the present study was to investigate the role of the endocannabinoid system in the ACC in FCA and fear in the presence of nociceptive tone in rats.

Methods: Guide cannulae were stereotaxically implanted into male Lister-Hooded rats (235-301g, n= 7-12), 1mm above the ACC under 2.5% isoflurane anaesthesia and rats allowed 5-7 days to recover. On the conditioning day, animals received footshock (10 x 1s, 0.4mA), while controls did not. 23.5 hours later, animals were briefly anaesthetised and received an intraplantar injection of formalin (2.5%) into the right hindpaw. 15 min later, rats received intra-ACC injection of vehicle (100% DMSO), URB597 (0.1mM/0.3μL DMSO), an inhibitor of the endocannabinoid-catabolising enzyme fatty acid amide hydrolase (FAAH), AM251 (2mM/0.3μL DMSO), a CB1 antagonist/inverse agonist, or a combination of URB597 + AM251 and were re-exposed to the arena in which they previously received footshock 15 minute later (24 hours post-footshock). Video footage was recorded for behavioural analysis. Rats were euthanized and brains harvested for histological verification of injector site placement. Data were analysed by ANOVA and Fisher's LSD post-hoc test ($p<0.05$ significant)

Results: Re-exposure to the context previously paired with footshock resulted in robust freezing behaviour in the first 10 minutes of the trial and reduced formalin-evoked nociceptive behaviour in the first 20 minutes of the trial, confirming the expression of FCA. Intra-ACC administration of AM251 increased the duration of freezing in the first 10 minutes of the trial and prolonged the expression of FCA towards the end of the trial, an effect that was not affected by co-administration of URB597. URB597 alone had no effect on freezing or FCA. Co-administration of AM251 and URB597 increased formalin-evoked nociceptive behaviour in non-fear conditioned animals in the first 10 minutes of the trial.

Conclusions: These findings suggest that blocking CB1 receptors in the ACC potentiates fear responding in the presence of nociceptive tone and prolongs FCA, suggesting a role for the endocannabinoid system in the ACC in the termination of FCA.

TLR3-INDUCED INFLAMMATORY RESPONSES IN THE HYPOTHALAMUS ARE ATTENUATED FOLLOWING FAAH, BUT NOT MAGL, INHIBITION; AN EFFECT INDEPENDENT OF SEX

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Introduction: Viral antigens are recognised by various toll-like receptors (TLRs) including TLR3, activation of which induces both systemic and central inflammatory responses to fight infection. Recent evidence has demonstrated that both synthetic cannabinoids such as WIN55212-2 and enhancing endogenous cannabinoid tone can modulate TLR3-induced inflammatory responses (*Downer et al., J. Biol. Chem. 2011; Henry et al., J. Neuroimmunol. 2014)*. However, it is unknown if sexual dimorphic effects exist in response to endocannabinoid modulation of TLR3-induced inflammatory responses. Thus, the aim of this study was to investigate if enhancing 2-AG or anandamide tone modulates TLR3-induced inflammatory responses and if these responses differ between males and females.

Methods: Male and Female Sprague-Dawley rats were systemically administered the MAGL inhibitor MJN110 (5mg/kg), or the FAAH inhibitor URB597 (1mg/kg), 1 hour or 30mins respectively, prior to the systemic administration of the TLR3 agonist poly I:C (3mg/kg). A further group received vehicle (ethanol:cremophor:saline; 1:1:18) followed 30min later by systemic poly I:C/saline administration. Animals were sacrificed 4hrs post-poly I:C/saline challenge, the spleen and hypothalamus excised and stored at -80°C. Concentrations of the endocannabinoids, anandamide and 2-AG, and the related N-acylethanolamines, N-oleoylethanolamine (OEA) and N-palmitoylethanolamine (PEA), were determined using LC-MS-MS. The expression of IFN and NFκB-inducible genes were determined using qRT-PCR. Data were analysed using One-way ANOVA followed by Fisher's LSD *post hoc* test. The level of significance was set at $P < 0.05$.

Results: Systemic administration of MJN110 increased 2-AG levels in the spleen and hypothalamus of both males and females; while URB597 increased anandamide, OEA and PEA levels. Poly I:C increased IP-10, IRF7 and TNF-α expression in the spleen of males and females; an effect not altered by MJN110 or URB597. In the hypothalamus, poly I:C increased IP-10, IRF7 and TNF-α expression in both male and female rats. URB597, but not MJN110, attenuated this poly I:C-induced increase in the hypothalamic expression of IRF7 and TNF-α expression in males and IP-10, IRF7 and TNF-α expression in females.

Summary and Conclusion: Enhancing 2-AG tone did not alter TLR3-induced inflammatory responses in the spleen or the hypothalamus of males or females. In contrast, increasing FAAH substrates, including anandamide, attenuated TLR3-induced inflammatory responses in the hypothalamus, but not the spleen, of male and female rats. These data support an important sex independent role of FAAH substrates in the modulation of TLR3-induced inflammatory responses in the hypothalamus, a key site involved in mediating the neuroendocrine and sickness response to infection.

PERIPHERAL INHIBITION OF FAAH ATTENUATES FORMALIN-EVOKED NOCICEPTIVE RESPONDING IN A MOUSE MODEL OF IFN-α-INDUCED HYPERALGESIA

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Introduction: Interferon-alpha (IFN-α) is a pro-inflammatory cytokine used to treat various cancers and infections. However, its use is associated with depression (Raison et al., CNS Drugs 2005;19(2):105-23) and painful symptoms (Shakoor et al., J Ayub Med Coll Abbottabad 2010;22(4):6-9). Studies in our lab have shown that repeated administration of IFN-α to mice results in depressive-like behaviour and enhanced nociceptive responding in the formalin test of persistent inflammatory pain. Anandamide (AEA) plays an important role in modulating emotional and nociceptive processing, however it is unknown if AEA tone is altered in response to chronic IFN-α administration. This study investigated if repeated IFN-α administration alters the expression of cannabinoid receptors, CB_1 and CB_2, levels of AEA or related *N*-acylethanolamines, *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA), in the descending pain pathway and paw tissue, in the presence and absence of inflammatory pain. The study also examined if inhibition of FAAH, the primary enzyme responsible for the metabolism of AEA, alters formalin-evoked nociceptive responding in this model.

Methods: Male C57Bl/6J mice were administered (h)IFN-α (Roferon-A: 8,000 IU/g s.c.) or saline daily for 8 days. 24 hours following the final treatment, a subset of animals were sacrificed and the periaqueductal gray (PAG), rostral ventromedial medulla (RVM), spinal cord and paw tissue dissected out, snap-frozen and stored at -80°C. A further subset received an intraplantar injection of formalin (1%; 20μl) into the left hind paw and nociceptive behaviour was recorded for 35 minutes, before sacrifice. In a separate experiment, IFN-α or saline treated animals received an intraplantar (1ug/10ul) or systemic (10mg/kg^{-1}) injection of the FAAH inhibitor PF-3845 or vehicle prior to formalin, and nociceptive behaviour was recorded for 60 minutes. Receptor expression was quantified using qRT-PCR and FAAH substrates with LC-MS-MS.

Results: IFN-α did not alter CB_1 or CB_2 receptor expression or AEA levels in the regions examined but reduced PEA levels in the spinal cord, when compared with saline-treated animals. IFN-α-treated mice exhibited enhanced late phase formalin-evoked nociceptive responding, associated with enhanced AEA levels in the RVM, an effect not observed in saline-treated counterparts. Formalin administration tended to increase FAAH substrate levels in the paw tissue of saline-treated animals, an effect not observed in IFN-α-treated. Systemic PF-3845 did not alter formalin-evoked nociceptive behaviour of saline- or IFN-α-treated mice. However, intraplantar PF-3845 reduced late phase formalin-evoked nociception and increased PEA and OEA levels in the paw tissue of IFN-α-treated animals, an effect not observed in saline-treated animals.

Conclusion: Repeated administration of IFN-α enhances nociceptive responding in the formalin test, an effect associated with impaired enhancement of FAAH substrate levels in the paw tissue. Enhancing FAAH substrate levels in the paw tissue, but not systemically, attenuated formalin-evoked hyperalgesia in IFN-α-treated mice. Taken together, these data highlight a possible role for peripheral N-ethanolamine(s) in mediating IFN-α-induced hyperalgesia.

ACTIVATION OF THE CB1 CANNABINOID RECEPTOR EVOKES CA^{2+} TRANSIENTS IN SPINAL ASTROCYTES BUT NOT IN C2C12 MYOTUBES AND SKELETAL MUSCLE FIBERS

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Introduction: Endocannabinoid signaling is one of the most abundant signaling mechanisms in the nervous system, and there are emerging evidences for its importance in other tissue types such as in skeletal muscle. The activation of cannabinoid-1 receptors (CB1Rs) activates G_i proteins in most cell types. However, it was also shown that in hippocampal astrocytes and in CB1R expressing HEK293 cells the activation of the receptor increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by a G_q protein and IP3-mediated way. We examined the possibility to release CB1-mediated Ca^{2+} transients in three model systems: astrocytes of the spinal cord, C2C12 myotubes and flexor digitorum brevis (FDB) skeletal muscle fibers.

Methods: 1 or 10 µM of different CB1-agonists were applied on the cells and changes in $[Ca^{2+}]_i$ were monitored by using the ratiometric fluorescent dye Fura-2. In astrocytes to determine the ratio of responding cells, confocal measurements were performed. In skeletal muscle cells the effects of the CB1 agonists and knockout of CB1R were tested on KCl-depolarization-evoked Ca^{2+} transients.

Results: In astrocytes all the tested drugs were capable of evoking Ca^{2+} transients. In cultures from wild-type mice 8±2% of the cells responded to WIN55,212 (WIN) and 18±3% responded to anandamide (AEA). Even in cultures derived from CB1-KO mice some cells responded to these drugs (3±1% and 5±1%, respectively) with smaller amplitudes.

In contrast, none of the examined agonists could evoke Ca^{2+} transients in C2C12 myotubes and in FDB fibers. Depolarization-evoked Ca^{2+} transients were significantly higher in FDB fibers isolated from CB1-KO mice (848±98 nM, n=47) compared to control (376±60 nM, n=32, p<0.01). On control FDB fibers the presence of WIN significantly reduced the amplitude of the KCl-evoked Ca^{2+} transients.

Conclusion: On the basis of our $[Ca^{2+}]_i$ measurements we can conclude that CB1R-mediated Ca^{2+} signaling is present in spinal astrocytes. However, the observed Ca^{2+} transients in CB1-KO cells raise the possibility of the presence of another, non-CB1-mediated endocannabinoid pathway in these cells. In contrast, in skeletal muscle CB1Rs did not evoke Ca^{2+} transients, but the activation of these receptors attenuated, while the absence of CB1Rs enhanced the KCl-depolarization-evoked Ca^{2+} transients.

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Program and Abstracts