



PROGRAM



***MNS MEETING 2015  
SANTA MARGHERITA DI PULA, 12-15 JUNE 2015***

ORGANIZED UNDER THE AUSPICES OF



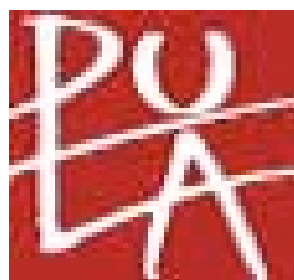
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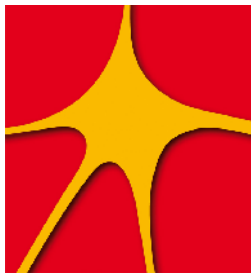
**Università degli Studi di Cagliari**



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CMST COST Action CM1103  
Structure-based drug design for all aspects and treatment of  
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function in the mammalian nervous system of the brain.



"A network for Improvement of cephalopod welfare  
and husbandry in research, aquaculture and fisheries"



Su biva biva cum sa 'entu' e su mari

## EXHIBITORS



**SANDOWN SCIENTIFIC**  
BEHAVIOURAL RESEARCH



charles river | every step  
of the way.



## LOCAL ORGANIZING COMMITTEE

Liana Fattore	(Cagliari)
Cristina Cadoni	(Cagliari)
Anna Carta	(Cagliari)
Marco Diana	(Sassari)
Miriam Melis	(Cagliari)
Annalisa Pinna	(Cagliari)
Valentina Valentini	(Cagliari)
Patrizia Campolongo	(Roma)
Carla Cannizzaro	(Palermo)
Paola Fadda	(Cagliari)
Patrizia Fattori	(Bologna)
Walter Fratta	(Cagliari)
Micaela Morelli	(Cagliari)

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Marc Landry	President Elect	France
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Hagai Bergman	Vice Secretary	Israel
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# **PROGRAM**

**Friday, June 12<sup>th</sup> 2015**

Santa Margherita di Pula, 12-15 June 2015

**Friday, June 12<sup>th</sup> 2015**

**13.00 - 19.00** Registration

Room: **NAUTILUS**

**13.45 - 14.00** MNS Presentation & Introduction to the Meeting

*Marie Mofteh, MNS President, Liana Fattore, President of the 2015 MNS Meeting*

**14.00 - 14.30** Presentation of COST ACTIONS

*Graziano Fiorito & Laura Della Corte*

**14.30-15.30** PLENARY LECTURE

**GAETANO DI CHIARA (Italy): Drugs of abuse as unconditioned homologs of conditioned rewards: the role of accumbens shell dopamine**

Host: Carla Cannizzaro

**15.30 - 17.15** SYMPOSIA

Room: **NAUTILUS**

**Neurocognitive function and emotion processing in psychopathology**

*Chairs: Adrianna Mendrek (Canada) & Walter Fratta (Italy)*

FLORINE DOLCOS (USA): "Neural mechanisms of emotion-cognition interactions in healthy functioning and disease: Evidence from brain imaging investigations".

ANDRIANNA MENDREK (Canada): "Functional cerebral connectivity in schizophrenia women during visuo-spatial processing".

ANGELA SIRIGU (France): "How oxytocin modulates the human brain and behavior".

HELENE POISSANT (Canada): "Transmission of fronto-parietal dysfunction during forethought in families with attention deficit/hyperactivity disorder".

Room **CYPREA****Emerging concepts in dopaminergic system development and regulation***Chairs: Stefano Espinoza (Italy) & Małgorzata Filip (Poland)*

ELIA DI SCHIAVI (Italy): “Genetic characterization of the dopamine system in the animal model *C. elegans*”

GIUSEPPE RONZITTI (France): “HDAC inhibition reduces dopamine-dependent neurodegeneration in a mouse model of DAT-deficiency syndrome”

DAMIANA LEO (Italy): “Pronounced dopaminergic dysregulation in dopamine transporter knock out rats”

DAVID MORENO (France): “Targeting dopamine D1-histamine H3 receptor heteromers reverts learning and long-term memory deficits in a mouse model of Huntington’s disease”.

MASSIMILIANO CAIAZZO (Italy): “Direct reprogramming of fibroblasts into functional dopaminergic neurons”.

Room **ASTREA****Glia in the nervous system: From housekeeping to processing functions***Chairs: Stéphane Oliet (France) & Anna R. Carta (Italy)*

STEPHANE OLIENT (France): “Surface dynamics of GLT-1 on astrocytes shapes excitatory transmission”.

STEPHEN D. SKAPER (Italy): “Co-ultramicrosized palmitoylethanolamide/luteolin promotes maturation of rat cortical oligodendrocytes in vitro”.

NATHALIE ROUACH (France): “Connexin 30: unconventional determinant of astroglial synapse coverage and memory”.

ESTEE KURANT (Israel): “Keeping the CNS clear: Glial phagocytosis of neuronal debris”.

ARNAU BUSQUETS-GARCIA (France): “Involvement of astrocytic CB1 receptors in synaptic plasticity and hippocampal-based memory”.

**Friday, June 12<sup>th</sup> 2015**

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Room **ALVANIA**

**Neurobiology of food intake regulation**

*Chairs: Carole Rovere (France) & Mohamed Najimi (Morocco)*

SERGE LUQUET (France): “Dietary triglycerides act on mesolimbic structures to regulate the rewarding and motivational aspects of feeding”.

ETIENNE CHALLET (France): “Timed feeding: impact on circadian clocks and metabolism”.

MOHAMMED ERRAMI (Morocco): “Molecular mechanisms underlying cannabinoids action on hypothalamic neuropeptide neurons”.

CAROLE ROVERE (France): “Role of chemokines in neuroinflammation and feeding behavior”.

NICOLAS CHARTREL (France): “26RFa, a novel neuropeptide regulating feeding behavior and glucose homeostasis”.

**17.15 - 17.45** Coffee break

**17.45 - 19.30** SYMPOSIA

Room **NAUTILUS**

**Depression, anxiety and addiction: is it all converge to impaired dopaminergic function?**

*Chairs: Rami Yaka (Israel) & Barry J. Everitt (UK)*

CHRISTINA DALLA (Greece) : “Sex differences in models of depression: is it all about serotonin?”

MIRIAM MELIS (Italy): “Effects of in utero exposure to cannabinoids on postnatal synaptic maturation of glutamatergic transmission in the VTA “.

FRANCOIS GEORGES (France): “In vivo homeostatic plasticity at the single-cell level in the BNST triggers persistent anxiolytic effect”.

RAMI YAKA (Israel): “Targeting drug-cue associations to prevent drug relapse”.

MARK UNGLESS (UK): “Functional diversity of midbrain dopamine neurons”.

**Friday, June 12<sup>th</sup> 2015**

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Room **CYPREA**

**Parkinson's disease - from neuroprotection to the treatment of motor and non-motor symptoms**

*Chairs: Gilberto Fisone (Sweden) & Moussa Youdim (Israel)*

ANNA CARTA (Italy): "Microgliosis in Parkinson's disease: can we harness it for good?"

NEVILLE VASSALLO (Malta): "Amyloid-lipid membrane interaction: a novel target for neurodegeneration".

RICCARDO BRAMBILLA (Italy): "Intracellular signalling mechanisms controlling L-DOPA induced Dyskinesia: a translational neuroscience perspective".

GILBERTO FISONE (Sweden): "Cognitive and affective dysfunctions in experimental Parkinsonism: modeling and mechanisms".

ROSARIO MORATALLA (Spain): "Activation of DREAM, a calcium-binding protein, reduces L-DOPA- induced dyskinesias in mice".

Room **ASTREA**

**Neuronal plasticity in health and disease: cognitive and clinical perspectives**

*Chairs: Said Boujraf (Morocco) & Driss Boussaoud (France)*

JULIEN DOYON (Canada): "Neural and physiological correlates of motor learning and consolidation".

HABIB BENALI (France): "Longitudinal functional network analysis for motor skill learning in humans".

MOHAMMED BENZAGMOUT (Morocco): "Brain plasticity induced by hemodialysis: neuroanatomical and BOLD-fMRI study".

DRISS BOUSSAOU (France): "Social learning: from behavior to neurons".

YVES JOANETTE (Canada): "Brain, communication and aging: a life-long adaptive process sustaining quality of life".

**Friday, June 12<sup>th</sup> 2015**

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Room **ALVANIA**

**Genes do matter: insights in the genetic vulnerability to nicotine addiction**

*Chairs: Cristina Cadoni (Italy) & Nataliè Thiriet (France)*

LAURIANE HARRINGTON (France): “The role of nicotinic acetylcholine receptor subunits in nicotine addiction”.

CRISTINA CADONI (Italy): “Strain differences in the long lasting effect of adolescent nicotine exposure on mesolimbic dopamine transmission of Lewis and Fischer 344 rats”.

JENNIFER J. WARE (UK): “Using biomarkers of tobacco exposure in genome-wide association studies”.

MORGANE BESSON (France): “The rs16969968 polymorphism predisposing to smoking confers midbrain- and hippocampus-dependent affective impairments that are alleviated by nicotine”.

**19.30 - 20.30**

**WELCOME COCKTAIL**

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## Saturday, June 13<sup>th</sup> 2015

Room **NAUTILUS**

### 8.30 - 9.30 PLENARY LECTURE

#### IDAN SEGEV – “The Human Brain Project: from Mouse to Man”

Host: Marco Diana

### 9.30 - 11.15 SYMPOSIA

Room **NAUTILUS**

#### **Interaction between dopaminergic and non-dopaminergic neurotransmission on Parkinson's disease: implication in therapy, dyskinesia and neuroprotection**

*Chairs: Marie-Therese Armentero (Italia) & Rosario Moratalla (Spain)*

ANNALISA PINNA (Italy): “Novel therapeutic strategy for the control of dyskinesia in the therapy of Parkinson's disease: acute and chronic studies”.

MARIANNE AMALRIC (France): “Bee venom and apamin, a small conductance calcium-activated potassium channel blocker, are potential new therapeutic targets for Parkinson's disease”.

RODRIGO A. CUNHA (Portugal): “ATP and adenosine modulation of striatal plasticity and Parkinson's disease”.

MARIE-THERESE ARMENTERO (Italy): “Neuroprotective and symptomatic potential of combined non- dopaminergic therapy in Parkinson's disease”.

KRYSTYNA GOLEMBIOWSKA (Poland): “The antioxidative and anti-inflammatory effects of adenosine A2A receptor antagonist as a new therapy of PD”.

**Saturday, June 13<sup>th</sup> 2015**

Santa Margherita di Pula, 12-15 June 2015

Room **CYPREA**

**Serotonin interaction with other neurotransmitters**

*Chairs: Giuseppe Di Giovanni (Malta) & Philippe De Deurwaerdère (France)*

PHILIPPE DE DEURWAERDERE (France): “A closer look at the pivotal role of serotonergic neurons in the mechanism of action of L-DOPA”.

GIUSEPPE DI GIOVANNI (Malta): “5-HT<sub>2C</sub> receptor control of generalized and focal seizures”.

MASSIMO PIERUCCI (Malta): “Role of the lateral habenula in nicotine addiction“. NELA PIVAC (Croatia): “Serotonin and dopamine in sleep disturbances in PTSD”.

FRANCESCO CRESPI (Italy): “Brain serotonin and nitric oxide (NO): linked in antidepressant activities? A voltammetry in vivo study with SSRI fluoxetine “.

Room **ASTREA**

**The GABA-B receptor: from molecular to behavioral regulation**

*Chairs: Maria Paola Castelli (Italy) & Bernhard Bettler (Switzerland)*

BERNHARD BETTLER (Switzerland) : “Deconstructing GABAB receptors”.

PHILIPPE RONDARD (France): “GABAB receptor complexes, crosstalk with other receptors “.

ALESSANDRA PORCU (Italy): “Thiophene derivatives: new allosteric modulators of the GABAB receptor”.

MALGORZATA FRANKOWSKA (Poland): “The role of GABAB receptors in substance use disorders”.

**Saturday, June 13<sup>th</sup> 2015**

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Room **ALVANIA****New horizons in nutrition, brain function and behavior***Chairs: Youssef Aboussaleh (Morocco) & Sebastiano Banni (Italy)*

YOUSSEF ABOUSSALEH (Morocco): “Nutritional backgrounds of memory loss in degenerative disease”.

MEHA FATIMA AFTAB (Pakistan): “Development of diabetes associated Alzheimer’s disease model in BALB/C mice”.

IMANE ACHOURI (Morocco): “Iron deficiency anaemia and cognitive performance of school children in Morocco”.

SEDA AISE ARTIS (Turkey): “Is Omega-3 rich diet a plausible alternative treatment for Alzheimer's Disease: A friend or foe?”.

MARIA SCHERMA (Italy): “The endocannabinoid system: possible role in the etiology of anorexia nervosa”.

**11.15 - 11.45** Coffee break

**Saturday, June 13<sup>th</sup> 2015**

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**11.45 - 13.15 SYMPOSIA**

Room **NAUTILUS**

**Novel Therapeutic Approaches to Neuropsychiatric Disorders**

*Chairs: John Bruno (USA) & Valentina Valentini (Italy)*

MARCO PISTIS (Italy): “Targeting the interaction between PPAR $\alpha$  and nicotinic receptors”.

JOHN BRUNO (USA): “Alpha7 nicotinic-glutamatergic interactions in prefrontal cortex: implications for cognition enhancement in schizophrenia”.

ASA KONRADSSON-GEUKEN (Sweden): “Age-dependent effects of ethanol on glutamatergic dynamics in the prefrontal cortex of freely moving rats using microelectrode amperometry”.

GIANLUIGI TANDA (USA): “The DAT, new therapeutic perspectives for an old neuropsychiatric target”.

Room **CYPREA**

**Novel approaches in treating cognitive deficits that accompany stress- and fear-related disorders**

*Chairs: Irit Akirav (Israel) & Viviana Trezza (Italy)*

IRIT AKIRAV (Israel): “Cannabinoids prevent the effects of an emotional trauma on extinction and plasticity”.

NICOLAS SINGEWALD (Austria): “Deficient fear extinction learning: novel treatment strategies”.

PATRIZIA CAMPOLONGO (Italy): “Stress and endocannabinoid modulation of aversive memory”.

JOZSEF HALLER (Hungary): “Coping, learning and cannabinoid signaling”.

**Saturday, June 13<sup>th</sup> 2015**

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Room **ASTREA**

**Exploring and treating the epileptic brain**

*Chairs: Anna Lisa Muntoni (Italy) & Merab Kokaia (Sweden)*

CHRISTOPHE BERNARD (France): “Novel diagnostic tools in epilepsy”.

ALON FRIEDMAN (Israel): “Blood-brain barrier dysfunctions as biomarker and target in the prevention of acquired epilepsy”.

ANNAMARIA VEZZANI (Italy): “Inflammatory targets in epilepsy treatment “.

MERAB KOKAIA (Sweden): “Optogenetic tools to study and treat epilepsy“.

ANNA LISA MUNTONI (Italy): “Targeting PPAR-alpha receptors in epilepsy”.

Room **ALVANIA**

**Novel methodologies for the analysis of drugs of abuse and endogenous biomarkers**

*Chairs: Umberto Spampinato (France) & Giorgio Pintore (Italy)*

GREGORY W. ENDRES (USA): “Designer drugs: detection, confirmation, and response”.

AMY PATTON (USA) : “Determination of metabolites and biomarkers utilizing mass spectral analysis”.

KATHRYN A. SEELY (USA): “Bedside to bench: how clinical analytical chemistry guides hypothesis- based drugs of abuse research “.

MARTIN EYSBERG (The Netherlands): “Analysis of DA and 5-HT under 2 min to further improve time resolution in on-line microdialysis”.

**13.30 – 14.30** Lunch

**Saturday, June 13<sup>th</sup> 2015**

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Room **NAUTILUS**

**15.00 - 16.00 PLENARY LECTURE**

**BARRY J EVERITT** - “Neural mechanisms underlying the development of compulsive drug seeking habits in addiction”

Host: Liana Fattore

**16.00 - 17.30 SYMPOSIA**

Room **NAUTILUS**

**How dopamine talks to the world-through the brain**

*Chairs: Hagai Bergman (Israel) & Suzanne Haber (USA)*

**SUZANNE N. HABER (USA)**: “The circuitry that underlies dopamine’s role in disease “.

**PAOLO ENRICO (Italy)**: “Ventral tegmental area dopamine neurons in opiate withdrawal: a computational perspective”.

**JOHN D. SALAMONE (USA)**: “Identification of a dopaminergic component of effort-related motivational symptoms in psychopathology: studies with animal models”.

**HAGAI BERGMAN (Israel)**: “Computational physiology of the basal ganglia and their disorders – from understanding to treatment”.

**Saturday, June 13<sup>th</sup> 2015**

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Room **CYPREA**

**Endocannabinoids and related endogenous lipid signaling molecules**

*Chairs: Stephen D. Skaper (Italy) & Vincenzo Di Marzo (Italy)*

SILVIA PONTIS (Italy): “Role of N-acyl ethanolamine-hydrolyzing acid amidase in (neuro)inflammation”.

FABIO IANNOTTI (Italy): “The non-psychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability “.

EVA DE LAGO (Spain): “Development of cannabinoid-based therapies for amyotrophic lateral sclerosis/frontotemporal dementia”.

LIVIO LUONGO (Italy): “Palmitoylethanolamide chronic treatment reduces sensory and cognitive dysfunction associated with mild traumatic brain injury”.

Room **ASTREA**

**Emerging drugs for treating alcohol use disorders: preclinical evidence**

*Chairs: Giancarlo Colombo (Italy) & Mercè Correa (Spain)*

ANA POLACHE (Spain): “Ethanol and metabolism: is it a target to develop new pharmacotherapies for alcoholism treatment?”.

MICKAEL NAASSILA (France): “Histone deacetylase inhibition as a potential therapeutic intervention to reduce alcohol intake and relapse”.

MIA ERICSON (Sweden): “Nucleus accumbens glycine receptors – a potential target for new treatment of alcohol dependence”.

GIANCARLO COLOMBO (Italy): “Preclinical evidence on the “anti-alcohol” properties of the positive allosteric modulators of the GABAB receptor “.

**Saturday, June 13<sup>th</sup> 2015**

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Room **ALVANIA**

**Multiple sclerosis: patient quality of life, physiopathology and therapeutic aspects**

*Chairs: Samir Ahboucha (Morocco) & Micaela Morelli (Italy)*

SAMIR AHBOUCHA (Morocco): “Multiple sclerosis as growing neurological disease: a historical and descriptive overview”.

MARKUS KIPP (Germany): “New insights into multiple sclerosis lesion formation”.

TIM CLARNER (Germany): “Myelin debris and inflammatory responses in MS: role of astrocytes”.

KLAUS G PETRY (France): “Combined molecular biology and bioinformatics tool developments for efficient peptide biomarker discovery in multiple sclerosis neurodegeneration”.

**17.30 - 18.30 POSTER SESSION I**

Wine & Cheese – Coffee break

**18.30 - 19.30 MNS General Assembly**

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## Sunday, June 14<sup>th</sup> 2015

Room **NAUTILUS**

### 8.30 – 9.30 PLENARY LECTURE

#### **CARLOS BELMONTE – “TRP Channels: A System for Early Detection of Environmental Menaces”.**

Host: Micaela Morelli

### 9.30 - 11.15 SYMPOSIA

Room **NAUTILUS**

#### **Neural circuits underlying motivated behaviors in physiology and disease**

*Chairs: Manuel Mameli (France) & Miriam Melis (Italy)*

RUI M. COSTA (Portugal): “Learning novel actions and shifting to automatic”.

RAFFAELLA TONINI (Italy): “Plasticity of corticostriatal endocannabinoid signaling in habit learning “.

ROSARIO MORATALLA (Spain): “Methamphetamine induces dopamine neurons degeneration evidenced by silver staining and causes motor defects in mice”.

ROGER A.H. ADAN (The Netherlands): “Combined use of the canine adenovirus-2 and DREADD- technology to activate specific neural pathways in vivo “.

MANUEL MAMELI (France): “Cellular substrates underlying aversive states “.

**Sunday, June 14<sup>th</sup> 2015**

Room **CYPREA**

### **K<sup>+</sup> Channels: structural features, physiological roles and channelopathies**

*Chairs: Mauro Pessia (Italy) & Stephen J. Tucker (UK)*

STEPHEN J. TUCKER (UK): “A structural and biophysical mechanism for polymodal gating of the TREK- 2 K2P channel”.

LUIGI CATACUZZENO (Italy): “Overexpression of large-conductance calcium-activated K channels in human glioblastoma stem-like cells and their role in the hypoxia-induced glioblastoma cell migration”.

PAOLA PEDARZANI (UK): “Multiple signalling roles of calcium-dependent K<sup>+</sup> channels in central neurons”.

MARIA C. D’ADAMO (Italy): “Genetically induced dysfunction of KIR2.1 channels: implications for short QT3 syndrome and autism-epilepsy phenotype “.

ANDREA H. NÉMETH (UK): “The role of the voltage gated potassium channel Kv3.3 in developmental disorders of the nervous system”.

Room **ASTREA**

### **Reward sensitivity from adolescence to adulthood**

*Chairs: Patrizia Campolongo (Italy) & Heidi M.B. Lesscher (The Netherlands)*

ERIKA ROMAN (Sweden): “Adolescent and adult behavioral profiles and neurobiology of relevance for voluntary alcohol intake in rats “.

VIVIANA TREZZA (Italy): “Rewarding value and neurobiological mechanisms of social play behavior in rats”.

HEIDI LESSCHER (The Netherlands) : “Social play deprivation in adolescence enhances alcohol consumption in adulthood“.

JEROME JEANBLANC (France): “Alcohol intake during adolescence induces alcohol addiction phenotypes in two animal models of schizophrenia “.

EVA MARIA MARCO (Spain): “Factors of vulnerability in alcohol addiction throughout life span”.

**Sunday, June 14<sup>th</sup> 2015**

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Room **ALVANIA**

**Innovative strategies for rescuing tissue injury induced by stroke**

*Chairs: Lucio Annunziato (Italy) & Ali Jahanshahi (The Netherlands)*

LUCIO ANNUNZIATO (Italy). “NCX expression and activity in microglia and oligodendrocytes after stroke”.

MAJED ALDEHRI (The Netherlands). “Cortical stimulation to enhance recovery after (experimental) stroke: a systematic review of neurobiological mechanisms”.

MILED BOUROUROU (France): “Is supplementation with alpha linolenic acid a preconditioning mechanism preventing mortality and cerebral damage, and improving motor and cognitive recovery post- stroke?”.

GIUSEPPE PIGNATARO (Italy): “Ionic homeostasis dysregulation and brain conditioning”.

**11.15 - 11.45** Coffee break

**Sunday, June 14<sup>th</sup> 2015**

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**11.45 - 13.15 SYMPOSIA**

Room **NAUTILUS**

**Drug addiction and epigenetic mechanisms**

*Chairs: Nathalie Thiriet (France) & Claudio D'Addario (Italy)*

JEAN ZWILLER (France): "Cocaine and epigenetic - effect of cocaine self-administered by rats on DNA methylation: from behavior to genes".

CLAUDIO D'ADDARIO (Italy): "Genetic association between variations in prodynorpin gene and alcoholism points up epigenetic regulation of gene activity".

VENETIA ZACHARIOU (USA): "HDAC5 modulates the actions of opiate and antidepressant drugs used for the treatment of chronic pain".

MAURIZIO GIUSTETTO (Italy): "Histone modifications and protein synthesis induced by morphine withdrawal".

Room **CYPREA**

**Parkinson's disease beyond the shaking palsy: the non-motor symptoms**

*Chairs: Abdelhamid Benazzou (France) & Ali Jahanshahi (The Netherlands)*

ALBERT LEENTJENS (The Netherlands): "Clinical psychiatric aspects of Parkinson's disease".

HAGAI BERGMAN (Israel): "The non-motor properties of the basal ganglia".

ABDELHAMID BENAZZOUZ (France): "The respective role of monoamines in the pathophysiology and therapy of Parkinson's disease".

YASIN TEMEL (The Netherlands): "The subthalamic nucleus-dorsal raphe nucleus link: a novel motor- limbic interface in the context of mood disorders in PD".

**Sunday, June 14<sup>th</sup> 2015**

Room **ASTREA**

**HOT TOPICS SESSION**

*Chairs: John Bruno (USA) & Liana Fattore (Italy)*

SIMONA CABIB (Italy): “Chronic stress fosters compulsion and D2R-dependent resistance to helplessness in genetically impulsive mice”.

GRAZIANO PINNA (USA): “Targeting neurosteroid biosynthesis for the treatment of stress-induced fear and anxiety”.

ANA INÉS ANSALDO (Canada): “Ageing, late bilingualism and neuroplasticity: Is it never too late to rewire the brain?”

PATRIZIA FATTORI (Italy): “Reaching a target in depth: a trend in superior parietal lobe”

ANDREW GUNDLACH (Australia): “DREADDing the uncertain’: Nucleus incertus network activation or silencing alters behavioural state – impact on arousal and spatial memory”.

Room **ALVANIA**

**From circadian clock to human health - PART I. Circadian clocks as an interface between the physiological processes and the environment**

*Chairs: Paul Pevet (France) & Nouria Lakhdar Ghazal (Morocco)*

JOHANNA H MEIJER (The Netherlands): “The effect of light on the circadian system”.

KHALID EL ALLALI (Morocco): “Ambient temperature cycle as a zeitgeber in desertic mammals”.

ETIENNE CHALLET (France): “Food intake, circadian clocks and metabolic disorders”.

HUGH D. PIGGINS (UK): “Scheduled voluntary exercise promotes circadian rhythms in neurons and behaviour”.

**Sunday, June 14<sup>th</sup> 2015**

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**13.30 – 14.30** Lunch

Room **NAUTILUS**

15.00 – 16.00 PLENARY LECTURE

**RENÉ HEN – “Hippocampal Neurogenesis: Impact on Mood and Cognition”.**

Host: Driss Boussaoud

16.00 - 17.45 SYMPOSIA

Room **NAUTILUS**

**New animal models of drug addiction: behavioral and neurobiological perspectives**

*Chairs: Marco Diana (Italy) & Veronique Deroche-Gamonet (France)*

SERGE AHMED (France): “Neuronal correlates of cocaine choice and addiction: focus on the orbitofrontal cortex“.

HEIDI LESSCHER (The Netherlands): “Compulsive cocaine and alcohol use and its limbic corticostriatal substrates”.

VERONIQUE DEROCHE-GAMONET (France): “Individual-based approach to the neurobiology of transition to cocaine addiction: focus on corticostriatal synaptic plasticity”.

ABRAHAM ZANGEN (Israel): “Repeated stimulation of the infralimbic cortex reduces cue-induced relapse to cocaine in the rat conflict model“.

NATHAN MARCHANT (USA): “Context-induced relapse to alcohol seeking after punishment-imposed abstinence: behavioural and neuronal mechanisms“.

**Sunday, June 14<sup>th</sup> 2015**

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Room **CYPREA**

**Ethanol and its metabolites: actions on dopamine, adenosine, opioid and endocannabinoid systems**

*Chairs: John Salamone (USA) & Giancarlo Colombo (Italy)*

MERCE' CORREA (Spain): "Adenosine as a potential neural modulator of some effects of ethanol and acetate on behavior".

ELIO ACQUAS (Italy): "Role of acetaldehyde, dopamine and salsolinol in the effects of ethanol".

LUCIA MARTÍ-PRATS (Spain): "Evaluation of the complex effects of ethanol and acetaldehyde on the activity of dopamine neurons in the posterior VTA: behavioral implications".

CARLA CANNIZZARO (Italy): "Role of the endocannabinoid system in the regulation of acetaldehyde motivational properties".

Room **ASTREA**

**Ultrasonic vocalizations in rodents: a tool for the investigation of psychoactive drugs and neuropsychiatric conditions**

*Chairs: Nicola Simola (Italy) & Viviana Trezza (Italy)*

NICOLA SIMOLA (Italy): "Similarities and differences in the effects of psychoactive drugs on the emission of 50-kHz ultrasonic vocalizations in the rat and role of non-dopaminergic receptors".

EWA TARACHA (Poland): "Rat 50-kHz ultrasonic vocalizations as a tool for the study of drug dependence: the example of amphetamine".

RAINER K.W. SCHWARTING (Germany): "Rat 50-kHz ultrasonic vocalizations as a tool for the study of anxiety and depression".

MICHELLE R. CIUCCI (USA): "Ultrasonic vocalization deficits in PINK1 knockout model of Parkinson's disease".

**Sunday, June 14<sup>th</sup> 2015**

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Room **ALVANIA**

**Neurobehavioral correlates of REM sleep disturbances**

Chairs: Franco Marrosu (Italy) & Marco Bortolato (USA)

PIERRE-HERVE' LUPPI (France): "Neuro-circuits controlling muscle atonia during paradoxical (REM) sleep and their role in RBD".

FRANCO MARROSU (Italy): "Effects of dopaminergic therapy on REM Sleep Behaviour Disorder in Parkinson's disease patients".

MONICA PULIGHEDDU (Italy): "Neuropsychiatric features associated with REM behavior disorder in Parkinson's disease patients".

MARCO BORTOLATO (USA): "Neurosteroids mediate the behavioral changes induced by REM sleep deprivation".

**17.45 - 18.45 POSTER SESSION II**

Wine & Cheese – Coffee break

**20.30 SOCIAL DINNER**

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## Monday, June 15<sup>th</sup> 2015

Room **NAUTILUS**

8.30 – 9.30 PLENARY LECTURE

ABDELJABBAR EL MANIRA – **“Neural circuits for Motor Behavior”**.

Host: Marc Landry

9.30 - 11.00 SYMPOSIA

Room **NAUTILUS**

### **Basal Ganglia microcircuits in action: from synapses to behavior**

*Chairs: Gilad Silberberg (Sweden) & Joshua Goldberg (USA)*

DANA COHEN (Israel): “Parallel information processing in striatal networks”.

RUI M. COSTA (Portugal): “Generating and shaping novel action repertoires”.

JOSHUA A. GOLDBERG (Israel): “Striatal cholinergic interneurons in Huntington’s disease”.

DAVID ROBBE (France): “The striatum constrains the execution of motor habits through continuous integration of contextual and kinematic information”.

GILAD SILBERBERG (Sweden): “Microcircuits underlying sensory integration in the striatum”.

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Room **CYPREA**

**From circadian clock to human health – PART II. Circadian clocks: keys for health**

*Chairs: Paul Pevet (France) & Monica Puligheddu (Italy)*

ANDRIES KALSBECK (The Netherlands): “Brain areas and pathways in the regulation of glucose metabolism”.

DEBRA J SKENE (UK): “Melatonin and circadian rhythm disruption in disease”.

DICK F. SWAAB (The Netherlands): “The human SCN in health and neuropsychiatric disorders: post- mortem observations”.

BEATRICE GUARDIOLA-LEMAÎTRE (France): “Circadian rhythms: a key for innovative therapeutics”.

Room **ASTREA**

**Personal genomics in psychiatric disorders**

*Chairs: Alessio Squassina (Italy) & Rady Ahmed (Egypt)*

GEORGE P. PATRINOS (Greece): “Development of an electronic pharmacogenomics assistant with emphasis to neuropsychiatric disorders”.

GAL HACHOHEN KLEIMAN (Israel): “Autophagy plays a key role in schizophrenia and beyond”.

GALILA AGAM (Israel): “A microarray and proteomics study of lithium-treated mice and knockout mice with lithium-like behavior reveals a common effect on mitochondrial function and autophagy – two potential targets for personalized medicine”.

ALESSIO SQUASSINA (Italy): “Mechanism of action of lithium: what have we learned from in-vitro studies in human-derived cell lines?”.



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Room **ALVANIA**

### **Neurolinguistics of Semitic languages**

*Chairs: Mireille Besson (France) & Nouria Laghdar-Ghazal (Morocco)*

SAID BOUIJRAF (Morocco): “Aspects of arabic representation in the brain of native speakers: BOLD-fMRI study”.

AHMED AHAMI (Morocco): “Different levels of deficits in children with dyslexia as revealed by LABEL”.

SAMI BOUDELAA (United Arab Emirates): “The effects of multiple sclerosis on morphological priming”.

MIREILLE BESSON (France): “Vowelling and semantic priming effects in Arabic”.

**11.00 - 11.30** Coffee break

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11.30 - 13.15 SYMPOSIA

Room **NAUTILUS**

**Monoamines in neuropsychiatric disorders**

*Chairs: Giuseppe Di Giovanni (Malta) & Marco Bortolato (USA)*

DUBRAVKA SVOB STRAC (Croatia): “Serotonin and side effects of antipsychotics”.

MARCO BORTOLATO (USA): “Role of serotonergic neurotransmission in the ontogeny of reactive aggression: focus on gene x environment interactions”.

ROBERTO FRAU (Italy): “Unraveling the contribution of dopamine receptor subtypes to the antipsychotic- like effects of 5 $\alpha$ -reductase inhibitors”.

EVGENI PONIMASKIN (Germany): “Role of 5-HT1A receptor palmitoylation in development and maintaining of depression”.

MARCELLO LEOPOLDO (Italy): “Role of 5-HT7 receptor in psychiatric disorders”.

Room **CYPREA**

**The juvenile brain: vulnerable period to the effects of stress and diet on neural plasticity and memory**

*Chairs: Guillaume Ferreira (France) & Irit Akirav (Israel)*

KATHARINA BRAUN (Germany): “Perinatal emotional experience interferes with the functional maturation of prefronto-limbic circuits: risk or benefit?”.

MOUNA MAROUN (Israel): “Stress differentially regulates prefrontal cortex plasticity and extinction in the juvenile and adult animals”.

NURIA DEL OLMO (Spain): “High-fats diets and behavioural disorders in adolescent mice: focusing on the role of hippocampus and prefrontal cortex”.

GUILLAUME FERREIRA (France): “Differential effects of juvenile and adult exposure to obesogenic diet on amygdala- dependent emotional memory: special emphasis on glucocorticoids

BERRİN ZUHAL ALTUNKAYNAK (Turkey): “Effects of different doses diclofenac sodium on developmental features of hippocampus in rats: a histopathological and stereological analysis”.

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Room **ASTREA**

**Neurobiology of pain**

*Chairs: Marc Landry (France) & Jenny M. Gunnensen (Australia) & Jacques Noel (France)*

JACQUES NOEL (France): “Role of the TREK potassium channels in cold and warm thermosensation and in pain perception”.

JENNY M. GUNNERSEN (Australia): “Sez6 is a neuropathic pain modulator and novel binding partner of  $\alpha 2-\delta$ ”.

ALEXANDRE FAVEREAUX (France): “Understanding microRNAs in pain mechanisms”.

DAVID BELIN (UK): “Interplay between pain and inhibitory control: relevance for impulsive / compulsive neuropsychiatric disorders”.

CLAIRE GAVERIAUX-RUFF (France): “Opioid receptors roles in pain: advances from genetic and pharmacology approaches”.

Room **ALVANIA**

**Joint Symposium with COST Action FA1301– CephInAction meets MNS2015**

*Chairs: Ildiko Kemenes (UK), Graziano Fiorito (Italy)*

LINDY HOLDEN-DYE (UK): “What does the future hold for invertebrate neuroscience?”

TAL SHOMRAT (Israel): "Conservation and convergence in the evolution of the octopus neural system mediating learning and memory"

SHUICHI SHIGENO (Japan): “Identifying molecular and connectivity architecture shared in mammalian and octopus brains”.

GIOVANNA PONTE (Italy): “Octopamine in Octopus brain: a long history of mapping a ‘neglected’ neuromodulator”.

BENNY HOCHNER (Israel): “Octopus motor control as inspiration for soft robotics”.

**13.30 – 14.30** Lunch

**Monday, June 15<sup>th</sup> 2015**

**15.00 - 16.45 SYMPOSIA**

Room **NAUTILUS**

**5-HT receptors and neuropsychiatric disorders: new pharmacological targets for old diseases**

*Chairs: Umberto Spampinato (France) & Małgorzata Filip (Poland)*

VÉRONIQUE SGAMBATO-FAURE (France): “Neurochemical and behavioral impact of a serotonergic lesion in the non-human primate”.

ANDREW C. McCREARY (The Netherlands): “5-HT<sub>1A</sub> receptor agonists for the treatment of L-dopa- induced dyskinesia in Parkinson’s disease: biased and mixed agonist actions, a translational perspective”.

CELINE DEVROYE (France): “Serotonin<sub>2B</sub> receptor-dopamine interaction: new opportunities for improved treatments of schizophrenia”.

MALGORZATA FILIP (Poland): “5-HT<sub>2C</sub> receptor control over substance use disorders”.

NASSER HADDJERI (France): “Serotonin<sub>7</sub> receptor antagonism as a fast acting antidepressant strategy”.

Room **CYPREA**

**Targeting phosphodiesterases for the treatment of brain disorders**

*Chairs: Gretchen L. Snyder (USA) & Raffaella Tonini (Italy)*

ANTHONY R. WEST (USA): “Impact of phosphodiesterase 9A inhibition on aberrant cortically-evoked spike activity in the striatum of rodent models of Huntington’s disease”.

EAMONN SHERIDAN (UK): “Characterization of mutations in PDE10A which lead to a hyperkinetic movement disorder in humans”.

FRANCESCA R. FUSCO (Italy): “Phosphodiesterase inhibitors as a therapeutic approach to Huntington’s disease”.

JOS PRICKAERTS (The Netherlands): “PDE4 as a target for cognition enhancement”.

GRETCHEN L. SNYDER (USA): “Preclinical profile of PDE1 Inhibitors for cognition enhancement”.

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Room **ASTREA**

**Multi-target compounds in neurodegenerative diseases**

Joint Symposium with COST CM1103

*Chairs: Massimo Valoti (Italy) & Rona Ramsey (UK)*

MOUSSA B.H. YODIM (Israel): “Designed multi target drugs targeting neuroprotection, neurorestoration and mitochondrial biogenesis via activation of HIF1 $\alpha$ , SIRT1, PGC-1 $\alpha$  and TFAM”.

JOSÈ MARCO-CONTELLES (Spain): “Multipotent drugs based on Donepezil for the potential treatment of Alzheimer’s disease”.

MERCEDES UNZETA (Spain): “New multifunctional metal-chelators molecules based on Donepezil for its potential use in Alzheimer’s disease therapy”.

HOLGER STARK (Germany): “Multiple targeting with histamine H3 receptor antagonists”.

Room **ALVANIA**

**ISIS – NEUREN Meeting**

**16.45 - 17.15** Coffee break

**Monday, June 15<sup>th</sup> 2015**

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Room **ALVANIA**:

**17.00-19.00** EARLY STAGE RESEARCHER TALKS

**WG Meeting**

**17.15-19.00** Oral Free Communications

Room **NAUTILUS**

**Regulation of reward, motivation, feeding and sexual behavior**

*Chairs: Mohamed Najimi (Morocco) & Paola Fadda (Italy)*

ANTONIA MANDUCA (Italy): "CB1 and Mu opioid receptors interplay in the nucleus accumbens core underlies social reward in adolescent rats".

LUCIA HIPÓLITO CUBEDO (Spain): "Inflammatory pain desensitizes mu opioid receptor in VTA: impact on opioid self-administration in rats".

JANA KUCEROVA (Czech Republic): "Female sex increases reinstatement of methamphetamine seeking behaviour after a forced abstinence in rats".

MARY TRESA ZANDA (Italy): "Characterization of the neuropsychopharmacological profile of the new psychoactive substance metoxetamine in rats".

GEORGE KEMENES (UK): "A two-neuron system for goal-directed decision-making in the defined feeding network of *Lymnaea stagnalis*".

LINDY HOLDEN-DYE (UK): "A multimodal framework for optimising *C. elegans* feeding behaviour to changing food availability".

FABRIZIO SANNA (Italy): "Involvement of dopamine in the differences in sexual behaviour between Roman High and Low Avoidance rats: behavioral, pharmacological and neurochemical findings".

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Room **CYPREA**

**Neurological and psychiatric diseases**

*Chairs: Maria Antonietta De Luca (Italy) & George Panagis (Greece)*

NOURIA LAKHDAR-GHAZAL (Morocco): “Effects of Manganese neurotoxicity on the circadian rhythm of locomotor activity in the rat”.

JOÃO CASACA-CARREIRA (The Netherlands): “Neuroanatomical alterations in a transgenic rat model of Huntington’s disease detectable by conventional histology but not with ultra-high-field MRI Running head: tgHD rat brain: a MRI and histological study”.

KARIM FIFEL (The Netherlands): “Modelling sleep alterations in Parkinson’s disease: Translational insights”.

YONATAN SERLIN (Israel): “Retinal fluorescent angiography predicts blood-brain barrier damage and neuropsychiatric morbidity in diabetic patients.

MARIA ANTONIETTA PILUDU (Italy): “ERK expression in the cerebral cortex and amygdala of Roman high- and low-avoidance rats during the acquisition of active avoidance behavior”.

YASSINE BENTEFOUR (Morocco): “Effects of paroxetine on PTSD-like symptoms in mice”.

KAITLYN ENRIGHT (Canada): “A novel neurophysiological marker for high-risk behaviour: Does the frontal N400 event related brain potential demarcate behavioural disinhibition towards dangerous social roles in DWI offenders?”.

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Room **ASTREA**

**Neurotransmission and brain plasticity**

*Chairs: Marie Mofteh (Egypt) & Patrizia Fattori (Italy)*

DAVIDE DULCIS (USA): “Activity-dependent Neurotransmitter Plasticity affecting Behavior”.

ILDIKO KEMENES (UK): “Evolutionary conserved mechanisms of associative learning in Lymnaea”

RAMON REIG (Sweden): “Sensory integration in the mouse striatum”.

SIMONA DEDONI (Italy): “Differential modulation of neurotrophin receptor expression and signalling by type I interferons”.

LAURA ROSEN (Canada): “Dopamine D3 receptor signaling and its downstream signaling targets are altered as a function of opiate exposure state in the basolateral amygdala”.

PIERPAOLO CERULLO (Italy): “Sodium/Calcium exchanger (NCX): a new druggable target in neuronal dysfunctions following hypoxic-ischemic injury in neonatal mice”.

**19.15 CONCLUSIONS**



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## **POSTER SESSION I**

## ***NEUROGENESIS, PLASTICITY & CELL DEATH***

**P 01.**

### **SOX-2 EXPRESSION IN SHEEP BRAIN: A POSSIBLE ROLE IN ADULT NEUROGENESIS**

Mahmoud Dahab<sup>1</sup>, Sherine Abdel Salam<sup>1</sup>, Hussein Khamis<sup>1</sup>, Marie Moftah<sup>1</sup>, Emmanuel Moyses<sup>2</sup>

**P 02.**

### **A CORDAL, NOT GANGLIONIC, PATTERN OF CEPHALOPOD BRAIN NEUROGENESIS**

Shuichi Shigeno<sup>1</sup>, Rahul Parnaik<sup>2</sup>, Caroline B. Albertin<sup>2</sup>, Clifton W. Ragsdale<sup>2</sup>

**P 03.**

### **OCTOPUS PALLIAL NERVE REGENERATION**

Pamela Imperadore<sup>1,2</sup>, Giovanna Ponte<sup>1</sup>, Graziano Fiorito<sup>3</sup>

**P 04.**

### **CELLULAR PLASTICITY AND REVERSIBILITY IN WATER STRESSED RATS: DP71 IMPLICATION**

Madina Sifi<sup>1</sup>, Sabrina Souttou<sup>1</sup>, Tiziana Annese<sup>2</sup>, Roza Benabdesselam<sup>3</sup>, Beatrice Nico<sup>2</sup>, Latifa Dorbani-Mamine<sup>1</sup>

**P 05.**

### **JAK4D, A FIRST-IN-CLASS THYROTROPIN-RELEASING HORMONE (TRH)-BASED COMPOUND, PROTECTS AGAINST FREE RADICAL RELEASE AND CELL DEATH INDUCED BY INTRASTRIATAL KAINATE**

Maria Alessandra Colivicchi<sup>1</sup>, Keith F. Tipton<sup>2</sup>, Julie A. Kelly<sup>2</sup>, Laura Della Corte<sup>1</sup>

## ***NEUROPROTECTION & TOXICITY***

**P 06.**

### **NOVEL INDOLE AND PYRIDOINDOLE DERIVATIVES WITH MULTI-TARGET-DIRECTED NEUROPROTECTIVE ACTION**

Jana Ballekova<sup>1</sup>, Magdalena Majekova<sup>1</sup>, Gerard Esteban<sup>2</sup>, Mercedes Unzeta<sup>2</sup>, Milan Stefek<sup>1</sup>

**P 07.**

### **NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF MDG548, A NOVEL PPAR $\gamma$ AGONIST**

Daniela Lecca<sup>1</sup>, Daniel K Nevin<sup>2</sup>, Giovanna Mulas<sup>1</sup>, Anna R Carta<sup>1</sup>

**P 08.**

### **CEMTIRESTAT, A NOVEL ALDOSE REDUCTASE AND MONOAMINE OXIDASE INHIBITOR, IN MULTITARGET DIRECTED NEUROPROTECTION**

Marta Soltsova Prnova<sup>1</sup>, Jana Ballekova<sup>1</sup>, Mercedes Unzeta<sup>2</sup>, Gerard Esteban<sup>2</sup>, Magdalena Majekova<sup>1</sup>, Milan Stefek<sup>1</sup>

**P 09.**

### **NEUROCHEMICAL MECHANISMS INVOLVED IN MDMA-INDUCED NEUROTOXICITY IN MICE**

Giulia Costa<sup>1</sup>, Nicola Simola<sup>1</sup>, Micaela Morelli<sup>1,2</sup>

**P 10.**

### **DIFFERENT NEUROTOXIC EFFECTS INDUCED BY COMBINED ADMINISTRATION OF MDMA PLUS CAFFEINE, IN ADULT AND ADOLESCENT MICE**

Pier Francesca Porceddu<sup>1</sup>, Lucia Frau<sup>1</sup>, Camilla Madeddu<sup>1</sup>, Maria Grazia Ennas<sup>1</sup>, M Paola Castelli<sup>1</sup>, Micaela Morelli<sup>1,2</sup>

**P 11.**

**THE CHANGES IN EXTRACELLULAR LEVEL AND TISSUE CONCENTRATION OF DA AND 5-HT INDUCED BY MDMA AND CAFFEINE GIVEN CHRONICALLY IN A “BINGE” MODEL IN THE MOUSE BRAIN**

Anna Maria Górska, Krystyna Gołombiowska

**P 12.**

**TOXICOLOGICAL RESEARCH 1,2,3,4-TETRAHYDROISOQUINOLINE AN EXO/ENDOGENOUS AMINE WITH ANTIDEPRESSANT-LIKE ACTIVITY – *IN VIVO*, *IN VITRO* AND *IN SILICO* STUDIES**

Edyta Mozdzeń<sup>1</sup>, Izabella Babińska<sup>2</sup>, Jacek Wójcikowski<sup>3</sup>, Paulina Rachwalska<sup>4</sup>, Lucyna Antkiewicz-Michaluk<sup>1</sup>

### ***MEMORY, COGNITION & BEHAVIOR***

**P 13.**

**NEURAL MECHANISMS INVOLVED IN PUNISHMENT-RELATED MEMORY-GUIDED ATTENTION**

Samuel Suárez-Suárez<sup>1</sup>, Kenia Correa<sup>1</sup>, Socorro Rodríguez Holguín<sup>1</sup>, Fernando Cadaveira<sup>1</sup>, Anna Christina Nobre<sup>2</sup>, Sonia Doallo

**P 14.**

**TIME COURSE OF THE EFFECTS OF PUNISHMENT AVOIDANCE-ASSOCIATED MEMORY-GUIDED ORIENTING ON PERCEPTION**

Sonia Doallo<sup>1</sup>, Samuel Suárez-Suárez<sup>1</sup>, Kenia Correa<sup>1</sup>, Socorro Rodríguez Holguín<sup>1</sup>, Fernando Cadaveira<sup>1</sup>, Anna Christina Nobre<sup>2</sup>

**P 15.**

**ENDOCANNABINOID SYSTEM AND CONSOLIDATION OF AVERSIVE MEMORY: BEYOND CANNABINOID RECEPTOR SUBTYPE 1**

Patrizia Ratano<sup>1</sup>, Fabrizio Forti<sup>1</sup>, Viviana Trezza<sup>2</sup>, Patrizia Campolongo<sup>1</sup>

**P 16.**

**THE EFFECT OF CO-TREATMENT WITH ANTIDEPRESSANTS AND RISPERIDONE ON THE MK-801-INDUCED CHANGE IN OBJECT RECOGNITION MEMORY IN MICE**

Zofia Rogóż<sup>1,2</sup>, Katarzyna Kamińska<sup>1</sup>

**P 17.**

**IMPROVING AGE-RELATED MEMORY IMPAIRMENTS WITH DONEPEZIL: WHY YOU CAN'T HAVE IT ALL**

Azza Sellami<sup>1</sup>, Laurent Brayda-Bruno<sup>1</sup>, Nicole Etchamendy<sup>2</sup>, Nicole Mons<sup>3</sup>, M Jabourian<sup>4</sup>, Xavier Nogues<sup>1,2</sup>, K Bernard<sup>4</sup>, Aline Marighetto<sup>1</sup>

**P 18.**

**DEEP BRAIN STIMULATION TO RESTORE MEMORY LOSS: TARGETS AND MECHANISMS**

Sarah Heschem<sup>1,4\*</sup>, Ali Jahanshahi<sup>1,4</sup>, Leewei Lim<sup>1,4</sup>, Arjan Blokland<sup>3,4</sup>, Yasin Temel<sup>1,2,4</sup>

**P 19.**

**A NOVEL, SELF-ADJUSTING DELAY DISCOUNTING PROCEDURE FOR THE STUDY OF CHOICE IMPULSIVITY IN RATS**

Mejda Wahab, Marcello Solinas

**P 20.**

**INFLAMMATORY PAIN DESENSITIZES MU OPIOID RECEPTOR IN VTA: IMPACT ON OPIOID SELF-ADMINISTRATION IN RATS**

L Hipolito<sup>1</sup>, AR Wilson-Poe<sup>2</sup>, Yolanda Campos-Jurado<sup>1</sup>, Elaine Zhong<sup>2</sup>, Jose Gonzalez-Romero<sup>2</sup>, Laszlo Virag<sup>2</sup>, Robert Whittington<sup>2</sup>, Sandra D Comer<sup>3</sup>, Susan M Carlton<sup>4</sup>, Michael R Bruchas<sup>5</sup>, Jose A Morón<sup>2</sup>

**P 21.**

**IN VIVO ACTIVATION OF SK CHANNELS REDUCES THE DOSE OF NMDA RECEPTOR ANTAGONIST NEEDED TO PRODUCE ANTINOCICEPTION**

L Hipólito<sup>1,2</sup>, A Fakira<sup>2</sup>, D Cabañero<sup>2</sup>, P Anand<sup>2</sup>, J-L Gonzalez-Romero<sup>2</sup>, S Carlton<sup>3</sup>, J Morón Concepción<sup>2</sup>, Z Melyan<sup>2</sup>

**P 22.**

**EXPLORING NOCICEPTION IN *Octopus vulgaris***

Giulia Di Cristina, Graziano Fiorito

***NEURODEGENERATIVE & NEUROPSYCHIATRIC DISORDERS***

**P 23.**

**ATTENTION DEFICIT HYPERACTIVITY DISORDER AMONG SCHOOL CHILDREN OF KENITRA CITY (NORTH WEST OF MOROCCO)**

F-Z Rouim<sup>1</sup>, F-Z Azzaoui<sup>2</sup>, A Ahami<sup>1</sup>

**P 24.**

**FORETHOUGHT IN YOUTH WITH ATTENTION DEFICIT/HYPERACTIVITY DISORDER: AN FMRI STUDY OF SEX-SPECIFIC DIFFERENCES**

Stephanie Chenail<sup>1</sup>, Hélène Poissant<sup>2</sup>, Lucile Rapin<sup>3</sup>, Adrianna Mendrek<sup>1,4</sup>

**P 25.**

**ASSOCIATION OF THE TNF $\alpha$ , TNFB AND TNFR2 GENETIC POLYMORPHISMS WITH PARANOID SCHIZOPHRENIA**

Oumaima Inoubli<sup>1</sup>, Siham Thabet, Ferid Zaafrane<sup>2</sup>, Anouar Mechri<sup>2</sup>, Lotfi Gaha<sup>2</sup>, Khaled Said<sup>1</sup>, Besma Bel Hadj Jrad<sup>1</sup>

**P 26.**

**ASSOCIATION OF THE IFN $\gamma$  AND IFNGR1 GENETIC POLYMORPHISMS WITH PARANOID SCHIZOPHRENIA**

Achraf Jemli<sup>1</sup>, Siham Thabet, Ferid Zaafrane<sup>2</sup>, Anouar Mechri<sup>2</sup>, Lotfi Gaha<sup>2</sup>, Khaled Said<sup>1</sup>, Besma Bel Hadj Jrad<sup>1</sup>

**P 27.**

**VGF PEPTIDES: A POSSIBLE ROLE IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) NEURODEGENERATIVE MECHANISMS**

Carla Brancia<sup>1</sup>, Barbara Noli<sup>1</sup>, Roberta Pilleri<sup>1</sup>, Andrea Boi<sup>1</sup>, Cristina Cocco<sup>1</sup>, Marina Boido<sup>2</sup>, Roberta Puddu<sup>3</sup>, Giuseppe Borghero<sup>3</sup>, Francesco Marrosu<sup>3</sup>, Alessandro Vercelli<sup>2</sup>, Gian-Luca Ferri<sup>1</sup>

**P 28.**

**THE EFFECT OF CO-TREATMENT WITH RISPERIDONE AND ANTIDEPRESSANTS IN AN ANIMAL MODEL OF THE NEGATIVE SYMPTOMS OF SCHIZOPHRENIA IN RATS**

Katarzyna Kamińska<sup>1</sup>, Zofia Rogóż<sup>1,2</sup>

**P 29.**

**INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN A NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA**

Tibor Štark<sup>1</sup>, Jana Kucerova<sup>1</sup>, Vladimír Pekařík<sup>1</sup>, Fabio Arturo Iannotti<sup>2</sup>, Teresa Aveta<sup>2</sup>, Katarina Tabiová<sup>1</sup>, Filippo Drago<sup>3</sup>, Vincenzo Di Marzo<sup>2</sup>, Alexandra Sulcova<sup>1</sup>, Vincenzo Micalè<sup>1,3</sup>

**P 30.**

**EVALUATION OF THE CB1 RECEPTOR DENSITY IN AN ANIMAL MODEL OF BINGE EATING DISORDER**

Roberto Collu<sup>1</sup>, Valentina Satta<sup>1</sup>, Maria Scherma<sup>1</sup>, Maria Paola Castelli<sup>1</sup>, Liana Fattore<sup>2,3</sup>, Walter Fratta<sup>1,2</sup>, Paola Fadda<sup>1,2</sup>

**P 31.**

**THE STANDARDIZED ROOT EXTRACT OF *WITHANIA SOMNIFERA* DUNAL, A PLANT OF THE SARDINIAN AND MEDITERRANEAN FLORA, COUNTERACTS MOTOR IMPAIRMENT IN A *DROSOPHILA* MODEL OF PARKINSON'S DISEASE**

Francescaelena De Rose<sup>1\*</sup>, Simone Poddighe<sup>2\*</sup>, Giuseppe Talani<sup>1\*</sup>, Maria Dolores Setzu<sup>2</sup>, Paolo

**P 32.**

**EVALUATION OF REWARDING PROPERTIES OF PRAMIPEXOLE IN A RAT MODEL OF PARKINSON'S DISEASE: ROLE OF GENETIC BACKGROUND**

Elena Espa<sup>1</sup>, Augusta Pisanu<sup>2</sup>, Carla Masala<sup>3</sup>, Gaetano Di Chiara<sup>1,2,4</sup>, Sandro Fenu<sup>1,4</sup>, Solla<sup>3</sup>, Francesco Marrosu<sup>3</sup>, Valentina Corda<sup>1</sup>, Elio Acquas<sup>1</sup>, Sanjay Kasture<sup>4</sup>, Enrico Sanna<sup>1</sup>, Anna Liscia<sup>1</sup>

**P 33.**

**PARKINSON'S DISEASE AND PATHOLOGICAL GAMBLING: ROLE OF NEUROSTEROIDS**

Romina Pes, Andrew T Fox, Laura J Mosher, Sean C Godar, Stephen C Fowler, Marco Bortolato

**P 34.**

**L-DOPA CHRONIC TREATMENT INCREASES REACTIVE MICROGLIA AND TNF- ALPHA PRODUCTION IN THE 6-OHDA LESIONED STRIATUM.**

Giovanna Mulas, Elena Espa, Sandro Fenu, Daniela Lecca, Elisabetta Pillai, Saturnino Spiga, Anna R Carta

**P 35.**

**INHIBITION OF 5ALPHA REDUCTASE COUNTERS LEVODOPA-INDUCED DYSKINESIA IN 6-OHDA-LESIONED RATS**

Silvia Fanni<sup>1</sup>, Roberto Frau<sup>1,2</sup>, Elisabetta Tronci<sup>3</sup>, Camino Fidalgo<sup>3</sup>, Carlo Lisci<sup>3</sup>, Alessandra Pardu<sup>1</sup>, Romina Pes<sup>1</sup>, Pierluigi Saba<sup>1</sup>, Paola Devoto<sup>1,2</sup>, Marco Bortolato<sup>2,4</sup>, Manolo Carta<sup>3</sup>

**P 36**

**CHARACTERIZATION OF GAIT DISTURBANCES IN EXPERIMENTAL HUNTINGTON'S DISEASE: A POSSIBLE RELATION WITH CHOREA?**

João Casaca-Carreira<sup>1</sup>, Yasin Temel<sup>1,2,3</sup>, Marloes van Zelst<sup>1</sup>, Ali Jahanshahi<sup>1,2,3</sup>

**P 37.**

**EARLY CHANGE OF THE DOPAMINERGIC SYSTEM IN AN EXPERIMENTAL MODEL OF HUNTINGTON'S DISEASE**

Melanie Lagiere<sup>1,3</sup>, Ali Jahanshahi<sup>1,2,3</sup>, Aldehri Majed<sup>1</sup>, Özlem Özsoy<sup>4</sup>, Yasin Temel<sup>1,2,3</sup>

**P 38.**

**PHARMACOLOGICAL EVALUATION OF NOVEL MULTI-TARGET COMPOUNDS DERIVED FROM DONEPEZIL+PROPARGYLAMINE+8-HYDROXYQUINOLINE (DPH) HYBRIDS FOR THE POTENTIAL USE IN ALZHEIMER'S DISEASE**

Gerard Esteban<sup>1</sup>, José-Luis Marco-Contelles<sup>2</sup>, Mercedes Unzeta<sup>1</sup>

**P 39.**

**PALMITOYLETHANOLAMIDE CONTROLS ASTROCYTE ACTIVATION AND THE AMYLOIDOGENIC PATHWAY IN AN *IN VIVO* MODEL OF ALZHEIMER'S DISEASE**

Maria Rosanna Bronzuoli, Samaneh Eivazpour, Silvia Bartoli, Liliana Ciconti, Luca Steardo, Caterina Scuderi

**P 40.**

**THE EFFECT OF ASS234 ON TAU PHOSPHORYLATION *IN VITRO***

Mirjana Babić<sup>1</sup>, Maja Jazvinščak Jembrek<sup>2,3</sup>, Ana Knezović<sup>4</sup>, Goran Šimić<sup>1</sup>

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**IN VITRO ELECTROTAXIS: LOW CURRENT DENSITIES ATTRACT NEURAL PRECURSOR CELLS**

Lisa-Maria Schönfeld<sup>1,2</sup>, Evi Lemmens<sup>2</sup>, Ali Jahanshahi<sup>1,3</sup>, Yasin Temel<sup>1,3</sup>, Sven Hendrix<sup>2</sup>

# **PLENARY LECTURE**

## **DRUGS OF ABUSE AS UNCONDITIONED HOMOLOGS OF CONDITIONED REWARDS: THE ROLE OF ACCUMBENS SHELL DOPAMINE**

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Two main subdivisions have been distinguished in the nucleus accumbens (NAc), a ventro-medial shell and a dorso-lateral core and have been attributed different functional properties and roles in behaviour. Microdialysis and voltammetry studies have shown that shell dopamine (DA) is preferentially activated by drugs of abuse after response-noncontingent (passive) and contingent exposure (self-administration). Non-contingent palatable food stimulates in vivo DA transmission in NAc shell and core. This response undergoes single-trial habituation by 2 hours specifically in the shell. Core DA instead is eventually potentiated. In contrast to food, drugs of abuse do not induce habituation of shell DA after repeated non-contingent as well as contingent exposure. Response contingency drastically affects the responsiveness of NAc shell and core DA transmission to repeated drug exposure. Thus, while response-contingent heroin and cocaine preferentially activate shell DA, non-contingent exposure blunts DA responsiveness in the shell while sensitizes it in the core. Recently we have investigated the role of response contingency in the changes of NAc shell and core DA induced in rats by sucrose feeding. FR1 as well as FR5 nose-poke responding for sucrose stimulates DA transmission selectively in the shell. In contrast, non-contingent sucrose presentation and feeding activates DA with a superimposable time-course in the shell and in the core. Thus, instrumental responding for sucrose seems to actually suppress the ability of sucrose to stimulate DA transmission in the core. This inhibition might serve to prevent impulsive and inappropriate responses, thus increasing the efficiency of goal-directed action. No habituation of shell responsiveness was observed under operant sucrose feeding. This observation, coupled to the fact that responding under extinction was also associated to selective stimulation of shell DA, suggests that activation of DA transmission during responding for sucrose is due to conditioned (CSs) rather than unconditioned sucrose stimuli. This lack of habituation also applies to the stimulation of DA transmission in the shell by non-contingent sucrose presentation and feeding. Thus, habituation of the unconditioned release of shell DA by sucrose is masked by activation of shell DA carried by CSs acquired as a result of either contingent or non-contingent sucrose feeding. These observations are consistent with the hypothesis that DA responsiveness in the NAc shell and core is driven by different stimuli and is differentially affected by response contingency. This in turn suggests that DA transmission in these two areas plays different roles in instrumental behaviour. The robust and selective activation of DA transmission in the shell during responding for sucrose and under extinction is consistent with a role of conditioned stimuli and with the property of encoding a prediction of the intrinsic rewarding value of the action outcome (sucrose). Because of this, activation of shell DA transmission might be intrinsically rewarding, while DA release in the NAc core might play more computational functions, eventually related to actions unrelated to the species-specific repertoire such as skill and habit learning. This hypothesis is consistent with the notion that cocaine-like psychostimulants strictly depend for their reinforcing properties from DA receptors in the NAc shell. Therefore, our observations suggest that the property of stimulating DA transmission in the NAc shell in a non-habituating fashion, typical of drugs of abuse, is homologous to that of incentive stimuli conditioned to a conventional reward like sucrose. Notably, while in the case of incentive CSs this property undergoes extinction if not reinforced by the primary reward, in the case of drugs of abuse is an unconditioned property and is therefore resistant to extinction. This property of mimicking unconditionally the NAc shell DA stimulant properties of incentive CSs might be critical for the ability of drugs of abuse to elicit addiction in predisposed individuals.

## **THE HUMAN BRAIN PROJECT**

Idan Segev

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The Human Brain Project (HBP) is one of two projects that were recently awarded as “EU Flagship Projects” for the decade to come. Many hundreds of scientists are involved in the HBP, with the overall goal of developing a novel model of ICT-based brain research to obtain: (i) A new shared platform to understanding the brain via theoretical and computational; (ii) New treatments for brain diseases; (iii) New brain-inspired computing technologies. In this presentation, I will briefly highlight the overall HBP and then discuss the key importance of using theoretical tools, modelling and computer simulations, in order to deepen our understanding of the healthy and the diseased brain.

## **NEURAL MECHANISMS UNDERLYING THE DEVELOPMENT OF COMPULSIVE DRUG SEEKING HABITS IN ADDICTION**

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I will discuss that drug addiction involves the transition from voluntarily or recreational drug use to compulsive drug seeking that reflects shifts in the control over behaviour from the ventral to the dorsal striatum and progressive loss of prefrontal cortical inhibitory control over maladaptive habits. This will be illustrated by experimental studies in animals and a growing body of clinical evidence. Not all individuals undergo this transition and the nature of the vulnerability to lose control over drug use will be discussed as endophenotypes for addiction, especially to stimulant drugs.

# **HARNESSING HIPPOCAMPAL NEUROGENESIS TO IMPROVE MOOD AND COGNITION**

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Although a role for adult neurogenesis in specific forms of learning and in mediating some of the effects of antidepressants has received considerable attention in recent years, much less is known about how alterations in this unique form of plasticity may contribute to neurologic or psychiatric disorders. One way to begin to address this question is to link the functions of adult-born hippocampal neurons with specific endophenotypes of these disorders. Recent studies have implicated adult born hippocampal neurons in pattern separation, a process by which similar experiences or events are transformed into discrete non-overlapping representations. Here, we propose that impaired pattern separation underlies the overgeneralization often seen in age-related memory impairments and in anxiety disorders and therefore, represents an endophenotype for these disorders. We will present evidence that strategies aimed at stimulating hippocampal neurogenesis result in improved pattern separation. The development of novel pro-neurogenic compounds may therefore have therapeutic potential for patients who display pattern separation deficits.

## NEURAL CIRCUITS FOR MOTOR BEHAVIOR

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Most processing in the nervous system is translated into motor actions. A salient feature of motor behavior is the orderly recruitment of motor pools and the coordination of their activity to produce movement with precise force, speed and timing. The executive component of the nervous system is the spinal cord that produces the activity to drive motoneurons in sequential pattern necessary to move in space. I will discuss the organization of circuit for motor behavior from the lens of the spinal circuits underlying locomotion. More specifically how these circuits are constructed to produce locomotor movements with versatile speed and force. Traditionally these circuits have been believed to be composed of a unit CPG that with increased activity produces faster movements. In this view, all motoneurons receive uniform inputs from premotor interneurons and their recruitment follows the size principle. Our recent results from adult zebrafish challenge this view. We show that slow, intermediate and fast motoneuron pools are connected to different premotor excitatory (V2a) interneurons and their order of recruitment is not dictated by their size or input resistance. The order of recruitment of the different motoneuron pools emerges from their selective wiring with the premotor circuit. Indeed, the spinal circuits can be deconstructed into three microcircuit modules. Each microcircuit encompasses a subset of interconnected excitatory V2a interneurons and a motoneuron pool that are recruited sequentially from slow, to intermediate and fast during swimming with increased speed. The connectomics, cellular properties, and function of each microcircuit are attuned to the properties of the muscle fibers they activate. Thus our findings reveal a circuit principle governing the recruitment of motor pools that is unrelated to the size principle. In addition we show that the spinal locomotor network display a specific connectivity map with ensemble microcircuit organization that acts as an intrinsic gearshift to change the speed of locomotion. Given the conserved organization of circuits across vertebrate species, the principles of circuit construction revealed in zebrafish could apply to other species including mammals where a similar a fine-grained circuit mapping has been difficult to achieve.

***SYMPOSIA***

*Neurocognitive function and emotion  
processing in psychopathology*

**NEURAL MECHANISMS OF EMOTION-COGNITION INTERACTIONS IN HEALTHY  
FUNCTIONING AND DISEASE: EVIDENCE FROM BRAIN IMAGING  
INVESTIGATIONS**

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Complex behaviour involves reciprocal influences between emotion and cognition. On the one hand, emotion is a “double-edged sword” that may affect various aspects of our cognition and behaviour, by enhancing or hindering them and by exerting transient and long-term influences. On the other hand, emotion processing is also susceptible to cognitive influences, typically exerted in the form of emotion regulation. Investigation of these relationships is fundamental not only for understanding the mechanisms underlying emotion-cognition interactions in healthy functioning but also for understanding changes associated with emotional disturbances, in which these interactions are dysfunctional. In my presentation, I will discuss evidence concerning the neural mechanisms of emotion-cognition interactions, as derived from brain imaging investigations in healthy and clinical groups. Aspects regarding individual differences that affect susceptibility to, or resilience against, symptoms of emotional dysregulation (anxiety and depression) will also be emphasized.

## FUNCTIONAL CEREBRAL CONNECTIVITY IN SCHIZOPHRENIA WOMEN DURING VISUO-SPATIAL PROCESSING

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Over the past few years we have established a research program devoted to examining neurocognitive, hormonal, and psychosocial factors implicated in sex/gender differences in psychoses. Our work to date revealed, among other things, intriguing disturbances of normal sexual dimorphism in visuo-spatial processing and associated brain function in clinically stable schizophrenia patients. The task involved mental rotation of three-dimensional figures and elicited superior performance in healthy men relative to healthy women, but the opposite effect was observed in patients (i.e., women performed better than men). In a similar manner, functional magnetic resonance imaging (fMRI) data showed greater activations in healthy males relative to females (mainly in the parietal and lateral prefrontal cortex), but the opposite pattern in patients. This effect was apparent mainly due to a significant deficit in male patients relative to controls, but normal performance in female patients relative to controls. In the present study we explored more thoroughly the fMRI data obtained in female participants. Thus, to evaluate differences in connectivity during the mental rotation task, we used the psychophysiological interaction (PPI) method, a multiple regression technique that allows the investigation of functional coupling between regions in relation to the experimental paradigm. Using PPI analyses, we found that healthy control women demonstrated significant positive connectivity between the right dorsolateral prefrontal cortex (DLPFC) and regions in the parietal, occipital, prefrontal and cingulate cortices, but no positive connectivity was observed in women with diagnosis of schizophrenia. In contrast, while there was no apparent negative coupling in healthy women, women with schizophrenia exhibited significant negative connectivity between the right DLPFC and regions in the temporal lobe and cingulate cortex. These results emphasize the importance of including women in studies of psychotic disorders, as well as the fact that similar performance on a given task may be accomplished by different physiological means.

## HOW OXYTOCIN MODULATES THE HUMAN BRAIN AND BEHAVIOR

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Social skills require specific cognitive and emotional competences. Individuals with High Functioning Autism (HFA) cannot engage in social interactions despite preserved cognitive abilities. Recently, it has been suggested that oxytocin, a hormone known to promote affiliation and mother-infant bonds, may be implicated in the social deficit of HFA. We investigated the effects of intranasal oxytocin administration on the social behavior of HFA patients<sup>1</sup>. We used a double-blind, placebo-controlled within-subject design to study social behaviour of thirteen patients diagnosed with HFA after intranasal inhalation of oxytocin compared to placebo. In one task inspired from the cyberball game, we developed a new social interaction paradigm, based on probabilistic algorithms, where participants interacted with fictitious partners. In a second task, we measured eye movement pattern, during examination of faces. Plasma oxytocin measures were performed using specific enzyme-immunoassay technique. When participants interacted with fictitious partners, we found that after oxytocin inhalation, patients exhibited stronger interactions with the most socially cooperative partner, and reported enhanced feelings of trust and preference. Also, during free viewing of pictures of faces, oxytocin selectively increased patient's gazing time on the socially-informative region of the face, i.e. the eyes. These findings suggest that oxytocin enhanced patients' capacities to process facial stimuli and to learn from social relevant cues to interact with other partners. Our results highlight the therapeutic potential of oxytocin through its action on core deficits of autism.

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## TRANSMISSION OF FRONTO-PARIETAL DYSFUNCTION DURING FORETHOUGHT IN FAMILIES WITH ATTENTION DEFICIT/HYPERACTIVITY DISORDER

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Only a few studies have investigated possible associations between familial kinship and neural abnormalities in ADHD<sup>1,2,3,4</sup>. Our aim was to further understanding of the intergenerational course of ADHD with a novel task involving anticipation of coming events: *forethought*.

We hypothesized that the presence of ADHD in families is associated with increased neural abnormalities in frontal and parietal regions. We formed nine biological parent-child dyads with (Group 1) and without ADHD (Group 2). Both groups, Control (parents and children) and ADHD (parents and children) made few incorrect answers suggesting that the *forethought* task did not discriminate groups at the behavioural level. However, for both ADHD children and their parents, we could identify decreased activations in the same three regions: 1) left-IPL/SMG, 2) right-SPL and 3) IFG. Among these regions, differences between Controls (both children and parents) and ADHD (both children and parents) are emphasized for the IPL/SMG for the contrasts Control children > ADHD children and Control parents > ADHD parents.

IPL is known as a multimodal associative region involved in sustaining attention. Left-IPL hypoactivation in both ADHD generations confirms previous findings of a deficit in sustained attention<sup>5,6,7,8</sup>. Right-SPL decreased activation in ADHD patients has also been observed in response inhibition and attention studies<sup>6,9</sup>. Decreased IFG activation has been a consistent fMRI finding in ADHD for various executive functions<sup>10</sup>. This pilot study confirms that the presence of ADHD in families is associated with increased neural abnormalities in frontal and parietal regions, specifically in the IPL region. This suggests that fronto-parietal dysfunctions in childhood ADHD may persist into adulthood.

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*Emerging concepts in dopaminergic system  
development and regulation*

## GENETIC CHARACTERIZATION OF THE DOPAMINE SYSTEM IN THE ANIMAL MODEL *C. ELEGANS*

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Dopaminergic (DA) neurons are known to be crucial for essential brain functions. Dysfunction of the DA system has been associated with a number of psychiatric and neurological diseases, including Parkinson disease. Most of the diseases associated with the DA system results from an imbalance in dopamine neurotransmission, with no apparent morphological changes. Moreover the DA system plays a pivotal role in drug addiction, as many drugs of abuse stimulate dopamine release. The molecular mechanisms underlying these diseases and dysfunctions are not completely understood and genetic investigations can provide insights into their pathogenesis. A deeper genetic analysis of DA system may therefore be crucial not only to develop novel therapeutic approaches, but also to understand the different functions played by DA neurons. To this end, researchers have to strive for a new level of innovation using animal models amenable for genetic manipulations. Our group uses the impressive experimental advantages offered by the animal model *C. elegans*, to understand how DA system develop, function, react and survive to various insults. The nematode, with its powerful genetics, has been used by researchers as a model to investigate neurodegeneration<sup>1</sup> and to understand the molecular mechanisms underlying dopaminergic system development and regulation<sup>2</sup>. *C. elegans* represents also a valuable model to study human diseases, since its genome encodes many human disease orthologs and the biological processes are well conserved, and thanks to its small size, rapid growth and simple food requirements, it is suitable for pharmacological screenings on whole living animals<sup>3</sup>. We will present *C. elegans* as an emerging animal model to study the dopamine system development and regulation and our recent data on the identification of genetic and chemical modifiers of DA function, obtained by genetic manipulations, drug treatments and detailed phenotypic analysis *in vivo*.

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## HDAC INHIBITION REDUCES DOPAMINE-DEPENDENT NEURODEGENERATION IN A MOUSE MODEL OF DAT-DEFICIENCY SYNDROME

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Dopamine (DA) is a neurotransmitter with important physiological functions in the brain and linked to different pathological states. A loss in dopaminergic innervations is a key feature of Parkinson's disease, while enhanced dopaminergic tone has been linked to schizophrenia and Huntington disease. Recently, it has been found that mutations leading to the loss of function of the DA transporter (DAT) cause a severe neurological disease called DAT deficiency syndrome (DTDS). The disease manifests initially as hyperkinetic movement disorder that evolves in hypokinetic parkinsonism-dystonia. DAT-KO mice present symptoms similar to DTDS patients thus it is reasonable to expect that the molecular mechanisms involved in dopamine-dependent postsynaptic neurodegeneration in this mouse line are similar to those involved in the human pathology. Therefore, the DAT-KO mouse is an ideal model to define the pathways involved in dopamine-mediated neurodegeneration and to test novel curative strategies for DAT-deficiency syndrome, thus meeting a precise medical need.

We used DAT KO mouse to mimic hyperdopaminergic tone and the dopamine-deficient DAT-KO mice (DDD) as an acute model of absolute dopamine deficiency. Two dimensional differential in-gel electrophoresis (2D-DIGE) followed by functional analysis of the identified proteins revealed acetylation as one of the pathways affected by the dopaminergic tone in striatum. Consistently, we found an up-regulation of several subtypes of histone deacetylase (HDAC) in DAT-KO mice, increase that was abolished in DDD-mice thus suggesting a dynamic regulation of these enzymes by the dopamine extracellular levels. Elevated dopamine levels in DAT KO mice have been related to the increase of tau phosphorylation and neurodegeneration, thus we decided to define the possible role of HDACs in this process. Pharmacological inhibition of HDAC activity decreased the level of phosphorylated tau in DAT-KO and the neurodegeneration associated to levels comparable to that of WT mice.

Our data indicate that the inhibition of HDAC represents a potential therapeutic target for dopamine-induced neurodegeneration, and suggest an intriguing role for dopamine in the regulation of HDACs.

## PRONOUNCED DOPAMINERGIC DYSREGULATION IN DOPAMINE TRANSPORTER KNOCKOUT RATS

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Dopamine (DA) plays an important role in the control of many physiological functions but many neuropsychiatric such as schizophrenia, Parkinson's disease and attention deficit hyperactivity disorder have a basis in a dysfunction of the dopaminergic systems. Concentrations of DA in the synaptic cleft have been suggested to be the primary determinant of the intensity of neuronal signaling. The major function of Dopamine Transporter (DAT) is the control of dopamine dynamics by rapid uptake of neurotransmitter into presynaptic nerve terminals. Therefore, DAT is an important regulatory element of both the synaptic action of DA and the intracellular stores of DA. Here, we present a newly developed strain of rats (DAT-KO rats) in which the gene encoding the DAT has been disrupted. DAT-KO rats develop normally but have lower weight in comparison to wild-type (WT) rats. Like in DAT-KO mice<sup>1</sup>, spontaneous locomotor activity is highly elevated in them. HPLC analysis showed that KO rats have decreased total tissue DA and increased levels of DA metabolites. Like in DAT-KO mice<sup>2</sup>. Fast Scan Cyclic Voltammetry analysis of DA dynamics in the dorsal striatum revealed that DA clearance in DAT-KO is much longer (over 60 seconds) when compared with WT, suggesting that the increase in the spontaneous locomotor activity is a direct consequence of the extended length of time that DA spends in the extracellular space. Moreover, bath applied cocaine had no effect on evoked basal DA efflux on DAT-KO rats but it showed the well know increase in DA level in control animals. In summary, lack of DAT in rats results in disrupted clearance of released DA that affects both the extracellular and intraneuronal concentrations of DA. DAT-KO rats could provide a novel translational model for several human diseases involving aberrant DA function and/or mutations affecting the DAT or DAT-related regulatory mechanisms.

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## TARGETING DOPAMINE D<sub>1</sub>-HISTAMINE H<sub>3</sub> RECEPTOR HETEROMERS REVERTS LEARNING AND LONG-TERM MEMORY DEFICITS IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

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In the early stages of Huntington's disease (HD) there is an excess of dopamine production and an over-activation of dopamine D<sub>1</sub> receptors (D<sub>1</sub>R) that can produce not only an imbalance in dopaminergic neurotransmission but can also directly lead to signaling cascades that induce cell death. Here we propose a new and provocative strategy to reduce the D<sub>1</sub>R over-activation effects in HD by targeting the recently described receptor complexes of D<sub>1</sub>R and the histamine receptors H<sub>3</sub> (H<sub>3</sub>R). We show the expression of D<sub>1</sub>R-H<sub>3</sub>R heteromers in a HD model of striatal neuronal progenitor cells and in different brain areas of mouse models of HD in the early but not in the late stages of the illness as well as in human control subjects and in grade 2 HD patients but not in grade 3 or 4 HD patients. Upon co-activation of D<sub>1</sub>R-H<sub>3</sub>R heteromers, H<sub>3</sub>R ligands act as a "molecular brake" for D<sub>1</sub>R signaling. D<sub>1</sub>R-induced cell death in cells and in brain slices and the signaling cascades responsible for this death are reduced by H<sub>3</sub>R ligands targeting D<sub>1</sub>R-H<sub>3</sub>R heteromers. Treatment of presymptomatic mouse models of HD with the H<sub>3</sub>R antagonist thioperamide can restore both cognitive and motor deficits of these animals and inhibits the loss of heteromer expression. Importantly, D<sub>1</sub>R-H<sub>3</sub>R heteromers are observed in human control subjects and grade 2 HD patients but not in grade 3 or 4 HD patients. Our results demonstrate that D<sub>1</sub>R-H<sub>3</sub>R heteromers play a pivotal role in controlling dopaminergic neurotransmission and indicate that D<sub>1</sub>R-H<sub>3</sub>R heteromers can be target for treating HD in the pre-symptomatic stages of the illness.

## DIRECT REPROGRAMMING OF FIBROBLASTS INTO FUNCTIONAL DOPAMINERGIC NEURONS

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Lineage-specific transcription factors, which drive cellular identity during embryogenesis, have been shown to convert cell fate when expressed ectopically in heterologous cells. Remarkably, developmental fate molecular switchers are sufficient to force neuronal conversion in differentiated somatic cells of other germinal layers. However, at present, it is unclear whether a specific neuronal type can be preferentially induced from direct reprogramming of fibroblasts. Thus, it remains uncertain whether neuronal subtype lineage choices occurring during brain morphogenesis can be reproduced *in vitro* starting from heterologous cells.

Herein, we screened the key molecular factors governing the dopaminergic (DA) neuronal specification during brain development for their ability to generate functional neurons directly from mouse and human fibroblasts. Remarkably, we found a minimal set of three factors *Ascl1*, *Nurr1* and *Lmx1a* (ANL) able to elicit such cellular reprogramming. Molecular and transcriptome studies showed reprogrammed DA neuronal cells to recapitulate gene expression of their brain homolog cells while lacking expression of other monoaminergic neuronal subtype's markers.

Induced DA (iDA) neuronal cells showed spontaneous electrical activity organized in regular spikes consistent with the pacemaker activity featured by brain DA neurons. The three factors were able to elicit DA neuronal conversion in prenatal or adult fibroblasts from healthy donors and Parkinson's disease patients. Moreover we found that our three DA factors are also able to differentiate human induced pluripotent stem cells (iPS) into dopaminergic neurons within only 3 weeks.

Overall we proved that ANL cocktail is able to convert mouse and human cells into DA neuronal. This finding might have significant implications in studies of neural development, disease *in vitro* modeling and cell replacement therapies.

***Glia in the nervous system: From housekeeping  
to processing functions***

## **SURFACE DYNAMICS OF GLT-1 ON ASTROCYTES SHAPES EXCITATORY TRANSMISSION**

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Glutamate is the major excitatory transmitter in the brain. Its concentration in the synapse and its diffusion in the extracellular space are tightly controlled by the astroglial transporter GLT-1. These transporters ensure point-to-point transmission and prevent excessive accumulation of glutamate that could be toxic to neurons. Although data are available on the regulated expression of these proteins in physiological and pathological conditions, as well as on its intracellular trafficking, nothing is known about its membrane dynamics. Using quantum dot imaging of single GLT-1 transporter, we here report that not only GLT-1 is highly mobile at the surface of astrocytes, but that this mobility depends strongly on neuronal and synaptic activity as well as on the activity of the transporter itself. Furthermore, GLT-1 membrane diffusion slows down dramatically at the vicinity of synaptic contacts, suggesting that an active process is engaged when the transporter senses glutamate. Finally, reducing experimentally GLT-1 membrane trafficking through cross-linking with anti-bodies modifies significantly the kinetics of the glutamatergic synaptic currents. Our results provide new insights on the astroglial glutamate transporter GLT-1 and the processes by which it shapes excitation and impacts synaptic transmission. It also offers open the door to new strategies to tackle neuronal disorders involving GLT-1 dysfunctions.

## CO-ULTRAMICRONIZED PALMITOYLETHANOLAMIDE/LUTEOLIN PROMOTES MATURATION OF RAT CORTICAL OLIGODENDROCYTES IN VITRO

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Oligodendrocytes are the myelin-producing cells of the central nervous system responsible for ensheathment of axons. They play an important role in maintaining integrity of axons in nerve transmission and neuronal survival, and participate in cross-talk with microglia<sup>1</sup>. Oligodendrocytes have limited ability to repair the damage to themselves or to other nerve cells, as seen in demyelinating diseases, such as multiple sclerosis (MS). MS lesions are characterized by the presence of undifferentiated oligodendrocyte precursor cells (OPCs), highlighting their inability to mature into myelin-producing oligodendrocytes<sup>2</sup>. Thus, an important strategy may be to replace the lost oligodendrocytes and/or promote their maturation or proliferation. N-palmitoylethanolamine (PEA) is an endogenous fatty acid amide belonging to the N-acylethanolamines family. Studies demonstrate PEA to possess analgesic, anti-inflammatory, and neuroprotective actions<sup>3</sup>. More recently, a composite of co-ultramicronized PEA and the flavonoid luteolin (co-ultraPEA/Lut, 10:1 by mass) was shown to be more efficacious than PEA alone in improving outcome in experimental models of spinal cord injury<sup>4</sup>, traumatic brain injury<sup>5</sup> and Alzheimer disease<sup>6</sup>. Here, we examined the ability of co-ultraPEA/Lut to promote progression of OPCs into a differentiated phenotype. OPCs were prepared from newborn rat cortical mixed glial cell cultures as described<sup>7</sup>, and treated the following day with 10  $\mu$ M co-ultraPEA/Lut. Cells were collected 1, 4 and 8 days later and analyzed for expression of myelin basic protein (MBP), a major structural component of myelin expressed exclusively in myelinating glia. Real-Time Polymerase Chain Reaction (qPCR) and Western blot analyses revealed a time-dependent increase in expression of both mRNA for MBP and MBP content by immunoblotting. Treatment with either ultramicronized PEA or luteolin was ineffective. Co-ultraPEA/Lut also promoted morphological development of OPCs and total protein content without affecting proliferation. Co-ultraPEA/Lut may represent a novel pharmacological strategy to promote OPC maturation.

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## CONNEXIN 30: UNCONVENTIONAL DETERMINANT OF ASTROGLIAL SYNAPSE COVERAGE AND MEMORY

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Astrocytes play active roles in brain physiology by dynamic interactions with neurons. Connexin 30, one of the two main astroglial gap-junction subunits, is thought to be involved in behavioral and basic cognitive processes. However, the underlying cellular and molecular mechanisms are unknown. We show here in mice that connexin 30 controls hippocampal excitatory synaptic transmission through modulation of astroglial glutamate transport, which directly alters synaptic glutamate levels. Unexpectedly, we found that connexin 30 regulated cell adhesion and migration and that connexin 30 modulation of glutamate transport, occurring independently of its channel function, was mediated by morphological changes controlling insertion of astroglial processes into synaptic clefts. By setting excitatory synaptic strength, connexin 30 plays an important role in long-term synaptic plasticity and in hippocampus-based contextual memory. Taken together, these results establish connexin 30 as a critical regulator of synaptic strength by controlling the synaptic location of astroglial processes.

## KEEPING THE CNS CLEAR: GLIAL PHAGOCYTOSIS OF NEURONAL DEBRIS

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In the developing and mature central nervous system (CNS), a large number of neurons die through apoptosis and are efficiently removed by phagocytic glia. The precise removal of apoptotic neurons is crucial for the formation and maintenance of a functional CNS and must be tightly regulated. Despite its great biological importance, the molecular mechanisms underlying the relationship between neuronal apoptosis and glial phagocytosis remain unclear. Since apoptosis and apoptotic cell clearance are highly conserved in evolution, we use *Drosophila Melanogaster* as a model system, which provides powerful tools to study the establishment and function of glia as primary phagocytes in the developing and adult CNS.

## INVOLVEMENT OF ASTROCYTIC CB1 RECEPTORS IN SYNAPTIC PLASTICITY AND HIPPOCAMPAL-BASED MEMORY

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Endocannabinoid signalling in the brain can have multiple effects on synaptic transmission and plasticity phenomena correlated with memory functions. However the cell-types and mechanisms through which the endocannabinoids system impacts memory processing in a physiological context remain largely unclear. Although the type-1 cannabinoid receptor (CB1R) is mainly expressed by neurons, recent evidence suggests that CB1R is expressed by brain astrocytes. Dynamic bidirectional communication between astrocytes and neurons is now thought to contribute to brain information processing, thereby modulating important aspects of synaptic transmission and plasticity. Noteworthy, it is now established that glial cells exert a sharp control over the activity of glutamate receptors and in particular the N-methyl-D-Aspartate types (NMDARs), which are central to memory formation in the hippocampus. Recently, the exogenous activation of hippocampal astroglial by CB1 agonists was shown to impair working memory in mice and to induce NMDAR-dependent alterations of synaptic plasticity (Han et al., 2012). However, the endogenous roles of astroglial CB1R in memory processes are unknown. Here, we will show published and unpublished data on how astroglial CB1 receptors are necessary to modulate cannabinoids induced effects and also to mediate physiological long-term memory and synaptic plasticity.

*Neurobiology of food intake regulation*

## **DIETARY TRIGLYCERIDES ACT ON MESOLIMBIC STRUCTURES TO REGULATE THE REWARDING AND MOTIVATIONAL ASPECTS OF FEEDING**

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Circulating triglycerides (TGs) normally increase after a meal but are altered in pathophysiological conditions, such as obesity. Although TG metabolism in the brain remains poorly understood, several brain structures express enzymes that process TG enriched particles, including mesolimbic structures. For this reason, and because consumption of high-fat diet alters dopamine signaling, we tested the hypothesis that TG might directly target mesolimbic reward circuits to control reward-seeking behaviors.

We found that the delivery of small amounts of TG to the brain through the carotid artery rapidly reduced both spontaneous and amphetamine-induced locomotion, abolished preference for palatable food and reduced the motivation to engage in food-seeking behavior. Conversely, targeted disruption of the TG-hydrolyzing enzyme lipoprotein lipase specifically in the nucleus accumbens increased palatable food preference and food-seeking behavior. Finally, sustained hypertriglyceridemia achieved through prolonged TG perfusion diet-induced obesity resulted in a return to normal palatable food preference despite continued locomotor suppression, suggesting that adaptive mechanisms occur. These findings reveal new mechanisms by which dietary fat may alter mesolimbic circuit function and reward seeking.

## TIMED FEEDING: IMPACT ON CIRCADIAN CLOCKS AND METABOLISM

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Biological functions display daily rhythms, including feeding/lipogenesis during the active period and fasting/lipolysis during the resting period. This temporal organization is controlled by a multi-oscillatory circadian timing system. Timed feeding is a potent synchroniser for secondary clocks in peripheral organs like the liver, but not for the master clock located in the suprachiasmatic nuclei of the hypothalamus which mainly reset by ambient light. However, timed calorie restriction (i.e., when only a hypocaloric diet is given every day) is able to modify the suprachiasmatic clock and modulate synchronisation to light, via increased phase-shifting effects of light. High-fat feeding also affects the suprachiasmatic clock and modulates synchronisation to light, via reduced phase-shifting effects of light. Timed feeding triggers a rhythmic behaviour so-called “food-anticipatory activity” that animals manifest prior to food access. This behaviour is controlled by the food clock, which is thought to be a network of coupled meal-entrainable brain oscillators, including the metabolic hypothalamus, metabolic brainstem and the cerebellum. Furthermore, timing of feeding modulates energy metabolism. For instance, diet-induced obesity and hepatic steatosis can be reduced by restricting high-fat feeding at certain times of day, independently of calorie intake. Therefore, dietary strategies to limit, or prevent, overweight and obesity could be optimized by taking into times of feeding.

## MOLECULAR MECHANISMS UNDERLYING CANNABINOIDS ACTION ON HYPOTHALAMIC NEUROPEPTIDE NEURONS

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The use of *cannabis* derivatives as recreational and therapeutic drugs can be traced back to the earliest civilization and today, extracts of cannabis are among the most commonly used drugs for psychotropic effects. Cannabinoid receptors (mainly CB1) are expressed at high levels in many brain regions and the anatomical distribution is consistent with behavioural effects of cannabinoids, including: euphoria, decreased motor activity, impairment of memory, antinociception and modulation of food intake. In the brain, the regulatory effect of cannabinoids on feeding behaviour is believed to be mediate at tow levels. First, it tonically reinforces the motivation to find and consume foods, through significant interactions with mesolimbic pathways involved in reward mechanism. Second, it transiently regulates the levels and/or action of hypothalamic orexigenic and anorectic neuropeptides. At peripheral level, the modulation effect of cannabinoids on feeding behaviour is believed to be mediate through CB1 receptors located in the gut, hepatocyte and adipocyte cells.

To date there are few studies investigating the mechanisms that underlie the effects of cannabinoids on feeding behaviour, and more specifically the involvement of hypothalamic neuropeptidrgic systems, particularly, NPY, Orexin and PMOC neurones in the cannabinoids effects. Our results suggest that one of the possible mechanisms allowing the stimulation of food intake by CB1 receptor agonists is through an inhibition (or delaying) of some ventromedial hypothalamic neurotransmitters implicated in the regulation of the satiety processes and the stimulation of hypothalamic neuropeptide systems implicated in the increase of feeding behaviour. As an example of this type of regulation, some of our results indicate that CB1 cannabinoid receptor positively regulates pro-opiomelanocortin (POMC) transcription through activation of the cAMP-response element.

## ROLE OF CHEMOKINES IN NEUROINFLAMMATION AND FEEDING BEHAVIOR

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What is the role of chemokines in the central regulation of feeding behavior and weight maintenance? Although the role of chemokine production has been long-time ignored, evidence is starting to accumulate indicating that answers to this question could be a key to a better understanding of eating disorders such as obesity or anorexia nervosa. Chemokines are well known to be produced after immune system activation in response to injury or infection. Interestingly, obesity is accompanied by low-grade inflammation, while anorexia nervosa patients are surprisingly free of infections. Our general objective is therefore to elucidate the neuroimmunological mechanisms modulating appetite and body weight regulation.

As a first step to this goal we have recently found that chemokines displayed changes in seric content that could reflect alterations in energy homeostasis associated with obesity and anorexia. We characterized thereafter chemokine production, regulation and function in animal models. In particular, we investigated which brain structures are activated and which chemokine-neuropeptide transcription patterns occur in these structures following peripheral or central administration of a general inflammatory inductor (LPS) or selected chemokines. More detailed aspects of brain cell activity on selected neuronal networks, particularly these expressing the orexigenic peptide melanin-concentrating hormone, have been addressed using eGFP-expressing mice. The possible mechanisms and their consequences in terms of modulation of neuro-endocrine systems and pathophysiology involved in feeding behavior will be discussed.

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## 26RFA, A NOVEL NEUROPEPTIDE REGULATING FEEDING BEHAVIOR AND GLUCOSE HOMEOSTASIS

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The neuropeptide 26RFa is a novel member of the RFamide peptide family initially isolated by our group from the frog brain. Characterization of the 26RFa precursor in various vertebrate species revealed that the primary structure of 26RFa is strongly conserved from fish to human. 26RFa was subsequently identified as the cognate ligand of the human orphan receptor GPR103. In the rat brain, 26RFa mRNA is primarily expressed in the ventromedial hypothalamic nucleus (VMH) and the lateral hypothalamic area, two structures involved in the hypothalamic control of feeding behavior. GPR103-expressing neurons are abundant in a number of brain nuclei associated with the regulation of food intake, and notably the arcuate nucleus (Arc). Consistent with this neuroanatomical distribution of the neuropeptide and its receptor, we found that i.c.v. administration of 26RFa induces a dose-dependent increase of food consumption in mice and rats. The mechanism of action of 26RFa in the control of feeding behavior was also investigated. Our data revealed that 26RFa exerts its orexigenic effect by modulating the activity of the NPY/Proopiomelanocortin neurons of the Arc.

In addition, recent data obtained by our group indicate that 26RFa is involved in glucose homeostasis. Indeed, we found that 26RFa and its receptor GPR103 are present in pancreatic  $\beta$  cells and in the gut. 26RFa attenuates the hyperglycemia induced by a glucose load, potentiates insulin sensitivity and increases plasma insulin concentrations. Consistent with these data, 26RFa stimulates insulin production by MIN6 insulinoma cells. Finally, we show, using *in vivo* and *in vitro* approaches, that a glucose load induces a massive secretion of 26RFa by the small intestine. Altogether, these new data suggest that 26RFa acts as an incretin to regulate glucose homeostasis. “Supported by grants from INSERM, the Fondation pour la Recherche Médicale, the LARC-Neuroscience network, the University of Rouen and the Conseil Régional de Haute-Normandie”.

***Depression, anxiety and addiction: is it all converge to impaired dopaminergic function?***

## SEX DIFFERENCES IN MODELS OF DEPRESSION: IS IT ALL ABOUT SEROTONIN?

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Psychiatric disorders, such as depression are characterized by sex differences in epidemiology, symptomatology and treatment response. Animal models have been widely employed for investigating the neurobiology of such disorders and the discovery of new treatments. However, mostly male animals were used in preclinical pharmacological studies<sup>1</sup>. During the last decade, our group has studied sex differences in behavior, as well as in neurochemical, neurobiological and neuroplasticity indices in different rodent models, such as the forced swim test (FST), the chronic mild stress (CMS) and others. At a behavioural level, depressive-like symptomatology in the FST is more evident in females than males, whereas this does not depend on sex differences in circulating corticosterone levels. Moreover, we have reported that CMS causes decreased serotonergic activity in the hippocampus and decreased dopaminergic activity in the prefrontal cortex of female rats. Following FST, serotonergic turnover ratio in the hippocampus is also decreased in female rats, whereas dopaminergic activity is enhanced in the hippocampus and the prefrontal cortex of male rats only (2). Regarding the role of gonadal hormones, we investigate the role of circulating hormones, as well as the role of locally synthesized estrogens in the brain by the enzyme aromatase. Our data indicate that estrogens originating from the gonads and the brain significantly affect the FST behavioral response and neurochemical indices<sup>3</sup>.

Overall, sex-differentiated neurobiological and behavioural indices in animal models of depression point to a key role of serotonin, but dopamine, as well. Lastly, we highlight the need for the inclusion of both males and females in experimental studies aiming at gender-oriented prevention, diagnosis and treatment of psychiatric disorders.

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## EFFECTS OF IN UTERO EXPOSURE TO CANNABINOIDS ON POSTNATAL SYNAPTIC MATURATION OF GLUTAMATERGIC TRANSMISSION IN THE VTA

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Despite the high prevalence of *Cannabis* use among pregnant women, its impact on the developing brain is still not accurate. However, the endocannabinoid system plays a key role in brain maturation and development in several structures including those relevant for mood, cognition, and reward, such as the mesocorticolimbic system. Thus, it is expected that exposure to *Cannabis* during early ontogeny might be detrimental, perturb fetal development and affect synaptic maturation in the offspring. 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-mediated short-term plasticity at excitatory synapses onto VTA DA cells. To this aim, the dams were administered either the psychoactive ingredient of *Cannabis*, D<sup>9</sup>-tetrahydrocannabinol (THC, 1 mg/kg s.c.) or a synthetic agonist at type-1 cannabinoid receptors (CB1), WIN55-212,2 (WIN, 0.5 mg/kg s.c.) once per day from GD 5 to GD 20. We found that in the off-spring of cannabinoid-exposed dams, during the third postnatal week, glutamatergic transmission was dominated by calcium-permeable AMPA receptors, which are immature. The delayed AMPA receptor switch in the offspring was accompanied by a paired-pulse facilitation and an increased AMPA to NMDA ratio. In addition, changes in endocannabinoid-mediated short term plasticity at these synapses was observed, which appear not to be related to CB1 receptor number/function.

Since prenatal *Cannabis* exposure has been linked to addiction vulnerability, and similar effects on glutamatergic transmission onto VTA DA neurons have been described following *in utero* exposure to cocaine, the present data suggest that maternal *Cannabis* use alters developmental regulation of mesolimbic DA system, thus prolonging the developmental critical period. Whether or not these changes are long lasting and/or might contribute to addiction vulnerability later in life has to be examined.

## ***IN VIVO* HOMEOSTATIC PLASTICITY AT THE SINGLE-CELL LEVEL IN THE BNST TRIGGERS PERSISTENT ANXIOLYTIC EFFECT**

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Anxiety is controlled by multiple neuronal circuits that share robust and reciprocal connections with the bed nucleus of the stria terminalis (BNST), a key structure controlling negative emotional states. However, it is unclear how BNST integrates diverse inputs to modulate anxiety. In this study, we evaluated the contribution of infralimbic cortex (ILCx) and ventral subiculum (vSUB) inputs in regulating BNST activity at the single-cell level. We combined *in vivo* single-cell recordings, tract-tracing, pharmacological approaches and behavioral testing to elucidate the network organization and the molecular machinery that contributes to changes in BNST synaptic function. We show that stimulation of the vSUB promotes homeostatic plasticity at specific BNST inputs and triggers persistent anxiolytic effect. We demonstrate that vSUB stimulation promotes *in vivo* CB<sub>1</sub>- and NMDA-receptors dependent homeostatic plasticity at the single-cell level in the BNST, which is instrumental for decreasing anxiety-like behavior.

## TARGETING DRUG-CUE ASSOCIATIONS TO PREVENT DRUG RELAPSE

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Preventing relapse to drug use is a major challenge in the treatment of drug addiction. Relapse is often initiated by exposure to the drug itself but mostly by drug-associated cues. Drug cues can be environmental or interoceptive. The risk of relapse is elevated when addicts encounter people, places or paraphernalia associated with earlier drug use. The phenomena of craving and cue-induced relapse appear to arise from long-term neuroadaptations within specific neuronal systems. Subcortical regions, such as the amygdala, the NAc and the mesolimbic dopamine system, have been shown in animal models to promote self-administration of drugs of abuse. Addictive drugs produce long-lived alterations in behavior largely by usurping normal mechanisms of associative memory.

In this talk, I will present biochemical and behavioral evidence for the molecular mechanisms that underlie the changes in proteins function and signaling pathways that occur in the NAc following prolonged cessation of repeated cocaine exposures. The talk will be focused on the alterations in the expression and function of glutamate receptors, key players in synaptic plasticity and learning and memory, which critically contribute to the development and expression of cocaine psychomotor sensitization. Furthermore, I will show that inhibition of PKM $\zeta$ , an important regulator of synaptic plasticity, in the NAc shell following cocaine-induced conditioned place preference (CPP), completely erases the expression and reinstatement of cocaine CPP. Together, these studies suggest that the molecular machinery that subserves normal learning and memory processes in the NAc is a critical target for the prevention of drug craving and relapse.

## FUNCTIONAL DIVERSITY OF MIDBRAIN DOPAMINE NEURONS

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Until recently dopamine neurons were commonly viewed as functionally homogeneous. However, emerging evidence now indicates that subgroups of dopamine neurons exhibit distinct information coding properties and synaptic adaptations in response to a range of motivationally-significant stimuli. For example, previous work from us and others has shown that dopamine neurons display differential responses to aversive events depending on their location within the midbrain and their dendritic structure. Moreover, it appears that distinct subgroups of dopamine neurons exhibit synaptic plasticity in response to different types of stimuli, including drugs and aversive events. I will present evidence for further diversity with respect to a subgroup of dopamine neurons in the dorsal raphe nucleus (DRN) and ventro-lateral periaqueductal grey (vlPAG). We find, for example, that these dopamine neurons exhibit synaptic adaptations 24 hours following a single dose of cocaine similar to those seen in many ventral tegmental area (VTA) dopamine neurons (i.e., an increase in the AMPA/NMDA ratio). However, in contrast to VTA dopamine neurons, DRN/vlPAG dopamine neurons also exhibit a similar adaptation in response to social isolation that is correlated with the number of previous cage mates. Moreover, similar to cocaine-induced synaptic adaptations in the VTA, it involves a change in AMPA receptor rectification, suggesting a switch in AMPA receptor subunit composition. We suggest that these neurons may play a role in social interactions that is distinct from other dopamine neuron subgroups of dopamine neurons. More generally, I shall consider how to integrate these findings within a broader working framework for understanding how diversity of firing activity relates to diversity of plasticity in these subgroups.

*Parkinson's disease - from neuroprotection to the treatment of motor and non-motor symptoms*

## MICROGLIOSIS IN PARKINSON'S DISEASE: CAN WE HARNESS IT FOR GOOD?

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Skewed microglia activation with pro-inflammatory prevailing over anti-inflammatory phenotypes may contribute to neurotoxicity in Parkinson's disease (PD), via the production of cytokines and neurotoxic species. Targeting the microglia polarization process is a proposed strategy for neuroprotection. The peroxisome proliferator-activated receptor (PPAR)gamma is expressed in microglia and peripheral immune cells, where they are involved in macrophages polarization. PPAR $\gamma$  agonists mediate neuroprotection in PD models. We investigated the neuroprotective activity of PPAR $\gamma$  agonists in the subacute MPTP and the chronic MPTP/probenecid (MPTPp) models of PD, and their action on microglia polarization via the evaluation of pro- and anti-inflammatory molecules. PPAR $\gamma$  agonists rosiglitazone and the novel compound MDG548, were neuroprotective in both MPTP models and reduced microglia activation and iNOS production in the substantia nigra compacta (SNc). In the MPTPp model we found a gradual increase of pro-inflammatory cytokines tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$ , over anti-inflammatory molecules such as transforming growth factor (TGF)- $\beta$ , IL-10 and CD206, within Iba-1-positive microglia, suggesting that a skewed polarization was associated with disease progression. Rosiglitazone administered during the full MPTPp treatment or for the last 10 days, reduced pro-inflammatory cytokines while increasing anti-inflammatory molecules as compared with the MPTPp treatment. Therefore, neuroprotective treatment with PPAR- $\gamma$  agonists exerts an anti-inflammatory action via a modulation of microglia polarization correcting the imbalance between pro- over anti-inflammatory molecules, offering a novel immunomodulatory approach to neuroprotection.

## AMYLOID-LIPID MEMBRANE INTERACTION: A NOVEL TARGET FOR NEURODEGENERATION

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Alzheimer's disease (AD) and Parkinson's disease (PD) are neurodegenerative disorders characterised by the misfolding of proteins into soluble prefibrillar aggregates. In our work, we have demonstrated that amyloid aggregates of the amyloid- $\beta$ (1-42) peptide,  $\alpha$ -synuclein and tau proteins robustly compromise the integrity of model membranes, hence mimicking the mechanism of pore-forming bacterial peptides like gramicidin. Eleven natural polyphenolic compounds and black tea extract were screened for protection against membrane damage by the amyloid aggregates. We identified a select group of potent inhibitory compounds which include baicalein, morin, nordihydroguaiaretic acid and black tea extract<sup>1,2</sup>. Since mitochondria are intimately involved in the pathophysiological cascades of both AD and PD<sup>3</sup>, the interaction of soluble amyloid aggregates with mitochondrial membranes was further explored. We made use of two reductionist models: (i) lipid vesicles with defined membrane compositions that mimic those of mitochondrial membranes, and (ii) isolated mitochondria from neuronal cells. Briefly, we found that aggregates induced a robust permeabilisation of mitochondrial-like vesicles and triggered cytochrome *c* release from isolated mitochondrial organelles. Importantly, the effect on mitochondria was shown to be dependent upon cardiolipin, an anionic phospholipid unique to mitochondria and a well-known key player in mitochondrial apoptosis<sup>4</sup>. Thus, we propose a generic mechanism of thrilling mitochondria in which soluble amyloid aggregates have the intrinsic capacity to permeabilise mitochondrial membranes, without the need of any other protein.

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## **INTRACELLULAR SIGNALLING MECHANISMS CONTROLLING L-DOPA INDUCED DYSKINESIA: A TRANSLATIONAL NEUROSCIENCE PERSPECTIVE**

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Hyperdopaminergic disorders include addictive behaviour in response to psychostimulants and L-DOPA induced dyskinesia in Parkinson's Disease patients. These disorders are characterised by abnormal cellular changes in the basal ganglia system and in particular in the striatum. Among the intracellular signalling cascades found altered in these two brain diseases, the Ras-ERK pathway seems to play a key role in their pathogenesis. Here it will be demonstrated that ERK and Ras-GRF1, as a striatal integrator of dopamine and glutamate signals to Ras, are not only essential for generating normal behavioural and electrophysiological responses in the striatum but also are implicated in both cocaine dependent alterations and aberrant motor symptoms associated to chronic L-DOPA treatment in a mouse model of Parkinson's Disease. Experimental evidence supporting combination therapies for these brain disorders targeting distinct components of Ras-ERK and related signal transduction pathways using small molecules, cell penetrating peptides and gene therapy approaches will be discussed.

## **COGNITIVE AND AFFECTIVE DYSFUNCTIONS IN EXPERIMENTAL PARKINSONISM: MODELING AND MECHANISMS**

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Cognitive and psychiatric disorders are increasingly recognized as a major challenge in the treatment of Parkinson's disease (PD). These non-motor symptoms, which often appear in the early stage of the disease, affect a large number of patients and are only partly resolved by conventional antiparkinsonian medications, such as L-DOPA. Here, we investigated memory deficits, as well as depressive- and anxiety-like behaviors in a mouse model of PD, based on bilateral injection of the toxin 6-hydroxydopamine (6-OHDA), in the dorsal striatum. This model displays reduced olfactory discrimination, decreased rearing and subtle gait modifications, which do not affect horizontal motor acitivity.

Bilateral 6-OHDA lesion reduced long-term, but not short-term, novel object recognition and decreased long-term potentiation specifically in the dentate gyrus. In addition, mice with a 6-OHDA lesion showed increased immobility in the forced swim test and tail suspension test, two behavioral paradigms of depression. The lesion exerted also anxiogenic effects, as shown by reduced time spent in the open arms, in the elevated plus maze test, and by increased thigmotaxis in the open-field test. L-DOPA rescued the deficit in long-term recognition memory, but did not modify depressive- and anxiety-like behaviors, which were instead counteracted by the dopamine D2/D3 receptor agonist, pramipexole. Reboxetine, a noradrenaline reuptake inhibitor, was also able to prevent the depressive and anxiogenic effects produced by the lesion with 6-OHDA. These data are discussed with regard to the possible mechanisms of action of dopaminergic and noradrenergic drugs.

## ACTIVATION OF DREAM, A CALCIUM-BINDING PROTEIN, REDUCES L-DOPA-INDUCED DYSKINESIAS IN MICE

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Previous studies have implicated the cAMP/PKA pathway as well as FosB and dynorphin-B expression mediated by dopamine D1 receptor stimulation in the development of L-DOPA-induced dyskinesia (LID). The magnitude of these molecular changes correlates with the intensity of dyskinesias. The calcium-binding protein DREAM binds to DRE sites in the DNA and represses transcription of target genes such as c-fos, Fos-related antigen-2 (fra-2) and prodynorphin. This repression is released by Ca<sup>2+</sup> and PKA activation. Dominant-active DREAM transgenic mice (daDREAM) and DREAM knockout mice (DREAM<sup>-/-</sup>) were used to define the involvement of DREAM in dyskinesias.

Dyskinesias were evaluated twice a week in 6-OHDA-lesioned mice during chronic L-DOPA (25 mg/kg). The impact of DREAM on L-DOPA efficacy was evaluated using the rotarod and the cylinder test after the establishment of dyskinesia and the molecular changes by immunohistochemistry and Western blot.

In daDREAM mice, LID was decreased during the entire treatment. In correlation with these behavioral results, daDREAM mice showed a decrease in FosB, P-Ach3, dynorphin-B and P-GluR1 expression. Conversely, genetic inactivation of DREAM potentiated the intensity of dyskinesia, and DREAM<sup>-/-</sup> mice exhibited an increase in expression of molecular markers associated with dyskinesias. Importantly, DREAM modifications did not affect the kinetic profile or antiparkinsonian efficacy of L-DOPA therapy.

DREAM decreases development of LID in mice and reduces L-DOPA-induced expression of FosB, P-Ach3 and dynorphin-B in the striatum. These data suggest that therapeutic approaches that activate DREAM may be useful to alleviate L-DOPA-induced dyskinesia without interfering with its therapeutic motor effects.

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*Neuronal plasticity in health and disease:  
cognitive and clinical perspectives*

## NEURAL AND PHYSIOLOGICAL CORRELATES OF MOTOR MEMORY LEARNING AND CONSOLIDATION

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For the last 20 years, research in my laboratory has focused on investigating the behavioral, neuronal and neurophysiological determinants of motor skill learning and consolidation (Albouy et al., 2013; Doyon, 2008; Doyon & Ungerleider, 2002; Doyon & Benali, 2005; Doyon et al., 2003, 2009, 2011, 2014; Fogel et al., 2012; King et al., 2013 for reviews). During this presentation, I will first briefly review some of our work focusing on motor sequence learning (MSL), which refers to the process by which movement elements come to be performed effortlessly as a unitary sequence through multiple sessions of practice. I will summarize the results of studies, which demonstrate that interactions between the cortico-striatal and cortico-cerebellar systems, as well as the spinal cord, are critical for establishing the motor routines used to acquire new sequence of movements. I will then present in more details other studies that aimed to identify the neural substrates and physiological correlates mediating the consolidation process of a newly acquired sequence of movements. More specifically, I will discuss our findings in healthy control subjects that either used behavioral night/nap protocols alone, or combined with functional magnetic resonance imaging (fMRI), electroencephalographic (EEG) recordings and/or a motor sequence-olfactory conditioning paradigm. I will argue that the consolidation of such a memory trace depends upon greater functional integration of the cortico-striatal system and N-REM sleep spindle activity measured during the post-training night.

## LONGITUDINAL FUNCTIONAL NETWORK ANALYSIS FOR MOTOR SKILL LEARNING IN HUMANS

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In a large number of imaging studies using fMRI studies in young<sup>1,2,3,4</sup> and older healthy subjects<sup>5</sup>, we have shown that both cortico-striatal (CS) and cortico cerebellar (CC) systems contribute differentially to motor skill learning. For example, we have identified the dynamic representational changes that occur within the cerebellar cortex and nuclei, in concert with the striatum and the motor cortical regions of the frontal and parietal lobes, during the fast and slow learning stages of a new motor skilled behavior<sup>6</sup>. We have then described the plasticity that occurs within the basal ganglia, and the putamen in particular, during the early and automatization process of MSL. Our results show that the motor representation acquired during this phase transfers from the associative to the sensorimotor region of the putamen<sup>3</sup>. We have also reported that the putamen is associated with improved kinematics measures, and particularly in the subjects' abilities to make transitions between finger movements when practicing a sequence, hence suggesting that the latter structure is involved in "chunking" elements together<sup>7</sup>. These findings are consistent with a model of motor skill learning that Dr. Julien Doyon first proposed in 2002<sup>6,8</sup>. The latter suggests that the cerebellum plays a critical role in the acquisition of new motor sequences, but that once subjects reach asymptotic performance, representational changes in motor cortical regions, in link with the striatum, may be sufficient for the long-term storage of the trained skill. This model also put forward that the reverse pattern of plasticity can be observed during motor adaptation (MA), where the cerebellum and motor cortical regions through glutamatergic modulation<sup>2</sup>, but not the striatum, contribute to the long-term representation of this form of learning. fMRI scanning during long rest periods and data-driven functional connectivity analyses has been conducted in order to explore the brain plasticity of task-related large-scale networks and their functional integrations.

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## BRAIN PLASTICITY INDUCED BY HEMODIALYSIS: NEUROANATOMICAL AND BOLD-FMRI STUDY

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Recent studies demonstrated that hemodialysis in the context of chronic renal failure originates oxidative stress that is a known factor contributing to long-term complications of dialysis. Despite recent progress on the choice of hemodialysis membrane, hemodialysis sessions are still causing side effects on general health especially on brain functioning. This includes plasticity and functional control organization of the anatomical entities involved in the control of given function.

The goal of this study is to demonstrate the basic neuroanatomical and neurophysiological changes induced by hemodialysis. Such impact on plasticity is studied in contexts of using two biocompatible hemodialysis membranes.

12 male volunteers following chronic hemodialysis for more than 6 months were recruited. Diabetic, smoking and patients with episodes of infection or treatment with iron or erythropoietin injection were excluded. Similarly, the BOLD-fMRI was performed before and after hemodialysis using motor paradigm immediately before and after hemodialysis sessions; the fMRI data was processed using SPM12 package.

The biological results of this study showed that hemodialysis increases the oxidative stress in these patients. [Malondialdehyde before hemodialysis =  $3,550 \pm 0,580\mu\text{M}$  vs. Malondialdehyde after hemodialysis =  $9,899 \pm 8,367\mu\text{M}$ ;  $p=0,002$ ].

BOLD-fMRI revealed significant activation of the motor cortex, the BOLD signal in the activated site is inversely correlated with level of oxidative stress.

Hemodialysis raises the inflammatory state of the brain tissue reflecting increased oxidative stress, while it was expected to decrease considering the removal of free radicals responsible of oxidative stress by hemodialysis procedure. Hence, particular care must be paid to hemodialysis patients considering the long term impact on general health and brain tissues in particular.

## **SOCIAL LEARNING: FROM BEHAVIOR TO NEURONS**

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While neuroscience research has tremendously advanced our knowledge about the neural mechanisms of individual learning, i.e. through trial-and-error, it is only recently that neuroscientists have begun to study observational learning, and thus little is known about its neural mechanisms. One limitation is that observational learning has been addressed under unconstrained experimental conditions, not compatible with neuronal recordings. We have first verified whether monkeys learn by observation under the constraining conditions of behavioral neurophysiology. Two animals sat in primate chairs facing each other, with their head fixed. A touch screen was placed face up between the chairs at arm's reach, and the monkeys were trained on an abstract visuomotor associative task. In one experiment, the monkeys alternated the roles of "actor" and "observer". The actor learned to associate visual cues with reaching targets, while the observer "watched" freely. Then, the observer was given the same cue-target associations just performed by the actor, or had to learn new, not previously observed ones. In experiment 2, one monkey learned from a human actor who performed the task with errors only, or with successes only in separate blocks. Monkeys learned from each other and from the experimenter, and gained more from the actor's errors than from his successes. We then recorded single neuronal activity from the observer's brain (dorsolateral prefrontal cortex, and anterior cingulate cortex) during three conditions: trial-and-error learning (TE); observation of new associations; execution of observed associations. Analysis on feedback related activity showed that the majority of neurons (n=115; 80%) were differentially active in TE versus observational learning. Most neurons responded to feedback information (on the actor's choices) during observation. These results show that prefrontal neurons that process feedback signals during individual learning are also involved in processing these signals when derived from others behavior.

**BRAIN, COMMUNICATION AND AGING:  
A LIFE-LONG ADAPTATIVE PROCESS SUSTAINING QUALITY OF LIFE**

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According to the World Health Organization, one of the determinants of an active aging is the ability to maintain social participation and to benefit from a rich social network. Communication abilities are crucial in this respect. Though communication abilities depend upon many cognitive processes that have a tendency to be affected in aging, the presence of some neurofunctional and neurocognitive adaptative processes makes it such that communication abilities are relatively preserved with age and able to support social participation. This presentation will summarize a number of studies by our group describing the neurofunctional and neurocognitive adaptative processes that allow the preservation of communication abilities in active aging. These neurofunctional and neurocognitive adaptative processes include changes in the characteristics of the neural network sustaining the semantic processing of words and discourse abilities. It also reflects the engagement of distinctive cognitive strategies based on the use of semantic knowledge, rather than on the purely executive functions. Taken together, these processes show how the brain is capable of adapting itself in aging in order to maintain social participation and networking as we become older and as we need them more and more.

***Genes do matter: insights in the genetic  
vulnerability to nicotine addiction***

## THE ROLE OF THE $\beta$ 4-CONTAINING NICOTINIC ACETYLCHOLINE RECEPTORS IN NICOTINE ADDICTION

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Nicotine is the chief neuroactive compound in tobacco that drives its repeated consumption. Nicotine exerts its neurological effects by activating nicotinic acetylcholine receptors (nAChRs), pentameric ligand-gated ion channels composed of  $\alpha$ , or  $\alpha$  plus  $\beta$  subunits ( $\alpha$ 2-10 and  $\beta$ 2-4). Human genetic studies have highlighted the polymorphic nature of the *CHRNA5-CHRNA3-CHRN4* genomic cluster, coding for subunits  $\alpha$ 5,  $\alpha$ 3 and  $\beta$ 4, and its implication in smoking behaviours. Variants in the  $\beta$ 4 regulatory domain reduce the age of tobacco initiation, whilst gain of function SNPs in  $\beta$ 4 (T91I and T375I) reduce the risk of nicotine dependence<sup>1,2,3</sup>. The role of  $\beta$ 4-containing ( $\beta$ 4\*) nAChRs has therefore been assessed in mouse models to elucidate their contribution to the pathophysiology of nicotine addiction. Over- expressing the *Chrna5-Chrna3-Chrnb4* cluster increases acquisition of nicotine self-administration, whereas over-expression solely of  $\beta$ 4 increases aversion to nicotine.  $\beta$ 4 knockout mice do not self-administer otherwise reinforcing doses of nicotine, a deficit that is reflected in the meso-accumbal network. The self-administration deficit is attenuated by viral-mediated re-expression of  $\beta$ 4 in the MHB, an effect that is reached only when above a critical threshold of  $\beta$ 4\* nAChR restoration. This identifies the MHB as host for  $\beta$ 4's regulation of nicotine reinforcement, and strengthens the importance of  $\beta$ 4 function and expression levels on rewarding properties of nicotine. Transgenic mice have therefore provided evidence of nAChRs' contribution to nicotine addiction processes, and the use of lentivirus technology has allowed us to stratify the role of nAChR subtypes in different neural networks.

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## STRAIN DIFFERENCES IN THE LONG-LASTING EFFECTS OF ADOLESCENT NICOTINE EXPOSURE ON MESOLIMBIC DOPAMINE TRANSMISSION OF LEWIS AND FISCHER344 RATS

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Numerous family, adoption and twin studies have outlined the significant role of heritable influence on individual vulnerability to addiction<sup>3</sup>. Results from twin studies have indicated that 33-74 % of the variance in liability to nicotine dependence can be accounted for heritable influences<sup>4,6</sup>. Similar contribution has been found for cocaine addiction<sup>5,7</sup>. Among factors influencing variation in heritability there is the developmental stage<sup>1</sup>. A significant overlap of genetic influences of alcohol, nicotine and other illicit drug addictions has emerged<sup>1</sup>, while results from genetic studies found only limited support for a contribution of specific genetic factors to individual vulnerability to specific drugs, thus supporting a role for common pathways in the vulnerability to different drugs of abuse (common liability theory)<sup>7</sup>. Given these premises, in order to investigate further the neurobiological mechanisms underlying common vulnerability to addiction, we studied the long-lasting effects of adolescent nicotine exposure on mesolimbic dopamine (DA) transmission by using a genetic animal model, the inbred Lewis (LEW) and Fischer 344 (F344) rat strains.

F344 and LEW male rats of 6 weeks of age (38-42 PND) were exposed once a day for 5 days to nicotine (0.4 mg/kg s.c.). At adulthood (10-12 weeks) animals were implanted with two microdialysis probes in the nucleus accumbens (NAc) shell and core<sup>2</sup>. The following day challenge with nicotine (0.4 mg/kg s.c.) showed that adolescent nicotine exposure potentiated DA response to nicotine in the NAc core of both strains, while in the NAc shell DA responsiveness was increased only in the vulnerable genotype (LEW) while unchanged in the F344 strain. Adolescent exposure to nicotine potentiated moreover DA increase induced by cocaine (10 mg/kg i.p.) in the NAc shell of LEW but not in F344 rats, while increasing DA response in the NAc core of this strain. This increased effect of cocaine in the NAc shell of LEW rats was paralleled by an increased rewarding effect of cocaine in this strain, as assessed by conditioned place preference studies. These results highlight the influence of the genetic background in the long-lasting effect of adolescent nicotine exposure and suggest that nicotine exposure during adolescence might increase nicotine rewarding properties in genetically vulnerable individuals, facilitating development of nicotine addiction but also functioning as a gateway toward abuse of other illicit drugs (cocaine).

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## USING BIOMARKERS OF TOBACCO EXPOSURE IN GENOME-WIDE ASSOCIATION STUDIES

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Tobacco use remains the leading cause of preventable death worldwide. Establishing the genetic aetiology of tobacco use and dependence is an important first step in understanding the neurobiological mechanisms of tobacco use, and in turn the development of effective treatments. Whilst genome-wide association studies (GWAS) have enjoyed considerable success in identifying genetic variants associated with complex behaviours such as cigarette smoking, the proportion of phenotypic variation explained remains modest. Given the need for large sample sizes in GWAS, behavioural phenotypes are often assessed using self-report measures (e.g., number of cigarettes smoked per day). These may be subject to reporting biases (e.g., a smoker may report smoking less than he or she actually smokes) or error. Objective assessment of behavioural phenotypes, using relevant biomarkers, can address these limitations and provide greater measurement precision, therefore improving statistical power. This may enable us to identify novel variants associated with tobacco use and other complex behaviours. We will discuss the results of a GWAS meta-analysis of levels of cotinine, the primary metabolite of nicotine, based on 4,548 daily smokers of European ancestry. Variants in two genomic regions were found to be associated with cotinine levels, including 15q25.1 (a region previously identified in association with self-reported smoking quantity) and a novel locus at 4q13.2. Further, we will discuss other GWAS employing alternative tobacco use biomarkers, such as exhaled carbon monoxide levels, and conclude with a discussion on the benefits and limitations of employing such phenotypes in genetic association studies.

**THE RS16969968 POLYMORPHISM PREDISPOSING TO SMOKING CONFERS MIDBRAIN- AND HIPPOCAMPUS-DEPENDENT AFFECTIVE IMPAIRMENTS THAT ARE ALLEVIATED BY NICOTINE**

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Although smoking prevalence has declined in recent years, certain subpopulations continue to smoke at high rates and show resistance to cessation treatments. Human genetic studies have reported a strong association between a haplotype encompassing a non-synonymous single nucleotide polymorphism (SNP), rs16969968, in the nicotinic acetylcholine receptor (nAChR)  $\alpha 5$  subunit gene ( $\alpha 5$ SNP), and predisposition to smoking. The  $\alpha 5$ SNP has been shown to confer decreased sensitivity to nicotine reward in a mouse model, which may appear paradoxical given the higher risk for addiction. We assessed whether the  $\alpha 5$ SNP confers ‘addiction-prone’ behavioural phenotypes, known to promote vulnerability to drug abuse and observable before exposure to the drug. We demonstrate that knockouts for the  $\alpha 5$  gene ( $\alpha 5^{-/-}$  mice) exhibit motivational and emotional deficits such as decreased exploratory and high anxiety-like behaviour. We reveal, using lentiviral vectors, that re-expression of  $\alpha 5$  in the ventral tegmental area (VTA) and hippocampus of  $\alpha 5^{-/-}$  mice rescues the respective phenotypes. Importantly, we show that expressing the  $\alpha 5$ SNP instead of the wild-type allele of  $\alpha 5$  results in a knockout-like phenotype for both behavioural parameters. Furthermore, we observe that exposure to nicotine alleviates the behavioural deficits identified in  $\alpha 5^{-/-}$  mice. Using *in vivo* microdialysis and electrophysiology, we identify altered spontaneous and nicotine-elicited serotonin and dopamine activity, in the dorsal raphe nucleus, hippocampus and VTA of  $\alpha 5^{-/-}$  mice, which underlie the observed behaviours. We demonstrate that expressing the rs16969968 SNP in specific brain areas of the mouse leads to midbrain- and hippocampus-dependent motivational/affective deficits that are alleviated by nicotine. We propose that, in addition to impaired sensitivity to nicotine, individuals carrying the haplotype of the *CHRNA5* gene exhibit altered cholinergic signalling through the  $\alpha 5^*$ -nAChRs associated with affective or cognitive distress favouring high levels of smoking as a form of self-medication.

*Interaction between dopaminergic and non-dopaminergic neurotransmission on Parkinson's disease: implication in therapy, dyskinesia and neuroprotection*

## NOVEL THERAPEUTIC STRATEGY FOR THE CONTROL OF DYSKINESIA IN THE THERAPY OF PARKINSON'S DISEASE: ACUTE AND CHRONIC STUDIES

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Recent report demonstrated that mixed serotonin 5-HT<sub>1A/B</sub> receptor agonist, eltoprazine, produces a near to full suppression of dyskinetic-like behaviors in animal models of Parkinson's disease (PD). However, eltoprazine resulted in a partial reduction of motility induced by L-dopa, both in rodents and in non-human primates. Moreover, in a recent clinical trial, the partial 5-HT<sub>1A</sub> agonist sarizotan has been found to be only partially effective. Preclinical and clinical studies showed that adenosine A<sub>2A</sub> receptor antagonists as preladenant, significantly increase L-dopa efficacy in PD, without exacerbating dyskinetic-like behaviors. On this basis, we hypothesize that combination of eltoprazine with preladenant may produce prevention or suppression of L-dopa-induced dyskinesia, without impairing the efficacy of L-dopa in relieving motor symptoms. Unilateral 6-hydroxydopamine-lesioned rats, L-dopa-naïve or rendered dyskinetic by repeated-L-dopa-treatment, were administered with eltoprazine (0.3 or 0.6 mg/kg) and preladenant (0.3 or 1 mg/kg), alone or in combination with L-dopa (4 or 6 mg/kg), and rotational behavior, as index of motility, and abnormal involuntary movements (AIMs) as index of dyskinesia, were evaluated.

Results show that combined administration of L-dopa (4 mg/kg) plus eltoprazine (0.6 mg/kg) plus preladenant (0.3 mg/kg) significantly prevented or reduced dyskinetic-like behaviors, as revealed by AIMs test without impairing the motor activity, as revealed by similar number of contralateral and ipsilateral rotations. Moreover, acutely, the combined treatment appears to prevent worsening of the motor performance induced in L-dopa-naïve animals by eltoprazine plus L-dopa in the adjusting step test and the initiation time of stepping, two tests with high predictive validity of PD associated motor disability.

Overall these data suggest that combination of L-dopa (4mg/kg) with eltoprazine (0.6mg/kg) and preladenant (0.3mg/kg) could be a new therapeutic strategy for treating motor symptoms and dyskinesia in PD.

**BEE VENOM AND APAMIN, A SMALL CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNEL BLOCKER, ARE POTENTIAL NEW THERAPEUTIC TARGETS FOR PARKINSON'S DISEASE**

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Patients with Parkinson's disease (PD) suffer from dopamine (DA) deficiency in the basal ganglia (BG) and are currently treated with the DA precursor L-DOPA. Long-term treatment alleviates motor symptoms but leads over the years to disabling dyskinesia. Search for new therapies is therefore crucially needed. Calcium-dependent potassium channels (SK) have emerged as alternative therapeutic targets due to their modulation of dopaminergic neuronal activity and their wide expression in the BG. The potential beneficial effects of bee venom treatment on PD symptoms and its mechanism of action in the BG were tested in rat PD models. Systemic bee venom administration alleviated akinesia in pharmacological and lesional models of PD. We then assessed the behavioural effects of the SK channel blocker apamin, extracted from bee venom, and compared them with those produced by bee venom in the same PD models. Both bee venom and apamin reversed haloperidol-induced catalepsy, while co-treatment with the SK opener CYPPA prevented this antiakinetik effect. After unilateral nigral 6-OHDA lesions, systemic administration of bee venom or apamin reduced forelimb asymmetry in the cylinder test and apomorphine-induced rotations revealing an antiparkinsonian action. SK blockade by apamin also increased DA extracellular level in the DA depleted striatum. The neural substrates of these effects were investigated by *in vivo* electrophysiological recordings of neuronal activity of the substantia nigra pars reticulata (SNr), the main BG output structure, after cortical stimulation. Bee venom restored the balance between the inhibitory and excitatory influence exerted by the trans-striatal direct and indirect pathways that were disrupted by the pharmacological blockade of DA receptors. These results suggest that bee venom restores the functional properties of the BG circuitry in PD conditions and emphasize the crucial role of SK blockade in the BG for an antiparkinsonian action. "Supported by CNRS, AMU, Fond. de France, Assoc. France Parkinson".

## **ATP AND ADENOSINE MODULATION OF STRIATAL PLASTICITY AND PARKINSON'S DISEASE**

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In animal models of Parkinson's disease (PD) (intra-nasal MPTP, intra-striatal 6-OHDA), there is a premotor modification of mood and memory that is mostly accompanied by alterations of synaptic plasticity in the medium prefrontal cortex, followed by an onset of motor dysfunction accompanied by alterations of dorsal striatum (DS) synaptic plasticity. Presynaptic adenosine A2A receptors (A2AR) are up-regulated in corticostriatal glutamatergic terminals at the onset of motor impairment and it is these presynaptic A2AR (rather than the more abundant postsynaptic A2AR) that control corticostriatal synaptic plasticity. Accordingly, forebrain A2AR deletion, but not the selective deletion of postsynaptic striatal A2AR, affords neuroprotection in the intra-nasal MPTP model of PD. In accordance with our previous findings that presynaptic A2AR are selectively engaged by adenosine derived from the extracellular catabolism of ATP (stored in synaptic vesicles) by ecto-5'-nucleotidase (5N), we now report that 5N inhibition was equi-effective to A2AR blockade to control synaptic plasticity and phenotypic modifications in a 6-OHDA model of PD. Finally, we enquired if ATP was only acting as a source of adenosine activating A2AR or if it might also act through P2 receptors and we found that non-selective P2 receptor (P2R) antagonists could also afford neuroprotection, in a manner mimicked by selective P2Y1R antagonists. Overall, these results prompt a role for ATP and adenosine in the control of corticostriatal plasticity and neurodegeneration associated with PD.

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## NEUROPROTECTIVE AND SYMPTOMATIC POTENTIAL OF COMBINED NON-DOPAMINERGIC THERAPY IN PARKINSON'S DISEASE

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L-DOPA has been the gold standard for the symptomatic treatment of Parkinson's disease (PD) for more than 40 years but long-term therapy is associated with the emergence of motor complications including L-DOPA-induced dyskinesia (LIDs) and response fluctuation. Adjunct and advanced therapies have been shown to reduce motor disabilities but may either worsen LIDS or show limited clinical applications due to elevated costs, complex management and stringent inclusion criteria for patients. While the relief of motor disabilities can be readily achieved at early PD stages, disease progression is inexorable. Nonetheless the development of therapeutic strategies that may slow down neurodegeneration AND have a beneficial impact on dopaminergic/nondopaminergic features of the disease is still an unmet need in PD research.

In the past decades nondopaminergic targets have attracted considerable attention for the treatment of PD. In particular, the adenosine A2 receptors (A2AR), metabotropic glutamate receptor 5 (mGluR5) and cannabinoid receptor 1 (CBR1) are strategically express in the brain where they may regulate basal ganglia motor-loop function. Furthermore, evidence indicates that they may intervene on excitatory mechanisms as well as glial activation that accompany neurodegenerative processes, and may modulate positive feedback mechanisms that thrive neuronal cell loss. Interestingly, in vitro and in vivo data indicates that these single G-protein-coupled-receptors may also exist in larger heteromeric complexes where they mutually interact and modulate the output activity of the receptor-complex.

We evaluated the neuroprotective and antiparkinsonian properties of several A2AR, mGluR5 and CBR1 antagonists, given alone or combined. Our findings suggest that combination of receptor antagonists provides better therapeutic benefits than those produced by the drugs alone. Our study sheds some light on the efficacy and advantages of combined non-dopaminergic PD treatment using low drug concentration and sets the base for in-depth studies to identify optimal doses at which these drugs reach highest efficacy.

## THE ANTIOXIDATIVE AND ANTI-INFLAMMATORY EFFECTS OF ADENOSINE A2A RECEPTOR ANTAGONISTS AS A NEW THERAPY OF PD

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Adenosine A2A receptor antagonists emerged as a new non-dopaminergic therapy of Parkinson's disease (PD). Behavioral studies revealed that these drugs alone or in combination with L-DOPA mitigated motor deficit in animal models of PD. Recently a neuroprotective potential of A2A receptor antagonists has been suggested but its mechanism is not fully understood. Since oxidative stress is regarded as the main factor contributing to etiology of PD it is crucial to find out whether A2A antagonists may protect dopaminergic neurons by counteracting free radicals generation. Two A2A antagonists belonging to xanthine and non-xanthine chemical classes, CSC and ZM 241385 increased production of hydroxyl radical when given acutely or repeatedly in normal rats. However, in low doses they decreased hydroxyl radical level induced by L-DOPA. CSC and ZM 241385 given repeatedly alone or in combination with L-DOPA decreased hydroxyl radical and glutamate extracellular level in striatum of rats with 6-OHDA damaged nigrostriatal DA neurons. CSC, unlike ZM 241385 given jointly with L-DOPA, increased reserpine-induced hydroxyl radical production. At the same time both A2A antagonists had no effect on an increase in extracellular glutamate in reserpinized rats. LPS-induced generation of hydroxyl radical, increase in glutamate, adenosine and decrease in extracellular DA in the rat striatum was reversed by caffeine and KW 6002. Thus, generation of reactive oxygen species originating from an increased glutamate, auto-oxidation of cytosolic DA or inflammation may be suppressed by A2A antagonists only at the stage corresponding to late phase of PD. The methylxanthine A2A antagonists bearing properties of MAO-B inhibition like CSC or KW6002 may cause risk of oxidative stress resulting from dysfunctional DA storage mechanism in early PD.

*Serotonin interaction  
with other neurotransmitters*

## A CLOSER LOOK AT THE PIVOTAL ROLE OF 5-HT NEURONS IN THE MECHANISM OF ACTION OF L-DOPA

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Numerous studies have pointed out serotonergic neurons as playing a pivotal role in the emergence of L-DOPA-induced dyskinesia (LID) in Parkinson's disease (PD). They are responsible for L-DOPA-induced dopamine (DA) release while noradrenergic neurons are involved in the clearance of DA extracellular levels. The nature of the release of DA and serotonin (5-HT) induced by L-DOPA together with the possible modifications induced in the dorsal raphe nucleus (DRN) are still unknown. To address the impact of L-DOPA at 5-HT cell bodies and terminals and its relevance to LID, we combined behavioural, electrophysiological and multi-site intracerebral microdialysis approaches in the 6-hydroxydopamine (6-OHDA) rat model of PD. We showed that acute L-DOPA treatment (6-12 mg/kg) did not modify DRN basal firing properties at odds with the multiple changes induced by L-DOPA (3-6-12-100 mg/kg) on 5-HT release monitored simultaneously in the striatum, prefrontal cortex (PFC), hippocampus (HIPP) and substantia nigra pars reticulata. Despite the induction of LID, chronic L-DOPA treatment (6 mg/kg/day) did not alter basal DRN neuronal activity or modify the sensitivity of 5-HT neurons to 5-HT<sub>1A</sub> receptor stimulation; however, it reduced the sensitivity of DRN activity to the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Using Ca<sup>2+</sup>-free medium or local application of the SSRI citalopram, we showed that L-DOPA (3-12 mg/kg) triggered both exocytotic (calcium-sensitive) and non-exocytotic (SERT-sensitive) mechanisms on in vivo 5-HT and DA releases whose proportions varied in the PFC and HIPP. The SERT-dependent mechanism is interesting in view of the variations reported in various rat brain areas after chronic treatment with L-DOPA. These data show that L-DOPA-induced changes of 5-HT and DA releases are due to an action at 5-HT terminals that can be independent from changes of 5-HT neuronal activity.

## 5-HT<sub>2C</sub> RECEPTOR CONTROL OF GENERALIZED AND FOCAL SEIZURES

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5-HT is an important neurotransmitter in the brain as it is involved in many neurological and psychiatric diseases including epilepsy. Serotonin receptors may directly or indirectly depolarize or hyperpolarize neurons by changing different ionic conductance. It is thus not surprising that 5-HT is able to change the excitability in most networks involved in epilepsy. The involvement of the serotonergic system in epilepsy was suggested in the late 1950s and all the areas involved in epilepsy receive 5-HT innervation and express different 5-HT-Rs including 5-HT<sub>2C</sub>-Rs. In general, agents that elevate extracellular 5-HT levels, such as 5-hydroxytryptophan and 5-HT reuptake blockers, inhibit both focal (limbic) and generalized seizures. Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically evoked convulsions. Nevertheless, the role of 5-HT<sub>2C</sub>-Rs in epilepsy modulation is far from being well established. Here we will focus on our recent experimental evidence on the effect of 5-HT<sub>2C</sub> system pharmacological manipulation on the focal temporal lobe epilepsy (TLE) and the idiopathic generalized absence epilepsy. Our data show a complex and different role of 5-HT<sub>2C</sub> in controlling focal and generalized epilepsy. 5-HT<sub>2C</sub> agonists might represent a new class of antiepileptic drugs (AEDs) potentially effective in mono- or polytherapy for the treatment of different epilepsies.

## ROLE OF THE LATERAL HABENULA IN NICOTINE ADDICTION

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Tobacco smoking represents a well known risks factor for health. So far, existing smoking cessation therapies have not been proven very successful at quitting this habit and a better understanding of the neurobiology of tobacco dependence is still needed. Nicotine is the neuro-active compound contained in tobacco that is responsible for its rewarding and reinforcing properties by acting on the midbrain dopaminergic system. The lateral habenula (LHb) is an epithalamic structure involved in pain, stress, depression and in encoding aversive stimuli. This structure is known to inhibit the DA system through activation of the RMTg, a GABA-ergic area located caudally to the VTA. The RMTg receives a strong glutamatergic input from the LHb and is activated by the systemic injection of nicotine in rats. Thus, the LHb might represent a possible target for the action of nicotine. Our data shows that systemic administration of nicotine modulates the LHb neuronal activity in vivo in rats. Following nicotine chronic treatment, this response is drastically decreased while after 1 day of withdrawal only low doses of nicotine are able to significantly affect the firing rate of the LHb neurons compared to controls. To further elucidate the role of the LHb in central nicotine effects, we recorded the activity of VTA putative-DA neurons in LHb electrolytic lesioned animals under different nicotine treatment regimens. Our evidences strongly suggest that the LHb might play an important role in mediating the effects of nicotine on the midbrain DA system thus participating to the mechanism of addiction to this drug.

## **BRAIN SEROTONIN AND NITRIC OXIDE (NO): LINKED IN ANTIDEPRESSANT ACTIVITIES? A VOLTAMMETRIC IN VIVO STUDY WITH SSRI FLUOXETINE**

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Amperometry (DCA) used together with electrically and chemically treated carbon fibre micro-electrodes ( $\mu$ CFE) allow selective monitoring of serotonin (5-HT) as well as nitric oxide (NO)<sup>1,2</sup>.

Preliminary in vitro studies have shown that the selective serotonin reuptake inhibitor (SSRI) antidepressant paroxetine inhibits constitutive nitric oxide synthase (cNOS) activity in animals and humans and that another SSRI such as fluoxetine reduced NO release in the media of synovial cells<sup>3</sup>. Successively, elevated plasma nitrate levels have been found in depressive states<sup>4</sup> while NOS inhibitor imidazole enhances the effects of antidepressants<sup>5</sup>.

The aim of this work was to verify by means of amperometry with  $\mu$ CFE the capability of fluoxetine to alter the in vivo release of central 5-HT and NO, in the attempt to confirm previous in vitro data on the monoamine as well as further clarify the putative antidepressant mechanism of action of this SSRI when acting on the NO system.

The in vivo results obtained in anaesthetised adult male rats following systemic injection of fluoxetine show significant increase to  $156 \pm 12\%$  of saline treated control rats of striatal levels of DCA current value monitored at +200 mV and corresponding to the oxidation of basal extracellular levels of 5-HT. In contrast, this treatment resulted in a significant decrease to  $75 \pm 6\%$  of striatal NO signal concomitantly monitored at +550mV.

These in vivo, in situ and in real time data support in vitro studies indicating influence of fluoxetine upon NO system and propose this methodology for monitoring interaction(s) between SSRIs and endogenous NO. It will also allow verifying the link between central serotonergic system and NO system in vivo i.e. in animal models of depression, useful step to elucidate the role of NO within the clinical antidepressant effect of SSRIs.

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***The GABA-B receptor: from molecular to  
behavioral regulation***

## DECONSTRUCTING GABA<sub>B</sub> RECEPTORS

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We are interested in the control of neuronal excitability by GABA<sub>B</sub> receptors. These receptors are promising drug targets for mental health disorders. Research over the past couple of years showed that the heterogeneity of native GABA<sub>B</sub> receptor signaling is not always faithfully reproduced in heterologous expression systems. We therefore initiated a search for proteins that regulate GABA<sub>B</sub> responses in their native context. In collaboration with Bernd Fakler (University Freiburg iBr) we used an unbiased proteomic approach that combines antibody-based affinity purification with high-resolution quantitative mass spectrometry to identify GPCR-associated proteins. Surprisingly, many of the identified proteins have not been implicated in GPCR signaling before. Finding the physiological functions of proteins that were identified in unbiased proteomic approaches is a challenging task. We adopted a strategy in which we systematically analyze the effects of proteins on receptor function and trafficking in a panel of assays in heterologous cells. We additionally probe the *in vivo* functions of these proteins in overexpression, knock-down and knock-out experiments. To address the molecular mechanism underlying functional effects of receptor-associated proteins we also identify their binding partners (besides the receptor) using reverse proteomics. This strategy is expensive and time-consuming but has enabled us to identify the physiological roles and mode of action of novel receptor-associated proteins, which provided novel and unexpected insights into GPCR signaling. During my presentation I will present data on GABA<sub>B</sub> receptor-associated proteins.

## **GABA<sub>B</sub> RECEPTOR COMPLEXES, CROSSTALK WITH OTHER RECEPTORS**

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Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, is fundamental to brain function and implicated in the pathophysiology of several neuropsychiatric disorders. In contrast to other neurotransmitters, GABA activates only one ligand-gated ion channel (GABA<sub>A/C</sub>) and the G protein-coupled GABA<sub>B</sub> receptor composed of GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits. GABA<sub>B</sub> receptor is involved in several processes such as cell survival, nerve growth cone guidance and migration of neurons. How one GABA<sub>B</sub> receptor induces multiple downstream functions remains to be determined. Here, we show these multiple functions can be generated from a single GABA<sub>B</sub> receptor through: (1) dimerization and oligomerization of the receptor; (2) cell surface expression and localization; (3) cross-talk with other GABA<sub>B</sub> receptor interacting proteins. First, we will illustrate how the oligomerization controls the activation and dynamics of the GABA<sub>B</sub> receptor. Then we will show how functional cross-talk between GABA<sub>B</sub> receptor and other receptors takes place during neuronal signaling. Finally, how these information could be used for the development of new drugs targeting the GABA<sub>B</sub> receptor will be discussed.

## THIOPHENE DERIVATIVES: NEW ALLOSTERIC MODULATORS OF THE GABA<sub>B</sub> RECEPTOR

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The identification of allosteric modulators of the GABA<sub>B</sub> receptor, involved in a wide range of pathophysiological processes, constitutes a novel approach to the pharmacological manipulation of the GABA<sub>B</sub> receptor that may lead to fewer side effects compared to those of baclofen (Lioresal<sup>®</sup>), the only GABA<sub>B</sub> agonist currently marketed and used.

Recently, our group identified two novel thiophene derivatives, methyl 2-(1-adamantanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate (COR627) and methyl 2-(cyclohexanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate (COR628), which act as GABA<sub>B</sub> positive allosteric modulators (PAMs) in *in vitro* and *in vivo* assays<sup>1</sup>. Both compounds potentiated GABA- and baclofen-stimulated [<sup>35</sup>S]GTPγS to native GABA<sub>B</sub> receptors, without activating it by themselves. *In vivo* experiments revealed that pretreatment with *per se* ineffective doses of COR627 and COR628 potentiated the sedative/hypnotic effect of a sub-threshold dose of baclofen. Recently, we developed a series of 2-(acylamino)thiophene derivatives, among which the 4-methylphenyl, the 4-*tert*-butylphenyl, the 4-chlorophenyl and the 4-(trifluoromethyl)phenyl compounds emerged as GABA<sub>B</sub> PAMs by potentiating [<sup>35</sup>S]GTPγS stimulation induced by GABA at 2.5 and 25 μM, with no intrinsic agonist activity. Contrary to other well known GABA<sub>B</sub> PAMs, such as CGP7930, GS39783, and *rac*-BHHF<sup>2</sup>, the new thiophene derivatives COR627 and COR628, as well as their 4-chlorophenyl and the 4-trifluorophenyl analogues, mainly affected the affinity/potency of GABA rather than its efficacy. Although displaying *in vitro* a lower potency than GS39783, the new 2-(acylamino)thiophene derivatives exhibited a higher efficacy *in vivo*<sup>3</sup>. The combination of these compounds with a *per se* non sedative dose of baclofen resulted in the potentiation of the sedative/hypnotic effect of baclofen in DBA mice. Moreover, these compounds showed cytotoxic effects at concentrations comparable to or higher than those of GS39783 or BHF177. In conclusion, our study discloses structurally novel and less cytotoxic GABA<sub>B</sub> PAMs, which may constitute additional tools for investigations on GABA<sub>B</sub> receptor function and physiopathology.

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## THE ROLE OF GABA<sub>B</sub> RECEPTORS IN SUBSTANCE USE DISORDERS

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Substance use disorder is a chronic and relapsing brain disorder and has immense health and societal impact. Preclinical and clinical studies carried out within the past few years have provided a premise that gamma-aminobutyric acid (GABA) transmission and GABA<sub>B</sub> receptors play a modulatory role in the mechanism of action of different drugs of abuse, with activation of the GABA<sub>B</sub> receptor being identified as a potential anti-addictive therapeutic strategy. Meanwhile, research has focused on evaluating the frequency of mood (e.g. depression) and substance use disorder co-morbidity.

This talk will focus on involvement of metabotropic GABA receptors and their ligands in animal models/tests of addiction. Progress in knowledge of role GABA<sub>B</sub> receptors has been troubled by the lack of appropriated tools what induced several controversial data, shown that modulation of that receptor might be useful in anti-addictive therapy. Discovery the new ligands of GABA<sub>B</sub> receptors called the positive allosteric modulators (PAMs) opened the possibility a better alternative for the therapeutic effects for GABA<sub>B</sub> receptor stimulation. In contrast to direct agonists, PAMs display side effects or tolerance after repeated treatment<sup>1</sup>.

We showed that acute pharmacological stimulation of GABA<sub>B</sub> receptors by agonists (baclofen, SKF 97541) and PAM (CGP7930) prevents or weakens the rewarding effects of cocaine in rat self-administration model and that observed effects are blocked by GABA<sub>B</sub> receptor antagonists. It is interesting to note that all studied GABA<sub>B</sub> receptor ligands prevent both the cocaine and the drug-associated conditioned stimulus-induced relapse. GABA<sub>B</sub> receptor ligands reduce immobility (as a measure of prodepressive behaviour) in forced swim test, both in naïve animals and in rats with depressive-like phenotypes (bulbectomized or withdrawn from cocaine self-administration). Stimulation of GABA<sub>B</sub> receptors limits rewarding properties of cocaine and cocaine-seeking behaviour in bulbectomized rats.

The current studies support usefulness of GABA<sub>B</sub> receptor ligands as novel therapeutic strategy for the treatment of cocaine use disorder, as well as their importance as modulators of depressive symptomatology linked to substance use disorders.

*1. Filip et al., Neuropharmacology. 2015; 88:36-47*

*New horizons in nutrition,  
brain function and behavior*

## **NUTRITIONAL BACKGROUNDS OF MEMORY LOSS IN DEGENERATIVE DISEASE**

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As human life span gains in years, with people over the age of 60, expected to double by 2050 the prevalence of Neurodegenerative disease such as Alzheimer's disease (AD) and Parkinson's disease (PD) is likely to increase, therefore having profound economical and social implications. Although the exact cause is not yet finally known, it has been postulated that the behavioural and neuronal declines associated with these disorders are amplified by many factors including nutritional deficiencies in some cases and nutritional overload in the others. The objective of this study is to relate neurocognitive disorders particularly memory in advanced age and its relation to nutritional status. For example, a large number of dietary elements like polyphenol rich foods or beverages have demonstrated beneficial effects on memory and learning in both animals and humans.

## **DEVELOPMENT OF DIABETES ASSOCIATED ALZHEIMER'S DISEASE MODEL IN BALB/C MICE**

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Dementia or memory loss is one of the hallmarks of Alzheimer's disease (AD). This memory loss is due to formation of amyloid beta plaques and neurofibrillary tangles present in the AD brains. Diabetes mellitus is one of the causes of Alzheimer's disease. Glycation frequently occurs in diabetes and advanced glycation end products are commonly found in the neurofibrillary tangles and plaques. Alzheimer's disease has been called type III diabetes since insulin resistance in brain has been suggested in the pathophysiology of Alzheimer's disease. Gene knockout models have been developed to study pathogenic mechanisms of Alzheimer's disease. However, no animal model has been developed yet to study diabetes related Alzheimer's disease. We have developed an ageing and diabetic mice model of Alzheimer's disease. Our model has shown cognitive deficits and histological findings relevant to Alzheimer's disease. Hence, this model can be used for the pre-clinical trials to study the effects of synthetic drugs and natural products for treatment of Alzheimer's disease.

## IRON DEFICIENCY ANAEMIA AND COGNITIVE PERFORMANCE OF SCHOOL CHILDREN IN MOROCCO

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Anemia and Iron deficiency are a public health problems in Morocco. More than 25 % of children are iron deficient. Thus, we study the association between anemia and iron deficiency with school children memory and psychological development.

342 students randomly chosen to evaluate the prevalence of iron deficiency. The sample represents school children of all educational levels and age ranged between 6-15 years. The level of haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration was measured in a group of 342 school children. The seric iron was assessed and anaemia was defined when haemoglobin < 11,5 g/dl.

A battery of cognitive tests were used: Memory image, bell test and numerical Elian. To obtain information about the daily food consumption, the educational status of the parents, socio-economic, and size of household a questionnaire was developed.

The prevalence of anemia was 16.2%. The mean hemoglobin concentration was 12.53 g/dl in boys and 12.52 g/dl in girls and the study showed a positive correlation between hemoglobin and ferritin and a strong positive correlation between hemoglobin and mean corpuscular volume suggesting that anemia in children surveyed is dominated by microcytic anemia by iron deficiency. The result suggests that iron deficiency is an important determinant of anaemia in this population, and it affects cognitive performance among school aged children.

Anaemia remains a common problem in the young children particularly the primary education school pupil of the households of low income. The result suggests that iron deficiency is an important determinant of anaemia in this population. Improving the economic status of the family, women education and health education about balanced animal and plant food consumption are recommended strategies to reduce the burden of anaemia.

## IS OMEGA-3 RICH DIET A PLAUSIBLE ALTERNATIVE TREATMENT FOR ALZHEIMER'S DISEASE: A FRIEND OR FOE?

Aise Seda Artis

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Alzheimer's disease (AD) results from a combination of genetic, lifestyle and environmental factors that affect the brain over time. The risk factors also involve lipoproteins, oxidative damage and inflammation. AD has a long preclinical phase in which amyloid and tau cerebral pathology accumulate without producing cognitive symptoms. Mild cognitive impairment has been suggested to represent a prodromal stage of AD with up to 80% of patients receiving an AD diagnosis within 6 years. It is unclear to what extent these degradations exist before the symptomatic onset.

One subtype of omega-3 fatty acids, namely docosahexaenoic acid (DHA), is heavily concentrated in neurons, its oxidation products are a good index of neuronal oxidative damage. Patients with AD have been reported to have decreased serum, brain, and neuronal DHA levels compared to controls. Recently studies suggest that a higher intake of omega-3 is associated with a reduced risk of dementia later in life. This reduced risk is thought to be more specifically a result of the omega-3 fatty acids. However high brain levels of omega-6 were associated with impaired cognitive function. Typical Western diets have <30% of the DHA recommended by expert panels.

We can presume that supplementation of omega-3 is the key solution for dementia/AD. Since it is relatively inexpensive, beneficial to decrease the cardiovascular diseases, working also in depression, and with high patient compliance, On the other hand, some research does not report this linkage. Studies suggest that effectiveness of Omega-3 limited by the genotype and it also has some unwanted effects.

So we have to discuss the right attitude and assessment of omega-3 intake and supplementation, comparisons between different combinations of PUFAs, whether there is a correct time to increase its consumption.

**THE ENDOCANNABINOID SYSTEM:  
POSSIBLE ROLE IN THE ETIOLOGY OF ANOREXIA NERVOSA**

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Anorexia nervosa (AN) is characterized by extreme body weight loss as consequence of rigid dietary restriction, often associated with excessive exercise<sup>1</sup>. In recent years, clinical evidence has shown disturbances in endocannabinoid signalling in patients affected by AN<sup>2</sup>. For example, enhanced plasma levels of anandamide (AEA) as well as a global cannabinoid receptor 1 (CB1R) up regulation were found in AN patients compared to healthy controls<sup>3,4</sup>. The “activity-based anorexia” (ABA) is one of the most validated animal model of AN in which food restriction together with voluntary access to a running wheel results in body weight loss and increased running wheel activity (RWA). In this study, we investigated whether pharmacological manipulation of endocannabinoid signalling may be effective in modulating the weight loss and the increase of RWA in the ABA paradigm. Our results show that subchronic treatment (6 days) with both the natural CB1/CB2 receptor agonist  $\Delta^9$ -tetrahydrocannabinol (0.5 and 0.75 mg/kg) and the synthetic CB1 receptor agonist CP 55,940 (0.03 and 0.06 mg/kg) at the higher doses tested, significantly reduced body weight loss, attenuated the RWA and produced a transient increase in food intake. However, subchronic treatment with the CB1 receptor inverse agonist/antagonist rimonabant (0.15 and 0.3 mg/kg) did not modify neither body weight loss or RWA. We have also found that plasma levels of leptin were significantly decreased in ABA animals, while ghrelin and corticosterone levels were increased. No effect was found on these levels after pharmacological treatments with both agonists and antagonists tested. Overall these results suggest that pharmacological therapies based on the modulation of the endocannabinoid signalling might be effective in the treatment of AN.

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*Novel Therapeutic Approaches  
to Neuropsychiatric Disorders*

## TARGETING THE INTERACTION BETWEEN PPAR $\alpha$ AND NICOTINIC RECEPTORS

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Nicotine is one of the drugs of abuse that causes addiction and relapse during abstinence. Its strong addicting properties result from its ability to enhance dopamine transmission, and to change synaptic plasticity. Preclinical studies have revealed that drugs targeting the peroxisome proliferator-activated-receptor- $\alpha$  (PPAR $\alpha$ ) show promise for the treatment of such an addiction. These drugs include synthetic agonists, such as the clinically available hypolipidemic fibrates, and drugs that increase the levels of fatty acid ethanolamides (FAEs), i.e. endogenous PPAR $\alpha$  agonists. We have shown the specific interaction between PPAR $\alpha$  and nicotine, and the molecular mechanisms whereby these intracellular receptors regulate nicotinic acetylcholine receptor (nAChR) functions in neurons. Hence, by negatively modulating nAChRs, FAEs appear to counterbalance the excessive cholinergic drive, thus providing a fine modulation of dopamine pathways at a single-cell level. Consequently, PPAR $\alpha$  activation may be suited to help resolve the disruption of dynamic balance of dopamine-acetylcholine systems, and prove beneficial in those disorders associated with dysfunction of such interplay, namely nicotine addiction, schizophrenia, depressive states and stress. The aim of the present study was to investigate on the effects of PPAR $\alpha$  activation on an animal model of depression derived from the learned helplessness paradigm. Rats were next fed a diet containing either a PPAR $\alpha$  synthetic agonist already marketed and clinically available (i.e. fenofibrate) or its vehicle. Electrophysiological, biochemical and behavioral results show an anti-depressant effect of the fenofibrate diet in this model of depression. Our results extend the field range of FAE roles to other neural circuit functions, and broaden the already wide capability of PPAR $\alpha$  to regulate not only nutrient metabolism and energy homeostasis, but dopamine systems. Consequently, the mutual influence between PPAR $\alpha$  and nAChRs might bear relevance for diverse neuropsychiatric disorders ranging from nicotine addiction to epilepsy.

## ALPHA7 NICOTINIC-GLUTAMATERGIC INTERACTIONS IN PREFRONTAL CORTEX: IMPLICATIONS FOR COGNITION ENHANCEMENT IN SCHIZOPHRENIA

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Schizophrenia (SZ) is a disorder involving maturational changes in brain circuitry. Symptoms emerge post-puberty; including core cognitive deficits that are predictive of patients' outcome yet ineffectively treated with current pharmacotherapies. Using animal models, we determined whether elevations in brain kynurenic acid (KYNA), a tryptophan metabolite and endogenous alpha7 nicotinic receptor (nAChR) and NMDA receptor antagonist, increased in the brains of patients with SZ, exacerbate a reduced expression of nACh and NMDA receptors and contribute to the observed cognitive executive deficits (e.g. attention; working memory). The search for effective cognition enhancers in SZ has focused on drugs that act as agonists at the nAChR or the glycine<sub>B</sub> site of the NMDAR. More recently, the effects of positive allosteric modulators (PAMs) of these receptors have also been studied as they, unlike direct agonists, are most effective under conditions in which cortical inputs have been activated to release ACh, choline, or glutamate. To identify potential cognition enhancers we fed pregnant rat dams kynurenine (100 mg/kg/day), the bioprecursor to KYNA, from ED15 to birth. Forebrain KYNA levels were elevated (470%) during gestation and again in adulthood (80%) when rats underwent cognitive testing. Rats with elevated KYNA exhibited reduced expression of mRNA for alpha7 nAChR (-11%) and mGluR2 (-15%) during development and as adults. They also displayed a much reduced ability to evoke ACh and glutamate release in PFC following mesolimbic stimulation. Behaviorally, they exhibited performance deficits in tasks measuring attentional set-shifting, working memory, and trace fear conditioning. In each case, cognitive deficits were attenuated or even completely reversed by administration of the partial alpha7 agonist SSR 180711 (1 or 3 mg/kg, i.p.) or by the administration of the PAM galantamine (3 mg/kg, i.p.). These results further justify the focus on alpha7 nAChR agonists and PAMs as potential cognition enhancers in several disease conditions.

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## AGE-DEPENDENT EFFECTS OF ETHANOL ON GLUTAMATERGIC DYNAMICS IN THE PREFRONTAL CORTEX OF FREELY MOVING RATS USING MICROELECTRODE AMPEROMETRY

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Alcohol abuse during adolescence may significantly alter vital functions of the PFC (e.g. decision making and inhibitory control). Glutamatergic neurotransmission plays an important role during brain maturation processes and is in turn known to be modulated by ethanol. In this study, we investigated glutamate dynamics with a second-by-second time resolution in the PFC of freely moving animals, using enzyme-based microelectrode amperometry. We analyzed the effects of an intraperitoneal ethanol injection (1g/kg) on cortical glutamate levels in adolescent (pre-pubertal and pubertal) and adult rats. Interestingly, basal glutamate levels decreased significantly with age. We also observed spontaneous glutamate release i.e. glutamate transients throughout the recordings. The frequency (transients/hour) and amplitude ( $\mu\text{M}$ ) of transients were significantly higher in adolescent animals compared to those of adults. In adolescent rats, the frequency and amplitude of glutamate transients were significantly decreased within the first hour following ethanol injection. The transients slowly recovered and in the third hour after the ethanol injection there was even a tendency to a rebound increase in the frequency of glutamate transients. In control animals receiving saline injection there were no significant changes in either frequency or amplitude of glutamate transients. Our data suggest that acute ethanol injections inhibit glutamatergic transmission in adolescent rats and may have relevance for the reported differential reaction to ethanol between adolescent and adult animals. The effects of ethanol exposure on prefrontal glutamate dynamics may disturb the maturation of the developing adolescent PFC and with this, inhibit the development of normal cortical functions such as behavior involved in controlling risk-taking and ethanol over-consumption.

## THE DAT, NEW THERAPEUTIC PERSPECTIVES FOR AN OLD NEUROPSYCHIATRIC TARGET

Gianluigi Tanda

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The dopamine transporter (DAT) controls synaptic dopamine concentrations. Genetic, pharmacologic or traumatic alteration of its function may result in development of brain disorders. The DAT is also the target for the development of medications for neurologic disorders. A main step in the evolution of DAT research has been the discovery of atypical DAT blockers that are devoid of cocaine-like behavioral effects, which conflict with the hypothesis from the early 1990's that all drugs inhibiting the DAT produce cocaine-like reinforcing effects. Recently it has been found that several of these atypical DAT inhibitors also block the harmful reinforcing actions of cocaine. In efforts to understand the molecular mechanisms of action of atypical DAT inhibitors, it has been found that, unlike cocaine and cocaine-like drugs, several atypical DAT inhibitors induce an intracellular oriented DAT conformation which might result in their slower onset of effects, and longer lasting activity compared to cocaine. More recently we have pointed out that several atypical DAT blockers possess antagonist activity at sigma receptors. We have shown that simultaneous blockade of DAT and sigma receptors may blunt the reinforcing effects of psychostimulants. We have also shown that modafinil and its isomers, have atypical dopaminergic neuroactivity, though the DAT is the only site at which these drugs bind. The DAT affinity of these compounds is ~2 micromolar, compared to >100 micromolar affinity for the closest secondary target. Interestingly, a recent comparison of modafinil or methylphenidate effects in combination with cocaine, while confirming the dopaminergic actions of methylphenidate in potentiating cocaine self-administration, ruled out the dopaminergic effects of modafinil in the same tests. Since clinical studies show a possible therapeutic action of modafinil in cocaine addicts, we are now developing new compounds based on R-modafinil as a parent-chemical structure, to explore their potential as a therapy for psychostimulant use disorders. "Support: NIDA/IRP"

*Novel approaches in treating  
cognitive deficits that accompany  
stress- and fear-related disorders*

## CANNABINOIDS PREVENT THE EFFECTS OF AN EMOTIONAL TRAUMA ON EXTINCTION AND PLASTICITY

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The formation of a fear memory following a traumatic event is an important mechanism for the subsequent development of post-traumatic stress disorder (PTSD). The consequences of exposure to trauma are affected not only by aspects of the event itself, but also by the frequency and severity of trauma reminders.

PTSD is different from other psychiatric disorders, in that it has a very clear point of onset. Hence, it seems that what we do in the first few hours after exposure to the traumatic event might have the potential to dramatically alter the trajectory of PTSD.

We aimed to examine whether intervention in the first few hours after trauma exposure using cannabinoids would prevent stress-induced alterations in extinction and plasticity. Maladaptive synaptic plasticity processes in response to specific external challenges are believed to underlie disorders such as PTSD. Growing attention has been focused on hippocampal-accumbens plasticity which regulates mood and motivation, as well as fear-related behaviors after stress exposure. We also examined alterations in CB1 receptors in a putative brain circuit that involves the amygdala, hippocampus and prefrontal cortex (PFC) (“the PTSD circuit”), as CB1 receptors are implicated in the etiology of PTSD.

Cannabinoid activation after trauma exposure prevented the stress-induced impairment in extinction and in hippocampal and accumbal plasticity. Additionally, cannabinoids prevented the development of PTSD-like symptoms and alterations in cannabinoid CB1 and glucocorticoid receptors (GR) in the fear circuit. Hence, exposure to an emotional trauma and reminders of the trauma caused lasting alterations in emotional processing associated with changes in GR and CB1r expression in brain areas dysfunctional in PTSD.

## DEFICIENT FEAR EXTINCTION LEARNING: NOVEL TREATMENT STRATEGIES

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Anxiety disorders including PTSD, phobia and panic are associated with an impairment in extinguishing learned fear, which contributes to treatment resistance and return of fear after treatment. Animal models of deficient extinction could be particularly useful to study underlying mechanisms and identify novel targets to inhibit pathological fear persistently, which is a major aim of extinction based cognitive behavioral therapy. We investigated different pharmacological and non-pharmacological treatments for their fear extinction-promoting effects using classical conditioning/extinction paradigms in a mouse model of impaired fear extinction, the 129/SvImJ (129S1) mouse (Holmes&Singewald, TINS 2013). Novel treatments targeting the zinc system, histone acetylation, mGluR7-mediated transmission or deep brain stimulation were identified to rescue the highly impaired fear extinction in this model. We observed that in particular multitarget approaches involving dopamine signalling, histone acetylation and brain zinc systems very efficiently promoted extinction and protected against spontaneous recovery and fear renewal in a novel context, hence revealing treatments helping to identify mechanisms leading to sustained fear inhibition. Quantifying gene expression changes in extinction-relevant brain areas such as the mPFC and amygdala following successful fear extinction revealed a restricted number of regulated genes, which indicate additional novel pathways important in rescue of impaired fear extinction. Taken together, these studies in a psychopathologically relevant animal model identified extinction-enhancing treatments that promoted sustained inhibition of fear and furthermore, revealed the neural target correlates and first insight into important affected signaling pathways of such interventions. These findings should provide a basis for the development of novel therapeutic adjuncts in extinction-driven therapy.

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**STRESS AND ENDOCANNABINOID MODULATION OF EMOTIONAL MEMORY**

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Enhanced memory for emotional events is a well-recognized phenomenon, which has obvious adaptive value in evolutionary terms. However, the efficient encoding of emotional memories can, in certain conditions, become maladaptive. An appropriate emotional response to an aversive event requires fine-tuned neurotransmitter release regulation and functional neuronal circuits. The endogenous cannabinoid system is a crucial modulator of these processes, playing an important role in the modulation of synaptic plasticity and memory function. Emerging evidence demonstrates that the level of stress associated to the environmental conditions plays a crucial role in modulating cannabinoid effects on emotional behaviors. Therefore, in this talk I will discuss to what extent the level of stress, associated to the level of training-induced emotional arousal, might have implications on cannabinoid effects on memory performances in rats. In particular, I will present data demonstrating that variations in the level of emotional arousal, associated to the experimental conditions, shape cannabinoid effects on memory functions. Given that, I will present results indicating that exogenous manipulation of the endocannabinoid system might differentially affect memory processes, thus being in certain conditions protective with regard to the development of long-term behavioral alterations partially resembling those seen in PTSD patients.

## **COPING, LEARNING AND CANNABINOID SIGNALING**

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Recent findings from our and other labs suggest that endocannabinoid signaling plays a major role in coping with environmental challenges. Particularly, we showed that anandamide signaling (studied by means of the FAAH inhibitor URB597) promotes active coping by decreasing the impact of less significant environmental stimuli on behavior, and by increasing the behavioral focus on the aversive stimulus. This not only affects ongoing behavior but may have long-lasting effects under particular conditions. E.g. URB597 administered once before shock exposure in the conditioned fear test ameliorated the emotional consequences of context exposure two weeks later. We recently identified the prefrontal cortex (but not the amygdala) as being the brain site where anandamide signaling affects coping, which is consistent with the role of this brain area in cognitive processing. Our ongoing experiments suggest that the coping-related roles of the other major endocannabinoid 2-AG (studied by means of the MAGL blocker JZL184) are different from those observed with anandamide. After presenting these data, we will discuss how the role of endocannabinoid signaling in coping may affect short-term and long-term memories related to aversive or traumatic experiences. Therapeutic implications will also be evaluated.

*Exploring and treating the epileptic brain*

## NOVEL DIAGNOSTIC TOOLS IN EPILEPSY

Christophe Bernard

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Seizures can be triggered in any “healthy” brain. They thus pertain to the normal repertoire of brain activities. In this presentation, I shall show that seizures obey simple and generic rules that are universal across brain regions and species. Biophysical parameters evolving slowly in time appear to be central to the mechanisms underlying seizure genesis and propagation. I shall show how many different paths can be travelled to the same endpoint, and how crossing seizure threshold can be influenced by multiple environmental factors, including stress.

## **BLOOD-BRAIN BARRIER DYSFUNCTIONS AS BIOMARKER AND TARGET IN THE PREVENTION OF ACQUIRED EPILEPSY**

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Traumatic, ischemic and infectious brain injuries are often followed by delayed neuro-psychiatric sequela, including epilepsy. Post-injury epilepsy (PIE) is frequently resistant to medications and can be associated with cognitive impairments. Data from clinical and animal studies strongly indicate the involvement of blood-brain barrier dysfunction in fostering brain inflammatory response and epileptogenesis. Furthermore, pharmacological interventions in experimental animal suggest that targeting blood-brain barrier pathology and associated inflammatory signaling may be promising new tools in preventing PIE. These experimental data call for the development of reliable methods to measure vascular dysfunction in vivo; Such methods will allow: (1) the documentation and follow-up brain vasculopathy that reflects a neuroinflammatory response and local epileptogenic modifications; (2) identification of patients at-risk for epilepsy; (3) assessment of patients' response to anti-epileptogenic treatment; and (4) a rationale decision making for dose and duration of treatment. We have thus developed imaging approaches to reliably and quantitatively detect blood-brain barrier dysfunction in vivo. We tested our approach in status epilepticus (SE)-exposed rats and found a higher abnormal signal in the amygdala, striatum and olfactory cortex 48 h after treatment in animals that were found to suffer from recurrent seizures 8-10 weeks after SE. The goals of this presentation are to: (1) demonstrate magnetic resonance (MR)-based imaging and analysis methods that allow a quantitative assessment of brain vascular dysfunction and a leaky BBB; (2) show the implementation of these methods in animal models of epileptogenesis; (3) establish feasibility in human studies; and (4) discuss the potential and limitations of blood-brain barrier imaging as a future biomarker of tissue at-risk for epilepsy and neurodegeneration.

## INFLAMMATORY TARGETS IN EPILEPSY TREATMENT

Annamaria Vezzani

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Specific immune and inflammatory processes play a key role in the mechanisms underlying seizure precipitation and recurrence. In particular, experimental brain injuries associated with the onset of acute seizures or the development of epilepsy in rodents, activate innate immunity mechanisms with the concomitant induction of cytokine-mediated signaling in neurons and glia, and the consequent downstream inflammatory cascade in brain cells and endothelial cells of the blood-brain barrier. A major trigger of this inflammatory cascade is the activation of the toll-like receptors (TLR4) and Interleukin-1 (IL-1) receptor (IL-1R1) by their endogenous ligands, namely High Mobility Group Box 1 (HMGB1) protein and IL-1beta. These molecules are released to activate homeostatic programmes of tissue repair; however, their protracted induction, as it occurs in epileptogenic tissue, leads to glial cells dysfunctions and neuronal hyperexcitability, underlying seizure generation and recurrence. Accordingly, drugs interfering with specific inflammatory mechanisms, such as P2X7 receptors or caspase-1 inhibitors, or complement inhibitors, have powerful anticonvulsive properties. Anticonvulsive effects have been attained also by blocking IL-1R1 with anakinra, an antiinflammatory drug used in rheumatoid arthritis. Neuroprotection has been attained in models of status epilepticus by antiinflammatory interventions, with a prominent neurotoxic role played both by PGE2 and IL-1beta. A protracted proinflammatory state in the brain may play a role in lowering seizure threshold and compromising cell survival, thereby contributing to the development of epilepsy. In this frame, anti-inflammatory interventions in rodent models of epilepsy, such as COX-2 inhibitors or anakinra, or their combination, when applied after the primary injury decreased cell loss, reduced spontaneous seizures frequency and generalization, and attenuated the comorbidities.

Importantly, some anti-inflammatory drugs targeting pathologic inflammatory signaling with demonstrated therapeutic effects in epilepsy models, are in clinical development or in medical use for other inflammatory disorders. This evidence highlights that pharmacological interventions targeting brain inflammation might provide new antiepileptic and disease-modifying drugs.

## OPTOGENETIC TOOLS TO STUDY AND TREAT EPILEPSY

Merab Kokaia

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Optogenetics allows for controlling activity of specific defined populations of neurons without affecting other neurons in the brain. Derived from micro-organisms, optogenetic tools comprise two main opsin genes encoding light-activated ion channels and pumps (channelrhodopsin-2 [ChR2]; halorhodopsin [NpHR], respectively). When exposed to light with appropriate wavelength, action potentials can be triggered in ChR2-expressing neurons, whereas inhibition of action potentials can be obtained in NpHR-expressing neurons, thus allowing for powerful control of neural activity. Optogenetics is now intensively used in laboratory animals, both in vitro and in vivo, for exploring functions of complex neural circuits and information processing in the normal brain and during various neurological conditions. In epilepsy, optogenetics allow for delineating mechanisms involved in ictogenesis and epileptogenesis, and can also be developed as a novel treatment strategy as has been demonstrated in recent rodent and nonhuman primate studies. In particular, we have shown that inhibiting principal neurons and activating inhibitory interneurons by optogenetics is as effective approach to suppress seizures, although some caveats need to be addressed specially. Our studies lay foundation for further exploring mechanisms, and novel treatment strategies for epilepsy.

## TARGETING PPAR-ALPHA RECEPTORS IN EPILEPSY

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Neuronal nicotinic acetylcholine receptors (nAChRs) play a key role in the pathophysiology of epilepsy. Based on genetic studies in epileptic patients and animal models of seizures, it has been demonstrated that nAChR activity is increased in some specific types of epilepsy, including nocturnal frontal lobe epilepsy (NFLE). We have recently discovered that activation of peroxisome-proliferator-activated receptors type alpha (PPAR-alpha), a family of nuclear receptor transcription factors, negatively modulates nAChRs in the brain. These results lay foundation for drugs targeting the PPAR-alpha as an adjunct therapy for resistant epilepsies in humans. Since PPAR-alpha agonists such as the fibrates have been already introduced in clinical practice from many years for the treatment of lipid metabolism disorders, the transition to the clinic is easy. Here we will present our most recent evidence on the efficacy of PPAR-alpha activation both in animal models and in a population of pharmaco-resistant NFLE subjects.

*Novel methodologies for the analysis of drugs  
of abuse and endogenous biomarkers*

## **DESIGNER DRUGS: DETECTION, CONFIRMATION, AND RESPONSE**

Gregory W. Endres

*Cayman Chemical, Ann Arbor, Michigan, USA, PinPoint Testing, Little Rock, Arkansas, USA*

Designer drugs represent an unprecedented challenge and danger as they are continually modified into countless new substances. Cayman Chemical (Ann Arbor, MI) provides products and resources to assist law enforcement in the detection, confirmation, and response to new substances of abuse. This presentation will focus on emerging drug trends including synthetic cannabinoids, stimulants, and opioids. New products and resources for the identification, quantitation, and reporting of new substances of abuse will be discussed.

## DETERMINATION OF METABOLITES AND BIOMARKERS UTILIZING MASS SPECTRAL ANALYSIS

Amy L. Patton

*PinPoint Testing, LLC, Little Rock, Arkansas, USA*

The recent emergence of synthetic drugs of abuse is just one example of an arena in which the use of mass spectrometry has increased dramatically. Basic mass spectral research is done around the world in an attempt to gain more toxicological information about these drugs, including metabolic profiles, human biomarkers for usage, and pharmacokinetic/pharmacodynamic properties. Using simple *in vitro* assays, such as enzyme kinetic reactions, prior to mass spectral analysis, a full metabolic profile of a new drug can be produced at a very fast rate; thus potentially allowing for human testing to be available for the new drug. This analysis also provide information about the ability of the body to metabolize and detoxify the drug. In addition, depending on the enzymes utilized in the reactions, a subset of the population can be studied for specific enhanced toxicological effects. *In vivo* pharmacokinetic and pharmacodynamic studies allow for an additional layer of information about the metabolic profiles of drugs in the living system, and mass spectral analysis is the backbone to these studies. Dosing an animal model with the drug of interest and measuring drug and/or metabolic levels over time in the brain and other tissues can be a valuable tool in gaining insight into pharmacological and neurological properties of that drug. Briefly, mass spectrometry can be a wealth of opportunity and information within the neuroscience and toxicology research communities.

## **BEDSIDE TO BENCH: HOW CLINICAL ANALYTICAL CHEMISTRY GUIDES HYPOTHESIS-BASED DRUGS OF ABUSE RESEARCH**

Kathryn A. Seely

*PinPoint Testing, Little Rock, Arkansas, USA*

Analytical chemistry, including mass spectrometry, is widely used in clinical laboratories to monitor a variety of human health targets, including therapeutic drug levels, enzymes, and markers for genetic abnormalities. Importantly, analytical chemistry can be used not only in a clinical laboratory, but readily utilized within the neuroscience research community. Since analytical chemistry can be used to explore an endless number targets, these techniques are a viable option to enhance basic neuroscience research. For example, the lack of necessary clinical information with emerging drugs of abuse has guided basic neuroscience research through toxicology, pharmacology, metabolomics, and pharmacokinetic studies. These analytical chemistry techniques are not only important for drugs of abuse research, but can be applied to a broader scope of research within the field of neuroscience. Hence, analytical chemistry is a robust option to be used in many laboratories to expand hypothesis-based neuroscience research.

## ANALYSIS OF DA AND 5-HT UNDER 2 MIN TO FURTHER IMPROVE TIME RESOLUTION IN ON-LINE MICRODIALYSIS

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Microdialysis of neurotransmitters *in vivo* has become an invaluable tool to study neurotransmission in the living brain. Extracellular fluid of the brain is sampled via a microdialysis probe and fractions are collected for further analysis. Typical flow rates in microdialysis are 1 - 2  $\mu\text{L}/\text{min}$ , decreasing the fraction size to a few microliters enables a temporal resolution of a few minutes. However, it also requires an analytical system that has the sensitivity for reliable quantification of neurotransmitters and the capability to handle samples of only a few microliters. In case of on-line analysis, the sample fractions are collected in a sample loop and analyzed immediately. For uninterrupted analysis in such a setup, the UHPLC analysis time should 'match' the time required to collect a sample.

We developed a fast and reliable method for analysis of dopamine (DA) and serotonin (5-HT). Small samples of less than 2  $\mu\text{L}$  were analyzed on a UHPLC system with a new electrochemical detector and a new flow cell, the DECADE Elite with Sencell. DA and 5-HT were quantified in less than 2 min total analysis time. An increased data rate was applied to analyze the fast chromatographic peaks and an elevated column temperature was applied to further facilitate the speed of separation. In the Sencell a proprietary Adjustable Spacer Technology (AST) is applied. The principle and feasibility of this set-up is shown with the analysis of dopamine (DA) and serotonin (5-HT). A detection limit of about 200 pmol/L has been obtained and a temporal resolution of about 1 min.

*How dopamine talks  
to the world-through the brain*

## THE CIRCUITRY THAT UNDERLIES DOPAMINE'S ROLE IN DISEASE

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The reward system is essential for associative learning, goal-directed behavior, and action selection. Reward circuit abnormalities are associated with many psychiatric and neurological illnesses, including major depressive disorder, obsessive-compulsive disorder, schizophrenia, Parkinson's disease, and addiction. One important question is how individual brain regions work together to produce both normal and abnormal reward-guided actions. A number of different brain regions respond to reward, but the core of this circuit is the midbrain dopamine system and its relationship to the striatum, and frontal cortex. The cortico-basal ganglia circuit also includes important modulators of the dopamine system such as the subthalamic nucleus and lateral habenula. This talk will address the complexity of the system and demonstrate regions through which the reward system impacts on cognition and motor control. These data are then used to predict which parts of the network are involved at various sites for deep brain stimulation for obsessive-compulsive disorder, depression, and Parkinson's disease.

## **VENTRAL TEGMENTAL AREA DOPAMINE NEURONS IN OPIATE WITHDRAWAL: A COMPUTATIONAL PERSPECTIVE**

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Dopamine (DA) neurons of the ventral tegmental area (VTA) play a key role in the neurobiological basis of goal-directed behaviors and addiction. Morphine (MOR) withdrawal induces profound changes in the morphology and physiology of VTA DA cells, but the mechanisms underlying these modifications are poorly understood.

Because of their predictive value, computational models are a powerful tool in neurobiological research, and are often used to gain further insights and deeper understanding on the molecular and physiological mechanisms underlying the development of various psychiatric disorders.

Here we present a biophysical model of a DA VTA neuron based on 3D morphological reconstruction and electrophysiological data, showing how opiate withdrawal-driven morphological and electrophysiological changes could affect the firing rate and discharge pattern.

The model findings suggest that changes in the balance of GABA/GLU inputs could explain the experimentally observed hypofunction of VTA DA neurons, while morphological changes could be responsible for their higher responsiveness to opiate administration observed during opiate withdrawal.

## **IDENTIFICATION OF A DOPAMINERGIC COMPONENT OF EFFORT-RELATED MOTIVATIONAL SYMPTOMS IN PSYCHOPATHOLOGY: STUDIES WITH ANIMAL MODELS**

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Motivational symptoms such as anergia/fatigue, apathy and psychomotor retardation are seen in people with depression and other disorders. Effort-related decision making is studied by offering a choice between a highly valued reinforcer that requires higher effort to obtain vs. a low reward/low effort option. Conditions associated with motivational symptoms (stress, proinflammatory cytokines, dopamine (DA) depletion) affect effort-related decision making in rats, causing animals to shift their choices away from tasks with high effort requirements, and instead select less effortful activities. These effects are not due to motor incapacitation, “anhedonia”, or changes in appetite or food preference. Tetrabenazine is a VMAT-2 inhibitor that blocks DA storage and induces depressive symptoms in humans. In rats, administration of tetrabenazine at doses that reduce accumbens DA transmission alters effort-related decision making, biasing animals towards low-effort choices. These effects can be reversed by coadministration of the catecholamine uptake blocker bupropion, an affect that depends upon stimulation of DA receptors. Moreover, the selective DA uptake blocker GBR 12909 fully reverses the effort-related effects of tetrabenazine. In contrast, the norepinephrine uptake blocker desipramine and the 5-HT uptake blocker fluoxetine fail to reverse the effects of tetrabenazine, and actually suppress lever pressing. Adenosine A2A antagonism is able to reverse the effects of tetrabenazine, an effect that is related to interactions between A2A and DA D2 receptors that are co-localized on accumbens medium spiny neurons. Blockade of DA uptake also enhances exertion of effort in rats tested on a progressive ratio/chow feeding choice task. These studies are consistent with the human literature indicating the involvement of DA in motivational symptoms, and the relative lack of effect of 5-HT uptake blockers on anergia/fatigue in humans. Furthermore, they suggest that potential therapeutic agents can be differentiated based upon their effort-related effects in animal models.

## COMPUTATIONAL PHYSIOLOGY OF THE BASAL GANGLIA AND THEIR DISORDERS – FROM UNDERSTANDING TO TREATMENT

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Our working hypothesis holds that the basal ganglia (BG) use actor/critic architecture enabling multi-objective optimization of behavioural policy. The dopamine neurons (and other BG modulators, critics) encode the mismatch between prediction and reality; whereas the BG main axis (actor) provides the connection between cortical and brainstem regions encoding state and action. Parkinson's disease (PD) is caused by the death of midbrain dopaminergic neurons (BG critics) and the consequent depletion of dopamine in the striatum – the input stage of the BG main axis (actor). The dopamine depletion in the striatum is leading to synchronous oscillations of the neurons in the BG main axis that is expressed as PD clinical symptoms (akinesia, rigidity, rest tremor, postural gait deficits and non-motor, emotional and cognitive deficits). Dopamine replacement therapy is therefore the first-line treatment of PD; but after 5-10 years most patients lose the pharmacological honeymoon, and are referred to Deep Brain Stimulation (DBS) procedures. Today, DBS parameters are intermittently adjusted. DBS methods are therefore poorly suited to cope with the fast neuronal and clinical dynamics of PD.

We suggest that we can adopt the multi-objective optimization strategies of the basal ganglia to treat their disorders. Our studies revealed that the Parkinsonian basal ganglia can be observed and controlled. Therefore, we hope that closed loop DBS would be an effective tool in the future treatment of basal ganglia related neurological and psychiatric disorders.

*Endocannabinoids and related endogenous lipid  
signaling molecules*

## 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 have shown that 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. PEA is preferentially degraded by the intracellular cysteine amidase, N-acylethanolamine acid amidase (NAAA). We have previously demonstrated that NAAA inhibition normalizes PEA levels, which are severely reduced in several inflammatory models. 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 upregulated in mice showing clinical signs of EAE. Immunofluorescence analysis demonstrated that NAAA levels are increased 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. When 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 greater clinical sign scores than did their wild type controls. Moreover the abrupt weight loss that accompanies EAE onset was greater in NAAA ki mice than in WT mice. The results suggest that modulation of NAAA activity might be beneficial for the treatment of MS.

**THE NON-PSYCHOTROPIC PLANT CANNABINOIDS, CANNABIDIVARIN (CBDV) AND CANNABIDIOL (CBD), ACTIVATE AND DESENSITIZE TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 (TRPV1) CHANNELS *IN VITRO*: POTENTIAL FOR THE TREATMENT OF NEURONAL HYPEREXCITABILITY**

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Epilepsy is the most common neurological disorder, with over 50 million people worldwide affected. Recent evidence suggests that the transient receptor potential cation channel subfamily V member 1 (TRPV1) may contribute to the onset and progression of some forms of epilepsy. Since the two non-psychoactive cannabinoids cannabidivarin (CBDV) and cannabidiol (CBD) exert anticonvulsant activity *in vivo* and produce TRPV1-mediated intracellular calcium elevation *in vitro*, we evaluated the effects of these two compounds on TRPV1 channel activation and desensitization and in an *in vitro* model of epileptiform activity. Patch clamp analysis in transfected HEK293 cells demonstrated that CBD and CBDV dose-dependently activate and rapidly desensitize TRPV1, as well as TRP channels of subfamily V type 2 (TRPV2) and subfamily A type 1 (TRPA1). TRPV1 and TRPV2 transcripts were shown to be expressed in rat hippocampal tissue. When tested on epileptiform neuronal spike activity in such slices exposed to a  $Mg^{2+}$ -free solution using multi electrode arrays (MEAs), CBDV reduced both epileptiform burst amplitude and duration. The prototypical TRPV1 agonist, capsaicin, produced similar, although not identical effects. Capsaicin, but not CBDV, effects on burst amplitude were reversed by IRTX, a selective TRPV1 antagonist. These data suggest that CBDV anti-epileptiform effects in the  $Mg^{2+}$ -free model are not uniquely mediated via activation of TRPV1. However, TRPV1 was strongly phosphorylated (and hence likely sensitized) in  $Mg^{2+}$ -free solution-treated hippocampal tissue, and both capsaicin and CBDV caused TRPV1 dephosphorylation, consistent with TRPV1 desensitization. We propose that CBDV effects on TRP channels should be studied further in different *in vitro* and *in vivo* models of epilepsy.

## DEVELOPMENT OF CANNABINOID-BASED THERAPIES FOR AMYOTROPHIC LATERAL SCLEROSIS/FRONTOTEMPORAL DEMENTIA

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Amyotrophic lateral sclerosis (ALS) is a degenerative disease produced by the damage of the upper and lower motor neurons leading to muscle denervation, atrophy and paralysis. The disease may be sporadic (the most abundant cases) or associated with some gene mutations (familial ALS). In familial cases and depending on the mutated gene, ALS could be accompanied by features of frontotemporal lobar dementia (FTLD), which supports the idea that, instead a unique disorder, ALS-related genes represent a spectrum of disorders. One of these dual genes is TARDBP, encoding TDP-43 protein, which represents a new type of proteinopathy characterized by the presence of this protein in the cytosol in the form of aggregates. The neuroprotective potential of cannabinoids has been investigated in ALS, so that different cannabinoid compounds, e.g.  $\Delta^9$ -tetrahydrocannabinol, cannabidiol, selective CB<sub>2</sub> receptor agonists or fatty acid amide hydrolase inhibitors, afforded neuroprotection (e.g. preserve motor neurons) in the superoxide dismutase-1 model (SOD-1, first gene that was identified in relation with ALS). By contrast, Sativex-like combination of phytocannabinoids was less effective in mutant SOD-1 mice, but we have preliminary evidence that WIN55,212-2 may afford neuroprotection in TDP-43 transgenic mice. Mutant SOD-1 mice have been also studied to identify the alterations caused by the disease in those elements of the endocannabinoid system targeted by the above treatments, whereas we have similar work in progress in TDP-43 transgenic mice. The most remarkable finding is a significant increase of CB<sub>2</sub> receptors in the spinal cord both in SOD-1 and TDP-43 transgenic mice, similar to the response found in other neurodegenerative disorders, which might become the CB<sub>2</sub> receptor in a promising therapeutic target for ALS. Collectively, our results support the idea that the endocannabinoid signaling system, in particular the CB<sub>2</sub> receptor, may serve for the development of a neuroprotective therapy in ALS and, by extension, in TDP-43-related disorders, e.g. FTLD. Anyway, more preclinical studies will be necessary before to go to the clinical evaluation of cannabinoid-based medicines as disease modifiers in ALS patients.

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## **PALMITOYLETHANOLAMIDE CHRONIC TREATMENT REDUCES THE SENSORIAL AND COGNITIVE DISFUNCTIONS ASSOCIATED WITH MILD TRAUMATIC BRAIN INJURY**

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Traumatic brain injury (TBI) represents a major public health problem. Traumatic brain injury (TBI) initiates a neuroinflammatory cascade that contributes to neuronal damage and behavioral impairment. Cannabinoids of all classes have the ability to protect neurons from a variety of insults that are believed to underlie delayed neuronal death after traumatic brain injury (TBI), including excitotoxicity and neuroinflammation.

We investigated the anti-neuroinflammatory properties of the palmitoylethanolamide (PEA), a commercially available compound with a pleiotropic mechanism of action.

We applied a model of mild TBI that develop sensorial and cognitive disfunctions. In particular, mice developed abnormal pain sensation (allodynia) and depression associated to repetitive, obsessive-compulsive behaviours. According to the literature, we found that TBI increased the number of proinflammatory/hypertrophic microglial cells in specific areas of the brain. We observed that PEA chronic treatment (10 mg/kg i.p.), significantly ameliorate the mechanical allodynia associated with TBI. Moreover, cognitive impairment associated with TBI such as depression and aggressiveness were reduced by PEA treatment. In particular, we measured the immobility time in sham, TBI and TBI treated animals in the tail suspension test and the results revealed that, while TBI animals showed an increased immobility time, PEA chronic treatment determined a reduction of depressive-like behaviour. Finally, we found that PEA, through a genomic mechanism PPAR- $\alpha$ -mediated, increased the expression level of CB2 cannabinoid receptor in primary microglial cells and, hence, could be responsible of the phenotype switch from pro to an anti-inflammatory/neuroprotective microglia.

Our results show a possible use of natural compounds such as PEA together with the already used drugs for the treatment of severe brain injury. Moreover, the discovery of new mechanisms in endogenous lipid compound could represent a new pharmacological tool to develop new molecules for the treatment of chronic neurological disorders.

*Emerging drugs for treating alcohol use disorders: preclinical evidence*

## ETHANOL AND METABOLISM: IS IT A TARGET TO DEVELOP NEW PHARMACOTHERAPIES FOR ALCOHOLISM TREATMENT?

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The rationale for the use of acetaldehyde (ACD) sequestering agents is conceptually equivalent to that characterizing the use of vaccines against drug addiction: these agents are designed to prevent the actions of ethanol-derived ACD on specific regions and pathways of the brain. These ACD-scavenging compounds (such as L-Cysteine and D-Penicillamine, DP) neither alter any neurotransmitter systems nor impede ethanol access to the brain; instead, they would act removing both hepatic and brain derived ACD, thus preventing the “priming” effect after ethanol consumption, i.e the activation of critical neuronal pathways involved in relapse. It is, therefore, assumed in this conception that the role of ACD in the reinforcing properties of ethanol is crucial. Effectively, as widely reported, ACD is involved in the ethanol-derived activation of the mesolimbic dopamine system, giving a mechanistic support to the potential use of ACD sequestering agents to prevent relapse. Our results show that DP is able to prevent ethanol-relapse-like drinking in Wistar rats, using a preclinical-model based on the Alcohol Deprivation Effect (ADE). We have also demonstrated that DP administration in the pVTA is sufficient to prevent the ADE, emphasizing the role displayed by this brain area in the relapse phenomenon. Moreover, recently, we have reported that the combination DP/naltrexone shows adequate anti-relapse preclinical efficacy and overcomes some therapeutic limitations of either drug alone. Thus, globally, our previous findings further support the role of ACD in the central effects of ethanol and suggest that sequestering agents of ACD, in general, and DP in particular, may represent a valuable therapy in the management of relapse in alcohol dependent patients. DP is currently approved by the *FDA* and *EMA* for other indications, which offers the additional advantage that, potentially, it could be easily implemented as a new therapeutic intervention for relapse prevention in alcoholism.

## HISTONE DEACETYLASE INHIBITION AS A POTENTIAL THERAPEUTIC INTERVENTION TO REDUCE ALCOHOL INTAKE AND RELAPSE

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**Background:** New strategies for the treatment of alcohol dependence are a pressing need and recent evidences suggest that targeting enzymes involved in epigenetic mechanisms seems to have great potential. Among these mechanisms, alteration of histones acetylation by Histone De-ACetylases (HDAC) is of great importance for gene expression and has also been implicated in addiction. In the present study, we examined whether intra-cerebroventricular (i.c.v.) administration of MS-275, a class I-specific HDAC inhibitor, could alter ethanol self-administration, motivation to consume ethanol and relapse in heavy drinking rats.

**Methods:** Male Long Evans rats trained to self-administer high levels of ethanol and implanted with a single cannula in the lateral ventricle received i.c.v. micro-infusions of MS-275 (250  $\mu$ M, 500  $\mu$ M and 1000  $\mu$ M) 3 hrs prior to the self-administration sessions.

**Results:** We demonstrated that MS-275 decreases ethanol self-administration by about 75%. We observed that 2 consecutive daily injections are necessary to decrease ethanol self-administration. Then, the dose-response curve test indicated that the MS-275 has a U-shape effect on ethanol self-administration with the dose of 500  $\mu$ M as the most efficient dose (75% decrease). In addition, we showed that MS-275 also diminished the motivation to consume ethanol (25% decrease) and we demonstrated that MS-275 reduced relapse (by about 50%) and postponed re-acquisition even when the treatment is stopped. Finally, we found that MS-275 increased the levels of acetylated-H4 specifically in nucleus accumbens and dorsolateral striatum.

**Conclusions:** Our study confirms the potential therapeutic interest of targeting epigenetic mechanisms in excessive alcohol drinking and strengthens the interest of focusing on specific isoforms of HDACs.

## **NUCLEUS ACCUMBENS GLYCINE RECEPTORS - A POTENTIAL TARGET FOR NEW TREATMENT OF ALCOHOL DEPENDENCE**

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The need for new and more efficient treatments of alcohol addiction is urgent all over the world. Since the understanding of the exact mechanisms underlying the development of alcohol addiction is unknown we need to simultaneously focus on ethanol's mechanism of action and mechanisms by which existing pharmacotherapies against alcoholism work. We have focused on determining how ethanol induce increased dopamine levels in the nucleus accumbens (nAc), an important part of the brain reward system that has heavily been implicated as an important node in the development of addiction. In this line of work we have found that ethanol primarily acts in the nAc where it directly, or indirectly, activates glycine receptors (GlyRs). Activation of nAc GlyRs will decrease a GABAergic tone present in the ventral tegmental area that, in turn, increases acetylcholine levels which activates nicotinic acetylcholine receptors on dopaminergic neurons producing an elevation of dopamine in the nAc. Interestingly using the same methodology, in vivo microdialysis in rats, we have found acamprosate to mimic the effects of ethanol. Thus we have suggested that acamprosate acts as a substitution when entering the mesolimbic pathway blocking its further activation by ethanol. In addition we have explored several glycine uptake inhibitors (GUIs) with regards to their effect on ethanol induced dopamine elevation as well as on voluntary ethanol consumption in the rat. These studies found that GUIs may be a promising new line of drugs in the treatment of addiction. In ongoing studies we continue to investigate the mechanisms by which ethanol increase dopamine and whether other drugs clinically demonstrated to decrease human alcohol consumption mechanistically interacts with nAc GlyRs. Thus far we can conclude that nAc GlyRs are an interesting target for new pharmacotherapies against alcoholism.

## PRECLINICAL EVIDENCE ON THE “ANTI-ALCOHOL” PROPERTIES OF THE POSITIVE ALLOSTERIC MODULATORS OF THE GABA<sub>B</sub> RECEPTOR

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Multiple lines of experimental evidence have demonstrated that pharmacological activation of the orthosteric GABA<sub>B</sub> receptor suppressed several alcohol-related behaviors in rodents and non-human primates. These studies have subsequently been translated to humans: the prototypic GABA<sub>B</sub> receptor agonist, baclofen, is currently undergoing extensive clinical evaluation as possible pharmacotherapy for alcohol use disorder (AUD). A recent, major step forward in the pharmacology of the GABA<sub>B</sub> receptor is represented by the positive allosteric modulators (PAMs): the presently available, *in vivo* effective GABA<sub>B</sub> PAMs appear to be preferable over baclofen, as they reproduce several psychopharmacological effects of baclofen displaying however a higher therapeutic index [i.e., larger separation between the “desired” (anxiolysis, “anti-addiction”) and “unwanted” (motor-incoordination, sedation) effects]. This ideal condition applies also to the “anti-alcohol” effects: acute and repeated treatment with non-sedative doses of all GABA<sub>B</sub> PAMs tested to date (namely CGP7930, GS39783, BHF177, *rac*-BHFF, and ADX71441) has indeed invariably resulted in marked and selective reductions in (a) acquisition and maintenance of high alcohol drinking in selectively bred Sardinian alcohol-preferring (sP) rats, (b) binge-like drinking in sP rats and alcohol-consuming C57BL/6J mice, and (c) operant, oral self-administration of alcohol, under the Fixed Ratio and Progressive Ratio schedules of reinforcement, in selectively bred Indiana alcohol-preferring (P), Finnish Alko Alcohol (AA), and sP rats. Because of the demonstrated, predictive validity of the experimental procedures used in these studies, the data collected to date suggest that GABA<sub>B</sub> PAMs may represent a novel class of agents with therapeutic potential for AUD. The recent transition of the first GABA<sub>B</sub> PAM (ADX71441) to the initial steps of clinical trials finally makes testing this hypothesis a feasible option.

***Multiple sclerosis: patient quality of life,  
physiopathology and therapeutic aspects***

## **MULTIPLE SCLEROSIS AS GROWING NEUROLOGICAL DISEASE: A HISTORICAL AND DESCRIPTIVE OVERVIEW**

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Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system (CNS) which commonly characterized by localized areas of myelin destruction, inflammation and axonal damage. Several body of evidence suggests that neuro-inflammatory and neurodegenerative diseases including MS are increasing in the south of the Mediterranean notably in Africa and middle Est. Although epidemiological knowledge of comorbidity in MS is less known about Africa, the MS incidence varies widely depending on latitude, race, and ethnic origin. For example, cases of MS are low in Africans, while confirming MS to be present in white and mixed-race communities. Notably, the frequency in white participants was high in emigrants from northern Europe and low in white population. In many of countries worldwide, MS shows increasing incidence and prevalence particularly in women who are affected within a range that may exceed 70% of MS patients, which suggest that sex hormones could play a role in MS. Symptoms encountered in MS patients were associated with fatigue, depression, visual disturbances, paresthesias, ataxia, muscle weaknesses and seizures. However, the cause of MS is unrevealed, and could be associated with infection in combination with genetic and environmental factors. The MS pathophysiologic mechanisms include inflammatory, systemic, blood brain barrier, immune system, vascular, neuromodulatory, and demyelinating processes, which make MS among degenerative disorders with multifaceted consequences.

## NEW INSIGHTS INTO MULTIPLE SCLEROSIS LESION FORMATION

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Histopathological characteristics of Multiple Sclerosis (MS) lesions, including oligodendrocyte death, demyelination, gliosis, axonal damage and peripheral cell invasion, are thought to represent a primary immune response against the central nervous system (CNS). Indeed, there is some evidence suggesting that the formation of new lesions begins with an immune dysregulation, with the consequence that the immune system targets the brain compartment, resulting in active demyelinating foci. Other studies, however, have proposed that neurodegenerative or neuroinflammatory events are the initial factors driving MS lesion formation, including oligodendrocyte death, focal microglia activation, or axonal damage, a series of events recently termed “inside-outside model”. This model suggests that oligodendrocyte degeneration, paralleled by a local, intracerebral innate immune response (herein referred to as “neuroinflammation”) is at the root of the disease, while adaptive immune involvement being a secondary phenomenon and not a cause of MS lesion formation. We will present experimental evidence that neuroinflammation is a potent trigger for peripheral immune cell recruitment into the CNS forebrain. In these studies, animals were fed cuprizone for three weeks followed by a period of two weeks on normal chow to induce the formation of neuroinflammatory foci within the forebrain. Subsequent immunization with MOG35-55 peptide, which induces the formation of myelin auto-reactive T-cells in the periphery, results in massive recruitment into the neuroinflamed forebrain regions. Remarkably, such infiltrates were found widespread within the forebrain including the cortex, white matter corpus callosum and subcortical regions, all of which are affected in MS. Furthermore, we will elaborate on possible mechanisms involved during immune cell recruitment into the pre-injured CNS.

## MYELIN DEBRIS AND INFLAMMATORY RESPONSES IN MS: ROLE OF ASTROCYTES

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In multiple sclerosis (MS) lesions, early and persisting activation of microglia occurs. Our knowledge regarding the factors triggering this activation is, however, incomplete. In this study, we investigated, whether the amount of myelin debris accumulating during demyelination is contributory to microglia activation. Furthermore, we aimed to characterize intercellular signaling mediating this activation. Therefore, we analyzed both the association between myelin debris levels and microgliosis, as well as the expression and source of potential signaling molecules within demyelinating lesions. Cortical areas with distinct and sparse myelination were analyzed for micro- and astrogliosis before and after cuprizone-induced demyelination in mice. In postmortem tissue of MS patients, leucocortical lesions were assessed for the type and level of inflammation in the cortical and white matter regions of the lesion. Genome-wide gene expression was performed on cuprizone-fed mice to investigate the temporal and causal relationship of demyelination and microglia activation. Our studies show that the magnitude of myelin loss positively correlates with microgliosis in the cuprizone model. In MS, the number of MHC class II-expressing cells is higher in the white compared with the grey matter part of leucocortical lesions. Furthermore, we observed a region-specific induction of distinct chemokines, among them Cxcl10, in cuprizone-fed mice. Early microglia activation was significantly reduced in CXCL10-deficient mice, resulting in an amelioration of cuprizone toxicity at later time points. Subsequent in vitro experiments revealed that CXCL10 induced migration and a proinflammatory phenotype in microglia. In situ hybridization analyses suggest that Cxcl10 mRNA is mainly expressed by astrocytes, indicating a modulating role of these cells during demyelinating events. Taken together, our data suggest (a) that myelin debris is an important variable in the inflammatory response during demyelinating events and (b) that astrocyte-derived chemokines are important mediators during the initiation of neuroinflammatory processes associated with demyelination.

**COMBINED MOLECULAR BIOLOGY AND BIOINFORMATICS TOOL  
DEVELOPMENTS FOR EFFICIENT PEPTIDE BIOMARKER DISCOVERY IN  
MULTIPLE SCLEROSIS NEURODEGENERATION**

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In Multiple Sclerosis (MS), myelin and axonal degeneration is caused by a proinflammatory autoimmune response involving molecular and cellular activity of the blood brain barrier (BBB). As the lesions are disseminated within the CNS, a major challenge is to target such lesions by specific biomarkers avoiding non-affected tissue. To streamline biomarker discovery in neurodegeneration pathology we developed a suppression subtractive DNA hybridization technique adapted for phage displayed combinatorial libraries derived peptide repertoires which generates enrichment of clones specific for one repertoire. We present the application in the MS rat model, experimental autoimmune encephalomyelitis (EAE) pathology and healthy control rats. Among the by *in vivo* selection recovered phage repertoire of EAE pathology, a large amount of peptide clones bind to surrounding non-affected healthy tissue. The phage clones of the *in vivo* selected Healthy repertoire serve as DNA subtractor from the EAE repertoire generating a Subtraction repertoire (“EAE minus Healthy”). The efficiency of the DNA subtraction technique was monitored by full Next Generation Sequencing (NGS) of the three repertoires. Quantitative and qualitative evaluation of the massive sequencing data revealed that there is very little chance to isolate pathology specific biomarker peptides in the EAE repertoire, while the probability is 70% in the Subtraction repertoire. Experimental binding studies with a series of newly defined peptide ligands in histology, BBB *in vitro* cultures and *in vivo* neuroinflammation MR imaging confirm the identification of specific molecular BBB alterations. The developed phage DNA subtraction technique in combination with NGS is of particular interest for complex repertoires produced by *in vivo* screening of small peptides combinatorial libraries for the discovery of biomarkers specific of molecular alterations untangled with healthy tissues, as it occurs in most CNS pathologies presenting neuroinflammatory activity.

*Neural circuits underlying motivated behaviors  
in physiology and disease*

## LEARNING NOVEL ACTIONS AND SHIFTING TO AUTOMATIC

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The process of perfecting an action through repetition may lead to changes in the neural circuits involved in executing the action, and to a bias towards a more automatic execution. This automatic execution can be reflected in changes in “how” the action is performed, as sequences of movements become faster, more precise and more accurate. We have uncovered that changes in specific striatal circuits seem to be important for the learning and shaping of novel actions. It can also be reflected in changes in “why” the action is executed. For example, extensive training on an instrumental task where animals lever press for particular outcomes can lead to a shift from goal-directed responding, that is sensitive to changes in the value of the outcome, to habitual responding, that is insensitive to outcome devaluation. We observed that shifts between goal-directed actions and habits are correlated with dynamic shifts in neural activity in corticostriatal neuronal ensembles. Furthermore, changing the activity in these circuits with optogenetic tools changes the behavioral strategy used. These studies suggest that different cortico-basal ganglia circuits dynamically encode different behavioral strategies, and that these circuits may compete for behavioral output.

## PLASTICITY OF CORTICOSTRIATAL ENDOCANNABINOID SIGNALING IN HABIT LEARNING

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The flexible and adaptive execution of new tasks - goal-directed behavior - and learning and automatization of regularly repeated tasks - habitual behavior - arise from changes in functional connectivity between cortical and sub-cortical brain areas. In particular, neuronal projections from the limbic and associative cortical areas to the medial part of the dorsal striatum (*cognitive system*) are involved in goal-directed actions and behavioral flexibility, while projections from sensory motor cortices to the dorsolateral striatum (*habit system*) in habit formation. Recent theories propose that synaptic neuromodulation of the cognitive control system and the habit system is crucial to determine action control. However, the precise physiological mechanism is still unclear. To address this question, we assessed the synaptic and behavioral 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). 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.

## **METHAMPHETAMINE INDUCES DOPAMINE NEURONS DEGENERATION EVIDENCED BY SILVER STAINING AND CAUSES MOTOR DEFECTS IN MICE**

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It is known that methamphetamine causes persistent loss of dopamine fibers. Our study addresses a long-standing question in the field: whether methamphetamine destroys not just the fibers, but also the cell bodies in the substantia-nigra, causing permanent damage.

We studied the neurotoxicity of methamphetamine by the integrity of dopamine neurons evaluated at different time-points after methamphetamine, using a marker to identify TH and amino-cupric-silver-staining to identify degenerating cell bodies and fibers.

Methamphetamine produces a progressive death of dopaminergic neurons in the substantia nigra, with 7-15% of these neurons degenerating 1 and 3 days post-methamphetamine, with further small loss of neurons at 7 and 30 days. The total loss of dopamine neurons was 20-25% at 7 or 30 days. Thus, this is the first demonstration of irreversible methamphetamine-induced neuronal loss. As shown previously, methamphetamine caused significant loss of striatal dopaminergic fibers with the greatest loss occurring 1 day posttreatment, followed by a progressive recovery. Despite partial recovery, some deficits in dopamine fibers persisted 30 days after treatment. This neuronal damage had functional consequences: mice exhibited a drastic decrease in movement and motor coordination 1 to 3 days after drug delivery, coincident with the peak nerve fiber loss. Motor activity and motor coordination recovered 7 days after methamphetamine, in parallel with the partial recovery of dopamine nerve fibers.

Our study is the first to provide conclusive evidence that methamphetamine kills dopamine neurons in the SN, along with destruction of dopaminergic terminals in the striatum.

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## **COMBINED USE OF THE CANINE ADENOVIRUS-2 AND DREADD-TECHNOLOGY TO ACTIVATE SPECIFIC NEURAL PATHWAYS IN VIVO**

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By combining CRE-recombinase expressing canine adenovirus-2 (CAV-2) and an adeno-associated virus (AAV-hSyn-DIO-hM3D(Gq)-mCherry) that contains the floxed inverted sequence of the designer receptor exclusively activated by designer drugs (DREADD) hM3D(Gq)-mCherry, we specifically targeted VTA projections in order to determine their role in reward associated behaviors. CAV-2 retrogradely infects projection neurons, which allowed us to specifically express hM3D(Gq)-mCherry in neurons that project from the ventral tegmental area (VTA) to the nucleus accumbens (Acb), prefrontal cortex (PFC) or amygdala. Activation of hM3D(Gq)-mCherry by intraperitoneal (i.p.) injections of clozapine-N-oxide (CNO) leads to increases in neuronal activity, which enabled us to specifically activate VTA to Acb projection neurons. The VTA to Acb pathway is part of the mesolimbic dopamine system and has been implicated in behavioral activation and the exertion of effort. Injections of all doses of CNO led to increases in progressive ratio (PR) performance only in the VTA-Acb targeted rats. We hereby validate the combined use of CAV-2 and DREADD-technology to activate specific neural pathways and determine consequent changes in behaviorally relevant paradigms.

## CELLULAR SUBSTRATES UNDERLYING AVERSIVE STATES

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The lateral habenula (LHb) is a structure located in the epithalamus. Its activity conveys negative-reward-related signals and its abnormal hyperactivity contributes to neuropsychiatric disorders such as depression and addiction, and in general to depressive-like phenotype. However, the cellular-level understanding of the initial processes leading to LHb dysfunction remains so far elusive. We combined electrophysiology in acute brain slices containing the LHb and behavioral approaches to understand whether aversive stimuli are capable to modify the cellular properties of LHb neurons. We found that, in mice, aversive stimuli such as exposure to foot-shocks (FsE) produce depressive-like behaviors, which occurs along with LHb neuronal hyperactivity. Indeed recording neuronal activity using patch-clamp recordings we found that the firing rate of neurons from FsE mice was significantly higher than in control mice. Combining pharmacology and ex-vivo approaches we find that FsE rapidly and persistently reduces baclofen-activated GABAB-GIRK currents in LHb neurons. This effect was specific as miniature excitatory and inhibitory currents amplitudes and frequency remained unchanged after FsE, indicating that excitatory and inhibitory GABA<sub>A</sub> mediated transmission was not affected. Mechanistically, we found that the reduction of GABAB-GIRK signaling relied on GABAB1 and GIRK2 internalization as indicated by immunogold labeling. In conclusion, the reduction of GABAB-GIRK signaling represents a novel rapid cellular mechanism shaping neuronal and potentially behavioral responses to aversive experience.

***K<sup>+</sup> Channels: structural features, physiological roles and channelopathies***

## **A STRUCTURAL AND BIOPHYSICAL MECHANISM FOR POLYMODAL GATING OF THE TREK-2 K2P CHANNEL**

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The K2P channel TREK-2 is an archetypal polymodal potassium channel which acts to couple a diverse range of regulatory stimuli to cellular electrical excitability. Alongside the other thermo- and mechano-gated K2P channels (TREK-1 and TRAAK) the TREK-2 channel is critical for discrimination of innocuous and noxious temperature and touch sensation. Guided by novel X-ray crystal structures, we have used a variety of electrophysiological, pharmacological and kinetic studies to demonstrate a mechanism of action for the state-dependent inhibition of TREK-2 by norfluoxetine, a biologically active metabolite of the anti-depressant Prozac. These studies also enable us to propose a structural basis for activation of TREK-2 by membrane stretch, temperature and arachidonic acid.

## OVEREXPRESSION OF LARGE-CONDUCTANCE CALCIUM-ACTIVATED K CHANNELS IN HUMAN GLIOBLASTOMA STEM-LIKE CELLS AND THEIR ROLE IN THE HYPOXIA-INDUCED GLIOBLASTOMA CELL MIGRATION

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Glioblastoma (GBM) is a brain tumor characterized by a short patient survival due to diffuse invasion of cancer cells into the healthy brain parenchyma. GBM cells abundantly express BK channels that promote volume changes during migration. Recent evidence suggests that the GBM high invasive potential mainly originates from a pool of stem-like cells, but the expression and function of BK channels in this subpopulation has not been studied. We therefore investigated whether BK channels are expressed in GBM stem-like cells, and are involved in their migratory process. In U87-MG cells, BK channels expression was markedly upregulated by culture conditions enriching GBM stem-like cells (U87-NS). FACS analysis further indicated that BK-rich cells also express the stem cell marker CD133. Finally, a similar correlation between BK channel subunit and CD133 expression was also found in cells derived from freshly resected GBM biopsies, suggesting the validity of our *in vitro* findings. Notably, the transwell migration of U87-NS cells was much more sensitive to BK channel block than the U87-MG cells, suggesting that the upregulated BK channels have a major role in GBM stem-cell migration.

GBM is characterized by extensive hypoxic areas strongly correlated with tumor malignancy. Since hypoxia may induce de-differentiation of GBM cells, we postulated that hypoxia increases BK channel expression through the expansion of BK-rich stem-like subpopulation, and that the upregulated BK channels have a role in the hypoxia-induced aggressiveness. In accordance, we found that U87-MG cells held in chronic hypoxia showed an increased BK channel expression compared to control cells. In addition, the hypoxia-induced BK channel upregulation promoted a highly migratory phenotype, as tested with the BK channel blocker paxilline in transwell migration assays. In conclusion, our data show that BK channels are highly expressed in GBM stem-like cells, where they participate to their high migratory ability.

## MULTIPLE SIGNALLING ROLES OF CALCIUM-DEPENDENT POTASSIUM CHANNELS IN CENTRAL NEURONS

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Hippocampal pyramidal neurons display a firing pattern characterized by a slow afterhyperpolarisation (sAHP) that underlies the late phase of spike frequency adaptation, mostly mediated by a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  current known as  $\text{sI}_{\text{AHP}}$ . This current has slow kinetics, is voltage-independent and depends on  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels during action potentials. Calcium-induced calcium release from ryanodine-sensitive stores further contributes to the activity-dependent potentiation of  $\text{sI}_{\text{AHP}}$ . In hippocampal neurons  $\text{sI}_{\text{AHP}}$  can be easily distinguished from the SK-channel mediated  $\text{I}_{\text{AHP}}$  based on their different kinetics, functional coupling to calcium sources and pharmacological profiles. A distinctive feature of  $\text{sI}_{\text{AHP}}$  is its modulation by several neurotransmitters and second messenger pathways. In particular, various monoamine transmitters suppress  $\text{sI}_{\text{AHP}}$  through a signaling pathway that involves cAMP and the activation of protein kinase A (PKA). Additionally, the  $\text{sI}_{\text{AHP}}$  is tonically modulated by the basal activity of PKA and a serine/threonine protein phosphatase, suggesting that the sAHP channels might be part of a signalling complex. To identify the components of the signaling domains underlying the monoaminergic and basal modulation of  $\text{sI}_{\text{AHP}}$ , we focused on specific adenylyl cyclases (AC), the calcium-stimulated AC1 and AC8. In CA1 pyramidal neurons from mice lacking both AC1 and AC8 (DKO), suppression of the  $\text{sI}_{\text{AHP}}$  by beta-adrenergic agonists and serotonin was similar to that observed in wildtype mice. Conversely, the NMDA receptor-mediated suppression of sAHP, observed in response to synaptic stimuli used for the induction of long-term potentiation, is abolished in DKO mice. The sAHP channels might therefore be part of distinct signalling domains in CA1 pyramidal neurons. The calcium-sensitive AC1 and AC8 do not seem to be an essential component of the signalling complex utilized by some monoaminergic transmitters to suppress  $\text{sI}_{\text{AHP}}$ , but are essential for the modulation of sAHP by NMDA receptors in response to synaptic stimuli that induce long-term synaptic plasticity.

## GENETICALLY INDUCED DYSFUNCTIONS OF KIR2.1 CHANNELS: IMPLICATIONS FOR SHORT QT<sub>3</sub> SYNDROME AND AUTISM–EPILEPSY PHENOTYPE

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Short QT<sub>3</sub> syndrome (SQT<sub>3</sub>S) is a cardiac disorder characterized by a high risk of mortality and associated with mutations in Kir2.1 (*KCNJ2*) channels. The molecular mechanisms leading to channel dysfunction, cardiac rhythm disturbances and neurodevelopmental disorders, potentially associated with SQT<sub>3</sub>S, remain incompletely understood. Here, we report on monozygotic twins displaying a short QT interval on electrocardiogram recordings and autism–epilepsy phenotype. Genetic screening identified a novel *KCNJ2* variant in Kir2.1 that (i) enhanced the channel's surface expression and stability at the plasmamembrane, (ii) reduced protein ubiquitylation and degradation, (iii) altered protein compartmentalization in lipid rafts by targeting more channels to cholesterol-poor domains and (iv) reduced interactions with caveolin 2. Importantly, our study reveals novel physiological mechanisms concerning wild-type Kir2.1 channel processing by the cell, such as binding to both caveolin 1 and 2, protein degradation through the ubiquitin–proteasome pathway; in addition, it uncovers a potential multifunctional site that controls Kir2.1 surface expression, protein half-life and partitioning to lipid rafts. The reported mechanisms emerge as crucial also for proper astrocyte function, suggesting the need for a neuropsychiatric evaluation in patients with SQT<sub>3</sub>S and offering new opportunities for disease management.

## THE ROLE OF THE VOLTAGE GATED POTASSIUM CHANNEL $K_v3.3$ IN DEVELOPMENTAL DISORDERS OF THE NERVOUS SYSTEM

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Cerebral palsy is a sporadic neurodevelopmental disorder with likely multiple aetiologies. Despite increasing evidence to the contrary, there is a common misconception that it is always caused by injury at or around the time of birth. However, due to the lack of proven causation in many cases, this prevailing view has remained.

We investigated several cases with a clinical diagnosis of the ataxic subtype of cerebral palsy using a combination of targeted- capture and exome sequencing and identified one patient who had a *de novo* mutation in the gene *KCNC3*.

Mutations in *KCNC3*, which encodes the voltage gated potassium channel  $K_v3.3$ , are known to cause a rare condition, spinocerebellar ataxia type 13 (SCA13) which is inherited as an autosomal dominant pattern trait<sup>1</sup>. Only a few families worldwide have been described and have two differing phenotypes: a later onset neurodegenerative disorder caused by the R420H mutation and an early onset congenital disorder caused by R423H and P448L which can be associated with mild intellectual impairment<sup>2,3</sup>.

Here we describe the clinical, genetic and electrophysiological characteristics of the case we have identified, and discuss the implications of the results for our understanding CP in general and more specifically the role of  $K_v3.3$  in neurodevelopment.

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*Reward sensitivity from adolescence  
to adulthood*

## ADOLESCENT AND ADULT BEHAVIORAL PROFILES AND NEUROBIOLOGY OF RELEVANCE FOR VOLUNTARY ALCOHOL INTAKE IN RATS

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Alcohol use disorder is a heterogeneous condition with regard to etiology, liability for addiction and response to treatment. The purpose of these studies is to examine the impact of behavioral traits, e.g. risk-related behaviors in the multivariate concentric square field™ (MCSF) test, and voluntary alcohol intake in adolescent and adult rats from different early life rearing conditions. Assessment of the consequences of being subjected to different early-life rearing conditions includes neurobiological analysis of central opioid and dopamine function, and behavior profiling. The MCSF test includes a variety of zones including sheltered, open and elevated areas, exploratory incentives, and areas with different illumination. Ongoing studies aim at characterizing adolescent explorative strategies in the MCSF test in more detail. Based on the MCSF trend analysis the animals can be classified into high and low risk-assessment, risk-taking and shelter-seeking behavior, respectively. Voluntary alcohol intake is investigated in adolescence or adulthood using a two-bottle free-choice paradigm. Endogenous opioids are important for social behavior early in life and results indicate that early life interference with social interactions impairs opioid function, and adolescent alcohol intake attenuates the protective effects of a beneficial early life rearing environment. Individual behavioral profiling reveals that risk-related behaviors are affected by disturbed early social interactions. Moreover, adolescent alcohol drinking affects adult drug-induced effects on dopamine networks. In adult animals, high risk-assessment behavior is associated with high voluntary alcohol intake and innate differences in dopamine dynamics. Ongoing studies point out the association between adult risk-related behaviors, voluntary alcohol intake and opioid and dopamine D2 receptor density. Taken together, these studies show the impact of early life events, behavioral characteristics and central opioid and dopamine function on end points of relevance to addiction.

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## **REWARDING VALUE AND NEUROBIOLOGICAL MECHANISMS OF SOCIAL PLAY BEHAVIOR IN RATS**

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Inbetween weaning and puberty, the young of all mammalian species, including humans, display a characteristic form of social interaction known as social play behavior or rough-and-tumble play. This form of social behavior is highly conserved throughout evolution and it is essential to develop behavioral and mental flexibility, and to acquire cognitive and social competence. Indeed, social isolation of rats during the juvenile/early adolescent phases, when social play is most abundant, produces long-lasting impairments in social capacities and cognitive control of behavior, even after prolonged re-socialization. Likewise, abnormalities in social play behavior are observed in childhood psychiatric disorders such as autism, early-onset schizophrenia and attention deficit/hyperactivity disorder. Therefore, identifying the neural underpinnings of social play behavior will increase our understanding of normal social development as well as of the aetiology of childhood and adolescent psychiatric disorders. In line with its importance for proper development of brain and behavior, social play is a natural reinforce, that can be used as incentive for place conditioning and operant responding. Our studies have revealed important roles for cannabinoid, opioid and dopaminergic neurotransmission in social play. This is in keeping with the rewarding properties of social play, as these neurotransmitter systems have been widely implicated in the positive subjective properties of food, sex and drugs of abuse. In-depth analysis of the underlying neural substrates has identified the nucleus accumbens and the amygdala as crucial brain areas involved in social play behavior. I will therefore present data showing that interacting opioid, cannabinoid and dopaminergic systems within the corticolimbic circuits underlying incentive motivation and reward modulate social play behavior in young rats.

## **SOCIAL PLAY DEPRIVATION IN ADOLESCENCE ENHANCES ALCOHOL CONSUMPTION IN ADULTHOOD**

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Social play behavior is a characteristic, highly rewarding form of social interaction that is abundantly expressed in between weaning and puberty. It is widely recognized that social play is of great importance for social, cognitive and emotional development. Interestingly, the rewarding effects of social play, other natural rewards and drugs of abuse are thought to be mediated by overlapping neural systems. Therefore, impaired or disrupted social play may cause long-lasting changes in brain reward circuits that increase the risk for psychiatric disorders in later life, such as drug addiction. Indeed, recent data from our group showed that deprivation from social play results in enhanced motivation for cocaine. To further delineate the impact of social play deprivation on later vulnerability to addictive behavior, we determined the effects of post-weaning social isolation on alcohol intake in adulthood. Comparable to our recent cocaine study, we used Lister Hooded rats that were socially isolated from postnatal day 21 until 42 and then re-socialized until early adulthood. Alcohol consumption was then determined using an intermittent every-other-day two-bottle choice paradigm in which the rats were given a choice between water and alcohol (20% v/v) for 3 days per week. Previously, we found marked individual differences in alcohol consumption using this paradigm. Here, we found a greater propensity to high alcohol consumption and preference in adult rats that were deprived from social play, particularly in the high drinking subgroup. Moreover, play deprivation enhanced the motivation to self-administer alcohol in an operant self-administration paradigm. Together, these findings underline the importance of social play for the adaptive development of brain reward circuitries, which confers resilience to addictive behavior.

## ALCOHOL INTAKE DURING ADOLESCENCE INDUCES ALCOHOL ADDICTION PHENOTYPES IN TWO ANIMAL MODELS OF SCHIZOPHRENIA

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Schizophrenia is a mental disorder characterized by a series of positive, negative or cognitive symptoms but with also the particularity of exhibiting high rate of comorbid use of drugs of abuse. While more than 80% of schizophrenics are smokers, the second drug the most consumed is alcohol with dramatic consequences on frequency and intensity of psychotic episodes and on life expectancy. Here we investigated the impact of alcohol intake during adolescence on the subsequent occurrence of alcohol addiction-like behavior in neonatal ventral hippocampal lesion (NVHL) rats, a neurodevelopmental model of schizophrenia and in STOP/MAP6 knock-out mice. Our findings demonstrated an increased liability to addictive behaviors in adult NVHL rats and STOP/MAP6 KO mice after voluntary alcohol intake during adolescence. NVHL rats displayed several signs of alcohol use disorder such as a loss of control over alcohol intake and high motivation to consume alcohol, associated with a higher resistance to extinction. In addition, once NVHL rats relapsed, they maintained higher drinking levels than controls. Concerning the STOP/MAP6 KO mice, we observed an increased consumption and preference for alcohol and a resistance to the negative effects of alcohol only when these mice were pre-exposed to alcohol during adolescence. Our results are in accordance with epidemiological studies underlying the particular vulnerability to alcohol addiction after adolescent exposure to alcohol and highlight the fact that schizophrenic subjects may be particularly at risk even after light alcohol consumption. Based on these results, it seems particularly relevant to prevent early onset of alcohol use in at risk subjects and thus to reduce the incidence of comorbid alcohol abuse in psychotic patients.

## FACTORS OF VULNERABILITY IN ALCOHOL ADDICTION THROUGHOUT LIFE SPAN

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Alcohol consumption, especially among adolescents, is a serious public health problem. In experimental animals, a protocol of intermittent access to alcohol appears to mimic the most common pattern of alcohol consumption among young people. We will present data showing that intermittent access to ethanol during adolescence causes, in rats, long-term deficits in recognition memory, as well as significant sex-dependent changes in hippocampal and frontal cortex dopaminergic, serotonergic and cannabinoid systems, presynaptic proteins and epigenetic markers. Effects on glia cells will be also discussed. Stress during childhood can be a factor of vulnerability in relation to alcohol dependence. By using a model of early life stress (maternal deprivation, MD) we found that MD did not alter baseline voluntary alcohol intake but induced an increase in voluntary alcohol consumption when the animals were subjected to a period of alcohol withdrawal and a protocol of restraint stress later in life. Finally we will show how a model of depression (chronic mild stress) in young adult animals resulted in sex-dependent behavioral alterations and a clear trend to increased alcohol consumption only in females. These results will be discussed in terms of 1) refinement of animal models of depression based on the sex of the animals and 2) the possible gender-dependent nature of the relationship between depression and alcohol dependence

*Innovative strategies for rescuing tissue injury  
induced by stroke*

## NCX EXPRESSION AND ACTIVITY IN MICROGLIA AND OLIGODENDROCYTES AFTER STROKE

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In the last few years, the development of neuroprotective strategies for brain ischemia has been primarily focused on targeting neuronal cell death. However, considering the role played by the different glial cells in neuronal survival, in debris removal and in functional remodeling after stroke, these cells are becoming a promising target to enhance an effective and additional therapy for brain ischemia. Alteration of intragial ionic homeostasis in response to ischemic injury plays a crucial role in inducing and maintaining glial responses in the ischemic brain. Therefore, glial transporters as potential candidates in stroke intervention are becoming promising targets to enhance an effective and additional therapy for brain ischemia. In particular, it has been reported that by targeting these transporters is possible to modulate regenerative processes occurring after stroke and other neurodegenerative disorders.

In particular, the initiation of microglial responses to the ischemic injury involves modifications of calcium homeostasis occurring through the  $\text{Na}^+/\text{Ca}^{2+}$  plasmamembrane exchanger NCX1. Changes in  $[\text{Ca}^{2+}]_i$  levels have also been shown to influence the developmental processes that accompany the transition of human oligodendrocyte precursor cells (OPCs) into mature myelinating oligodendrocytes and are required for the initiation of myelination and remyelination processes. Interestingly, functional studies, as well as mRNA and protein expression analyses, revealed that the brain isoform of sodium calcium exchanger, NCX3, is fundamental for OPC differentiation into oligodendrocyte. Indeed, the knocking down of the NCX3 isoform in OPCs prevented the upregulation of the myelin protein markers CNPase and MBP. Furthermore, NCX3 knockout mice exhibit not only a reduced size of spinal cord but also a marked hypomyelination, as revealed by the decrease in MBP expression and by the accompanying increase in OPCs number. These findings indicate that calcium signaling mediated by NCX3 plays a crucial role in oligodendrocyte maturation and myelin formation.

Undoubtedly, future studies devoted to unravel the importance of ionic transporters as key factors in the processes of glia survival and death will have a strong impact in setting on new therapeutic strategies.

## **CORTICAL STIMULATION TO ENHANCE RECOVERY AFTER (EXPERIMENTAL) STROKE: A SYSTEMATIC REVIEW OF NEUROBIOLOGICAL MECHANISMS**

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Stroke is a leading cause of disability worldwide. Recently, open-label clinical trials have shown that motor cortex (MC) stimulation promotes motor recovery in patients with ischemic stroke. The underlying neurobiological mechanisms are investigated to a certain extent. However, a comprehensive overview of this topic is lacking. Here, we have performed a systematic review of the potential mechanisms of recovery induced by electrical current applied to the ischemic cortical areas. Most of the studies were done in animal models of stroke. Ischemia was generally induced by either surgical or pharmacological methods. Electrical stimulation was applied epidurally or subdurally, using different stimulation parameters. In most of the studies, a profound behavioural recovery was observed of the neurological motor deficit. With respect to the mechanisms, at least three major ones were identified: angiogenesis, neurogenesis and electrotaxis of newborn cells. In our presentation, we will further specify these pathways. Based on existing data we conclude that electrical stimulation of the cortex has the potential to activate a number of key mechanisms of neurobiological recovery.

## IS SUPPLEMENTATION WITH ALPHA LINOLENIC ACID A PRECONDITIONING MECHANISM PREVENTING MORTALITY AND CEREBRAL DAMAGE, AND IMPROVING MOTOR AND COGNITIVE RECOVERY POST-STROKE?

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Stroke is a worldwide major cause of mortality and morbidity. Preclinical studies have identified over 1000 molecules with brain-protective properties. More than 200 clinical trials have evaluated neuroprotective candidates for ischemic stroke yet, to date almost all failed, leading to a re-analysis of treatment strategies against stroke. An emerging view is to seek combinatory therapy, or discovering molecules able to stimulate multiple protective and regenerative mechanisms. A pertinent experimental approach to identify such candidates is the study of brain preconditioning, which refers to how the brain protects itself against ischemia and others stress stimuli. The recent discovery that nutrients like alpha-linolenic acid (ALA is an essential omega-3 polyunsaturated fatty acid required as part of our daily diet) may be an efficient brain preconditionner against stroke fosters the novel concept of brain preconditioning by nutraceuticals.

This talk will stress the underestimated role of nutrition in preventing and combating stroke. Although there is a consensus that increased consumption of salt, fatty foods and alcoholic beverages may promote pathologies like hypertension, obesity and alcoholism - all of which are well known risk factors of stroke - few risk factors are attributed to a deficiency in an essential nutrient in the diet. The ALA deficiency observed in the Western modern diets might itself constitute a risk factor.

This talk will outline how ALA supplementation by modification of the daily diet prevents mortality and cerebral damage, and improves motor and cognitive recovery in rodent model of ischemic stroke. It will also describe the pleiotropic ability of ALA to trigger responses that are multicellular, mechanistically diverse, resulting in neuronal protection, stimulation of neuroplasticity, and brain artery vasodilation. Finally, It will also underline the capacity of ALA as a non-ischemic preconditioner able to induce brain tolerance to excitotoxicity-driven neuronal death in rat models of global ischemia and kainic acid-induced epileptic seizure and in a mouse model of transient focal ischemia. The temporal window of brain protection and the protective pathways triggered by ALA preconditioning paralleled preconditioning by sublethal insults and by adenosine and  $K_{ATP}$  channel agonists that are acknowledged as gold standards in chemical preconditionings. Overall, this talk proposes a promising therapeutic opportunity by integrating a nutritional-based approach focusing on enriching the daily diet in ALA to prevent the devastating damage caused by stroke.

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## IONIC HOMEOSTASIS DYSREGULATION AND BRAIN CONDITIONING

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Most of the current focus on developing neuroprotective therapies is aimed at preventing neuronal death. However, these approaches have not been successful despite many years of clinical trials mainly because the numerous side effects observed in humans and absent in animals used at preclinical level. Recently, the research in this field aims to overcome this problem by developing strategies which induce, mimic, or boost endogenous protective responses and thus do not interfere with physiological neurotransmission. Using a mild insult to induce endogenous neuroprotective mechanisms is known as preconditioning. When followed by a severe insult, or challenge, preconditioning results in a state of tolerance in which the injury inflicted by the challenge is mitigated. Tolerance is observed in ischemia, seizure, and infection and can be induced when the severe insult is preceded by a mild insult of the same or different type. Classical tolerance requires new protein synthesis and confers delayed and temporary neuroprotection, taking hours to develop, peaking over the course of 1-3 days, and then abating. A new promising approach for neuroprotection derives from postconditioning, in which neuroprotection is achieved by a modified reperfusion subsequent to a prolonged ischemic episode. Many pathways have been proposed as plausible mechanisms to explain the neuroprotection offered by preconditioning and postconditioning. Although the mechanisms through which these two endogenous protective strategies exert their effects are not yet fully understood, recent evidence highlights that the maintenance of ionic homeostasis plays a key role in propagating these neuroprotective phenomena. In this presentation the role of protein transporters and ionic channels involved in the control of ionic homeostasis in the neuroprotective effect of ischemic preconditioning and postconditioning, will be reviewed. In particular, it will be illustrated the role of  $\text{Na}^+/\text{Ca}^{2+}$  exchangers (NCX), the plasma membrane  $\text{Ca}^{2+}$ -ATPase (PMCA), the  $\text{Na}^+/\text{H}^+$  exchange (NHE), the  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  cotransport (NKCC) and the acid-sensing cation channels (ASIC).

*Drug addiction and epigenetic mechanisms*

## **COCAINE AND EPIGENETICS - EFFECT OF COCAINE SELF-ADMINISTERED BY RATS ON DNA METHYLATION: FROM BEHAVIOR TO GENES**

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Regulation of gene expression is known to contribute to the long-term adaptations taking place in response to drugs of abuse. Recent studies highlight the regulation of gene transcription in neurons by chromatin remodeling, a process governed by the interplay of DNA methylation and post-translational modifications of histones. To test the involvement of DNA methylation on drug reinforcing properties, we submitted rats to the cocaine intravenous self-administration paradigm. Using the fixed-ratio 5 schedule, we found that i.c.v. injection of the DNA methyl-transferase inhibitors, 5-aza-2'-deoxycytidine (5-aza) and zebularin, dose-dependently increased cocaine self-administration. The cocaine dose-response curve indicates that the inhibitors actually increased the reinforcing properties of cocaine. The same inhibitors did not significantly affect the breaking point under the progressive ratio schedule, indicating that they did not modify the motivation of animals for cocaine.

We then investigated genome-wide alterations in DNA methylation patterns in the median prefrontal cortex of rats self-administering cocaine and treated with 5-aza. The study allowed the identification of 188,926 differentially methylated genomic regions in response to cocaine treatment, in association or not with 5-aza. Most of these regions were found inside and downstream of genes, while only 9% were located in promoter regions. It is noteworthy that 99% of differential methylation occurred outside CpG islands. Cocaine was found to globally reduce DNA methylation. In contrast, repeated 5-aza treatment of rats that also self-administered cocaine actually increased the overall methylation. The differentially methylated regions were found to belong to 18,351 genes. The correlation between gene expression and gene methylation is presented, whether the methylation took place in the promoter regions or inside genes. The methylome approach was further validated by analyzing the methylation and expression of genes already known to be involved in neuronal plasticity.

## GENETIC ASSOCIATION BETWEEN VARIATIONS IN PRODYNORPIN GENE AND ALCOHOLISM POINTS UP EPIGENETIC REGULATION OF GENE ACTIVITY

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Alcohol induces changes in the central nervous system by altering the function of several neurotransmitter and neuromodulatory systems, including the endogenous opioid system (EOS). Increasing evidence shows that the EOS is implicated in the development and/or maintenance of alcoholism, but molecular mechanisms underlying ethanol-induced adaptive transformations in the EOS are not sufficiently well understood.

Genome-wide association studies have shown associations of EOS genes with alcoholism, however evidence for how they exert an effect on target genes remains unclear. We analyzed genetic association of the variations in the prodynorphin (PDYN) gene with the disease in 744 alcoholic Swedish patients with a definite current DSM-IV diagnosis of alcohol dependence. We have observed that SNP rs2235751 and rs10854244 are associated with alcohol-dependence. It is of relevance that the same variants belong to a candidate haplotype recently associated to alcohol dependence and negative craving in an independent cohort from US alcohol dependent subjects<sup>1</sup>.

Moreover, after methylation analysis in a subset of individuals, data we found an increase in DNA methylation at PDYN gene promoter in alcoholics compared to healthy controls. By analyzing the connection between genetic variants and DNA methylation, we have observed that the presence of the minor allele in both SNPs is linked with a higher DNA methylation of PDYN gene promoter.

We could thus suggest the impact of genetic control on DNA methylation shows that a higher DNA methylation may be due to a possible predisposition effect of the SNPs and thus increase the risk of alcoholism

Our results provide new insights of the relevance of genetic and epigenetic interactions for individual differences in susceptibility to alcohol abuse. We believe that alcohol effects on PDYN function might be of relevance for better understanding the role of EOS in alcoholism and in searching for new treatment strategies.

1. Karpyak VM et al. Association of the PDYN gene with alcohol dependence and the propensity to drink in negative emotional states. *Int J Neuropsychopharmacol.* 2013 Jun;16(5):975-85

## **HDAC5 MODULATES THE ACTIONS OF OPIATE AND ANTIDEPRESSANT DRUGS USED FOR THE TREATMENT OF CHRONIC PAIN**

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The epigenetic modifier Histone deacetylase-5 (HDAC5) is expressed in several brain regions involved in mood, reward and motivation. HDAC5 has been shown to regulate responses to psychostimulants, as well as emotional responses. Here, we use genetic mouse models, viral mediated gene transfer and several biochemical approaches to understand the role of HDAC5 in the acute and chronic actions of opiate analgesics and tricyclic antidepressants, in models of acute and chronic pain. Our findings suggest that while HDAC5 does not affect analgesic responses to morphine, or morphine dependence, it promotes the development of analgesic tolerance. Genetic ablation of the HDAC5 gene does not affect thermal or mechanical nociceptive thresholds, or the level of mechanical allodynia following nerve injury. HDAC5 plays a potent negative modulatory role in the actions of tricyclic antidepressants such as desipramine. Specifically, knockout of HDAC5 accelerates the onset of the antiallodynic action of DMI in models of neuropathic pain and also enhances DMI efficacy. Part of this phenotype is related to HDAC5 actions in the brain reward center, as overexpression of HDAC5 in the nucleus accumbens prevents the antiallodynic actions of DMI. Importantly, the nuclear shuttling of HDAC5 upon DMI administration is controlled by RGS9-2 and G protein beta subunits. Furthermore, our studies show that DMI reduces the phosphorylation of HDAC5 and promotes the nuclear translocation of this protein. Together these data reveal an important role of HDAC5 in both opiate and tricyclic antidepressants used for the treatment of pain.

## HISTONE MODIFICATIONS AND PROTEIN SYNTHESIS INDUCED BY MORPHINE WITHDRAWAL

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The activation of the extracellular regulated kinase (ERK) is a necessary step for several cognitive processes such as learning and memory formation. In addition to its role in plasticity and behavior, it has been shown that the ERK pathway is crucial in the cellular and molecular mechanisms underlying the compulsive nature of addictive habits. Indeed, *in vivo* treatment with cocaine, opioids and other addictive substances potently activates ERK in several mouse brain regions. In the context of opiates abuse how ERK signalling is recruited by morphine administration and the identity of the downstream effectors of ERK are still open questions. Putative ERK substrates are present in distinct neuronal compartments, and the precise subcellular location in which ERK activation occurs is therefore a major determinant of its function. In the nucleus, a large number of studies have shown that ERK signaling plays an important role in stimuli-induced modifications of epigenetic marks, such as histone H3 phosphorylation. In the cytoplasm, one target of ERK activation is the ribosomal protein S6 (rpS6), a component of the 40S ribosomal subunit which phosphorylation levels correlates with the rates of protein synthesis. The phosphorylation of rpS6 is also strictly dependent on the activation of the AKT/mTOR pathway. I will present data on histone modifications induced during withdrawal after chronic morphine administration in rats, and the involvement of the MAP kinase ERK in this phenomenon. Moreover, I will illustrate how epigenetic changes occurring in brain regions critically involved in drug addiction are orchestrated with new protein synthesis.

*Parkinson's disease beyond the shaking palsy:  
the non-motor symptoms*

## CLINICAL PSYCHIATRIC ASPECTS OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disease, characterized primarily by motor symptoms. In addition, a range of neuropsychiatric symptom, among which depression, anxiety, hallucinations, and cognitive decline are common and form an important determinant of everyday functioning and quality of life. Such neuropsychiatric symptoms are the result of a complex interaction of multiple risk and protective factors. Research addressing the aetiology of psychopathology in PD is often focused on biological factors related to the pathophysiology and treatment of PD. However, contextual factors and general population risk factors should also be taken into account.

Biological, PD related, risk factors for psychopathology can be easily understood against the background of the Braak staging system. Different cerebral regions that are part of various functional neuro-anatomic circuits are affected sequentially, with degeneration first affecting the olfactory tract and lower brainstem regions, then proceeding upwards to the midbrain, and next to the basal forebrain and cerebral cortex. The diversity of systems affected, and the fact that some of these systems are affected before involvement of the nigral substance, may explain the diversity of psychiatric symptoms as well as the fact that some of the non-motor symptoms may precede motor symptoms.

Recently there has been more focus on the influence of general population risk factors, which are not related to PD, as well as on contextual factors. For depression and anxiety, PD specific factors appear to be less important than general, not PD-related, risk factors. These findings underscore the importance of understanding psychopathology in PD within a complex multifactorial framework that is not restricted to the pathophysiology of PD. A restricted approach obscures the complex nature of psychopathologic comorbidities encountered in PD, and may subsequently lead to wrong conclusions about what might be salient targets for prevention and treatment.

## THE NON-MOTOR CHARACTERISTICS OF THE BASAL GANGLIA

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Previous models of human behavior assume a serial events starting with limbic (wanting), cognitive (planning) and motor (action) domains leading to reward/punishment and positive/negative reinforcement learning. On the other hand, a popular view of the basal ganglia networks was of parallel segregated loops that control independently the different cortical domains of limbic, cognitive and motor performance.

Our working hypothesis holds that the critics/actor network of the basal ganglia aim at multi-objective optimization (MOO) of gain and cost functions. To do so, they process in parallel limbic, cognitive and motor information (e.g., in the ventral striatum, caudate and putamen respectively).

During deep brain stimulation (DBS) of human patients with advanced Parkinson's disease we had recorded the spontaneous and evoked neural (spiking) activity at different locations inside the subthalamic nucleus (STN). We found that the STN dorso-lateral domains display synchronous oscillatory activity (in the tremor and beta frequency band) as well as robust responses to manipulation of the patient joints. On the other hand, the spiking activity in the STN ventro-medial areas is non-correlated and Poisson-like. The ventro-medial sites were in general not responsive to manipulation of the patient's joint, but show strong responses to emotional voices (mainly on the right side) and to the deviant stimuli in a Go-NoGo task.

We therefore suggest that parallel processing of motor, cognitive and emotional domains is carried in the input stages of the basal ganglia (striatum and STN). These parallel domains are integrated by the central and output structures of the basal ganglia (e.g., the external and internal segments of the globus pallidus).

## THE RESPECTIVE ROLE OF MONOAMINES IN THE PATHOPHYSIOLOGY AND THERAPY OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by the manifestation of motor symptoms associated with the degeneration of dopamine (DA) neurons in the substantia nigra pars compacta. Furthermore, PD is also characterized by the non-motor symptoms, such as anxiety and depression. However, depletion of DA alone in animal models has failed to simultaneously elicit both the motor and non-motor deficits of PD, because the disease is a multi-system disorder. Non-motor symptoms of PD are under-studied and therefore not well treated. Here, we investigated the role of combined depletions of monoamines in the manifestation of motor and non-motor deficits in the rat. Then, we studied the impact of these depletions on the efficacy of deep brain stimulation of the subthalamic nucleus (STN-DBS).

We performed selective depletions of dopamine, norepinephrine and serotonin, and the behavioral effects of different combined depletions were investigated using the Open field, elevated plus maze and the forced swim test.

Bilateral dopamine depletion alone induced locomotor deficits associated with anxiety and mild "depressive-like" behavior. Although additional depletions of norepinephrine and/or serotonin did not potentiate locomotor and anxiety disorders, combined depletions of the three monoamines dramatically exacerbated "depressive-like" behavior. STN-DBS markedly reversed locomotor deficits and anxiety behavior in animals with bilateral dopamine depletion alone. However, these improvements were reduced or lost by the additional depletion of norepinephrine and/or serotonin, indicating that the depletion of these monoamines may interfere with the antiparkinsonian efficacy of STN-DBS. Furthermore, our results showed that acute STN-DBS improved "depressive-like" disorder in animals with bilateral depletion of dopamine and also in animals with combined depletions of the three monoamines.

Our data highlight the key role of monoamine depletions in the pathophysiology of anxiety and depressive-like disorders. They provide the first evidence that combined depletions of monoamines alter the efficacy of STN-DBS on the motor and anxiety disorders.

## **THE SUBTHALAMIC NUCLEUS-DORSAL RAPHE NUCLEUS LINK: A NOVEL MOTOR-LIMBIC INTERFACE IN THE CONTEXT OF MOOD DISORDERS IN PD.**

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Depression is the most common neuropsychiatric co-morbidity in Parkinson's disease. The underlying mechanism is complex and involves biological, psychosocial and therapeutic factors. The biological mechanism can be sought in changes in monoamine systems, in particular the serotonergic (5-hydroxytryptamine, 5-HT) system. It is well established that the 5-HT system is seriously affected in the Parkinsonian brain, with loss of 5-HT markers and substantial damage to the dorsal and median raphe nuclei that contain the largest clusters of 5-HT neurons. However, it remains to be resolved whether these pathological alterations of the 5-HT system are substantial enough to develop depression. Insights suggest rather a combination of altered network activity and low 5-HT as a cause of depression in PD. The latter hypothesis has been derived from studies investigating depression as a side-effect of deep brain stimulation (DBS) of the subthalamic nucleus (STN), a treatment option in advanced Parkinson's disease. Interestingly, it has recently been demonstrated that STN DBS in animal models inhibits 5-HT neurotransmission, which might be responsible for the post-operative behavioural side effects. This highlights the close interaction between the basal ganglia and the 5-HT system, not only in motor but also limbic functions. In this review we will give an overview of 5-HT alterations in Parkinson's disease and its role in depression. Moreover, we will evaluate the influence of an altered basal ganglia network activity on 5-HT neurotransmission, as occurring during STN DBS.

## **HOT TOPICS SESSION**

## **CHRONIC STRESS FOSTERS COMPULSION AND D2R-DEPENDENT RESISTANCE TO HELPLESSNESS IN GENETICALLY IMPULSIVE MICE**

Valentina Colelli, Sonia Canterini, Paolo Campus, Maria Teresa Fiorenza, Stefano Puglisi-Allegra,  
Simona Cabib

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Psychopathogenesis is supported by aberrant neuroplasticity building up through the interplay of a large number of genes with adverse environmental conditions.

In these experiments we tested whether mice of the inbred DBA/2J strain, -characterized by genetic impulsivity- develop compulsion-associated neural and behavioral phenotypes following adverse housing condition.

Mice exposed to 12 days of restricted feeding (FR) showed perseveration, a proxy measure of compulsive behavior, in different testing conditions. Moreover, FR mice showed low mRNA levels of the dopamine D2 receptors (D2R) in the left dorsolateral striatum (DLS), a neural phenotype associated with compulsion in humans, and elevated expression of deltaFosB immunostaining in the Nucleus Accumbens (NAc) a marker of neuroadaptive changes associate with compulsive drug- and food-seeking. A 10 minutes experience of forced swim (FS) promoted helplessness in both FR and continuously free-fed (FF) mice. Instead, only FF mice showed FS-induced c-fos expression in the NAc and in left DLS and 24 h persistence of the acquired helplessness. The fast decay of helplessness in FR mice was due to the reduced availability of D2R in the left DLS because post-FS infusion of the D2/D3 antagonist (-) sulpiride in the DLS, but not of the D1 antagonist SCH23390, reproduced the behavioral effects of restricted feeding in continuously free-fed mice.

## TARGETING NEUROSTEROID BIOSYNTHESIS FOR THE TREATMENT OF STRESS-INDUCED FEAR AND ANXIETY

Graziano Pinna

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Clinical studies have suggested that the pharmacological action of selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine may include the ability of these drugs to normalize decreased brain levels of neurosteroids in patients with depression and post-traumatic stress disorder (PTSD), in particular the progesterone derivative allopregnanolone, which potently, positively, and allosterically modulates the action of GABA at GABA<sub>A</sub> receptors.

Allopregnanolone biosynthesis is also decreased in association with the emergence of PTSD-like behaviors, which results from protracted social isolation stress in mice. Similar to PTSD patients, socially isolated (SI) mice also exhibit changes in the frontocortical and hippocampal expression of GABA<sub>A</sub> receptor subunits, resulting in resistance to benzodiazepine-mediated sedation and anxiolysis. Allopregnanolone acts at a larger spectrum of GABA<sub>A</sub> receptor subunits than do benzodiazepines, and increasing corticolimbic allopregnanolone levels in SI mice by injecting allopregnanolone, administering synthetic analogs of allopregnanolone, such as ganaxolone, or stimulating allopregnanolone biosynthesis with a *selective brain steroidogenic stimulant (SBSS)*, such as fluoxetine, at doses far below those that block serotonin reuptake, improves anxiety, fear, and other PTSD-like behaviors in the SI mouse model. Hence, the neurosteroidogenic action of SSRIs is a novel and more selective mechanism than 5-HT reuptake inhibition, which for several decades has been thought to be the main molecular mechanism for the therapeutic action of SSRIs.

Thus, new SBSS molecules that stimulate allopregnanolone biosynthesis in corticolimbic neurons offer a novel non-traditional therapeutic approach. These drugs exert potent non-sedating pharmacological effects for the treatment of cognitive deficits in a number of stress-induced emotional disorders but also for conditions, including premenstrual dysphoria and catamenial epilepsy, which are the result of a rapid drop of progesterone and its metabolite levels.

**AGEING, LATE BILINGUALISM AND NEUROPLASTICITY:  
IS IT NEVER TOO LATE TO REWIRE THE BRAIN?**

Ana Inés Ansaldo

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Ageing brings a series of anatomical and neurofunctional changes that can either sustain or deteriorate cognitive abilities. As well, a number of socio-demographic factors may contribute to support cognition in the old age. Thus, life-long bilingualism has been considered to boost interference control, and delay dementia onset by enhancing cognitive reserve. However, the neural correlates of the bilingual advantage have been poorly studied. Moreover, this potential advantage has been shown in early bilinguals, whereas little is known about the impact of late bilingualism on the ageing brain's potential to rewire. This paper reports our work on late bilingualism and the ageing brain. Elderly late bilinguals (ELB) and their monolingual peers (ELM) were tested on the Simon Task -- known to tap on interference control abilities -- during fMRI scanning. Both groups showed equivalent behavioural performance, but the bilingual group showed a modulation of brain activity according to the evolution of response times across trials. Thus, ELB showed decreased BOLD-fitted responses in the right-frontal cortex, concurrently with decreasing response times, as the Simon Task progressed. Moreover, ELB activated the left inferior parietal lobule, known for its contribution to spatial processing, whereas ELM recruited the right prefrontal gyrus, which is part of the inhibitory control circuit. The fact that ELB do not resort to the prefrontal cortex to solve interference, represents a bilingual advantage, given that this circuit is particularly vulnerable to ageing. Moreover, the use of control strategies induced by late bilingualism concerns not only verbal, but also non-verbal stimuli.

## REACHING A TARGET IN DEPTH: A TREND IN SUPERIOR PARIETAL LOBUE

Patrizia Fattori, Kostas Hadjidimitrakis, Giulia Dal Bò, Annalisa Bosco, Rossella Breveglieri, Michela Gamberini, Claudio Galletti

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In real life, almost all the reach-to-grasp actions we perform are directed towards targets located in different positions around us, often at different depth. Although behavioural studies have studied reaching mechanisms in realistic situations, single cell recordings have neglected the depth dimension of reaching movements till recently. We studied reaching actions performed towards targets placed at different lateralities and distances in the two most caudal areas of the monkey superior parietal lobule: areas V6A and PEc, both involved in the control of reaches.

Single unit activity was recorded from two *Macaca fascicularis* monkeys performing foveal arm reaching towards visual targets placed at eye level at different depths and directions in the peripersonal space, in a dark environment. In V6A, direction and depth information are jointly encoded in the majority of cells during both target fixation and movement execution. In PEc, neurons are affected by direction and depth, in some cases by both of them, but these modulations are not equally distributed over the several phases of the reaching task. In fact, the effect of direction is more common than depth when the monkey is fixating the target without performing any arm movement. The opposite occurs after the onset of arm movement, when depth and jointly depth and direction signals become predominant. It is interesting to note that, while both PEc and V6A cells process depth information during the arm movement, in V6A, but much less in PEc, this type of information starts to be encoded at fixation onset, that is well before the onset of arm movement. These data suggest that while both superior parietal areas are involved in the visuomotor transformations for reaches in 3D space, there is a posterior-to-anterior gradient from a oculo-visuomotor representation in V6A, to a predominantly arm-motor encoding of the reaching action in PEc.

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**‘DREADDING THE UNCERTAIN’: *NUCLEUS INCERTUS* NETWORK ACTIVATION OR SILENCING ALTERS BEHAVIOURAL STATE – IMPACT ON AROUSAL AND SPATIAL MEMORY**

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Arousal and attention/vigilance are essential for survival and relevant brain circuits within the brainstem, hypothalamus and forebrain are still being characterised. A putative substrate is the *nucleus incertus* (NI) in the pontine periventricular grey, which consists primarily of large GABA/peptide neurons with strong ascending projections to limbic forebrain in rat, mouse and primate. Efferent/afferent connections implicate NI in behavioural planning, hippocampal/cortical activity in attention, and oculomotor control (Goto M *et al.*, 2003). NI is a site of corticotropin-releasing factor (CRF) action, and forms a neurocircuit positioned to modulate arousal/stress responses and de/synchronization of hippocampal theta rhythm (4-12 Hz activity), during exploration and memory processing (Ma *et al.*, 2013). NI is also a key locus of relaxin-3-producing neurons, which contribute to theta rhythm generation and associated spatial working memory, via actions on RXFP3 receptors in the septohippocampal system. As NI function remains unclear, we used DREADDs (i.e. designer receptors exclusively activated by designer drugs) to modulate NI neuron activity in rats and assessed behavioural and physiological effects. An adeno-associated viral (AAV) vector was used to transduce excitatory hM3Dq and inhibitory (hM4Di), DREADDs into NI neural networks of male Sprague-Dawley rats. Activation of hM3Dq by CNO (n=16, 3 mg/kg, i.p.) produced sustained locomotor activity (persistent horizontal/vertical plane activity, but not increased velocity,  $P<0.05$ ) and associated cortical desynchronisation, consistent with effects related to increased arousal and impairment of habituation/rest. Activation of hM4Di (n=5/group; CNO, 10 mg/kg, i.p.) produced NI inhibition and a significant reduction in performance in a Morris water maze ‘probe trial’, with time in the target quadrant reduced to chance, *c.f.* controls, >50% time in target quadrant ( $P<0.01$ ). These data suggest NI is an integrative neural network regulating behavioural state, associated with arousal, cortical oscillations and spatial working memory, and associated mechanisms and additional roles of NI in affective behaviours are under investigation.

*From circadian clock to human health –  
PART I. Circadian clocks as an interface  
between the physiological processes and the  
environment*

## THE EFFECT OF LIGHT ON THE CIRCADIAN SYSTEM

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In mammals, the central pacemaker coordinating 24-hour rhythms is located in the suprachiasmatic nucleus (SCN). Individual neurons of the SCN function as cell autonomous oscillators and have a molecular basis for rhythm generation. Communication and synchronization among these neurons is crucial for obtaining a coherent rhythm at the population level. This population rhythm is signaled to other brain areas, rendering a 24-hour rhythm in the electrical activity of many brain areas, and in bodily functions.

Synchronization of the SCN rhythm to the environmental light-dark cycle is mediated by melanopsin containing retinal ganglion cells, and additionally by opsin based pigments of rods and cones. The different photopigments sense different intensities and colour and collectively mediate photoentrainment. Rods are most important for photoentrainment at low light intensities, cone contribute at intermediate intensities, and melanopsin detects light at high intensities and integrates light information over longer periods of time.

Synchronization among SCN neurons is mediated by electrical synapses and neurotransmitters such as GABA and VIP. Plasticity in the degree of synchronization is essential for adaptation to the changing seasons. A high degree of synchronization results in a narrow population waveform, representing short days, a low degree of synchronization results in a broad waveform, and represents long days. This finding may have relevance for modern society, in which we create artificial long days throughout the year.

In aging, the robustness of overt 24-hour rhythms is reduced, and elderly suffer from loss of alertness during day and reduced sleep pressure at night. Recording in the SCN reveal that in aging, a decrease in phase synchrony occurs leading to a decrease in the robustness of the SCN rhythm. Treatments aimed to restore circadian function should thus be aimed at enhancing cellular communication. Possible ways to enhance communication include the use of proper light levels and exercise.

## AMBIENT TEMPERATURE CYCLE AS A ZEITGEBER IN DESERT MAMMALS

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The dromedary (*Camelus dromedarius*) is known by its well adaptation to the hostile conditions of arid and desert areas. In such habitat, besides photoperiod which marks each season, we assumed that other environmental factors, such ambient temperature cycle, may be strong enough to entrain the biological clock and to allow animals to anticipate seasonal changes in their physiological functions. We thus studied the effect of the ambient temperature cycle. This cue is a rhythmic and seasonal signal and under dehydration, it affects thermoregulation. We first demonstrated that the rhythm of body temperature in camel is under control of the circadian clock (thus a true output of the clock) but also is depending of the photoperiod. Then after, we established that the body temperature rhythm can be entrained by ambient temperature. We also studied this 'entraining' capacity of the 24h ambient temperature cycle on another known output of the clock, the melatonin rhythm. The data obtained demonstrate that after a shift in the ambient temperature cycle, not only body temperature but also the melatonin rhythm was shifted. It appears thus, in the Dromedary, that the daily cycle of ambient temperature is a true synchronizing cue. With such important results, our hypothesis were that this particular physiology is may be not specific to camel but is common of animal living under this desert area. Thus, we checked this hypothesis in another desert mammal: the goat (*Capra hircus*). By the same experimental design used before, the results show a surprising important effect of ambient temperature in the goat and like in camel, the body temperature rhythm and the endogenous clock were synchronized by this environmental cue. These new data suggest that, in desert, the ambient temperature is a major environmental cue capable of affecting the physiology of animals living in this biotope.

## FOOD INTAKE, CIRCADIAN CLOCKS AND METABOLIC DISORDERS

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Daily rhythms in physiology (e.g., hormonal secretions) and behavior (e.g., feeding/fasting and sleep/wake cycles) are controlled by a network of endogenous circadian clocks. The master clock located in the suprachiasmatic nuclei of the hypothalamus is synchronized by ambient light perceived by the retina. The suprachiasmatic clock adjusts the phase of peripheral clocks, present in many parts of the brain and peripheral organs, such as liver and white adipose tissue, using neural pathways (autonomic nervous system) and hormonal cues (internal time-givers: melatonin and glucocorticoid rhythms). The molecular clock machinery involves 24-h oscillations of specific genes, called clock genes such as *Bmal1*, *Clock* and *Rev-erba*. Numerous metabolic processes, like adipogenesis, are regulated by transcriptional networks interconnected with molecular clocks. Circadian dysfunction, whether due to endogenous mechanisms disorders (e.g., knock-out of clock genes) or synchronization defects (e.g., chronic jet-lag or shift work), is a newly identified determinant of metabolic risk factors. Notably, mice mutated or knock-out for a clock gene display at least one feature of the metabolic syndrome (obesity, diabetes, and/or hypertension). For instance, *Rev-erba*<sup>-/-</sup> mice fed with chow diet are obese and hyperglycemic, without hyperphagia or hypoactivity. Furthermore, chronic desynchronisation in wild-type rodents leads to fat overload and/or impaired glucose tolerance, as well as premature cellular aging. Together, these findings reveal that circadian dysfunction is a potential cause for the metabolic syndrome.

## SCHEDULED VOLUNTARY EXERCISE PROMOTES CIRCADIAN RHYTHMS IN NEURONS AND BEHAVIOUR

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The suprachiasmatic nuclei (SCN) function as the master circadian pacemaker, coordinating daily rhythms throughout the brain and body. Environmental light signals, as well as stimuli that promote arousal, act on the SCN to synchronise circadian rhythms to the natural world. The SCN itself contains thousands of cell autonomous clocks and vasoactive intestinal polypeptide (VIP) acting via the VPAC<sub>2</sub> receptor is a key intercellular signal that enables these cellular clocks to coordinate their activity. Animals deficient in VIP (*Vip*<sup>-/-</sup>) or VPAC<sub>2</sub> (*Vipr2*<sup>-/-</sup>) exhibit profound disruptions in their SCN cellular activity as well as behavioural and physiological rhythms. Interestingly, the response of *Vip*<sup>-/-</sup> and *Vipr2*<sup>-/-</sup> mice to light is aberrant and these mice cannot entrain their circadian rhythms to light-dark cycles. In this study, we used C57BL6, *Vip*<sup>-/-</sup>, *Vipr2*<sup>-/-</sup>, and *Vip*<sup>-/-</sup> x *Vipr2*<sup>-/-</sup> (DKO) mice to investigate the effects on circadian rhythms in behavior of ~21 days of daily exposure to an arousal-promoting stimulus of 6h/day of scheduled voluntary exercise (SVE) in a running-wheel. Prior to SVE, all C57BL6 mice sustained ~24h rhythms in locomotor activity, while no *Vip*<sup>-/-</sup>, *Vipr2*<sup>-/-</sup>, or DKO animals did so. Following SVE, all C57BL6 animals continued to express ~24h behavioral rhythms, while the majority of *Vipr2*<sup>-/-</sup> mice now also showed ~24h rhythms in behavior. For both *Vip*<sup>-/-</sup> and DKO mice, ~24h rhythms were present post-SVE, but in a reduced proportion of individuals. This indicates that the absence of VIP reduces the effectiveness of SVE in restoring ~24h rhythms in behavior. In brain slices, the SCN of *Vipr2*<sup>-/-</sup> mice exposed to SVE showed an increase in the proportion of rhythmic clock cells as well as an increase in their synchrony compared to these parameters in slices from non-SVE *Vipr2*<sup>-/-</sup> animals. Thus, scheduled exercise can circumvent intercellular signaling deficits to improve SCN function and circadian rhythms in behavior.

*New animal models of drug addiction:  
behavioral and neurobiological perspectives*

## NEURONAL CORRELATES OF COCAINE CHOICE AND ADDICTION: FOCUS ON THE ORBITOFRONTAL CORTEX

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Mainstream experimental research on drug addiction involves nonhuman animals – most frequently rats – that are provided with ready access to drugs for intravenous self-administration but without access to other rewarding options. Under this standard setting, most rats readily self-administer most drugs that lead to addiction in humans, including cocaine, and eventually escalate their intake when given extended access to the drug. Many important discoveries in the neurobiology of drugs of abuse have been made using the standard setting. However, the lack of other options during drug access may limit its validity for understanding the neurobiology of drug addiction as a psychiatric disorder. Indeed, when there is no valuable alternative to the drug, drug self-administration may merely reflect a normal reaction to the lack of choice as opposed to reflecting a disordered behavior caused by an underlying brain dysfunction. For instance, if some individual rats self-administer a drug when no other options are available and stop self-administering it when other valuable options become available, then their drug self-administration behavior can hardly be considered a compulsion, and is more likely a normal response to the lack of valuable alternatives to drug self-administration. Inversely, if some rats continue to take the drug despite and at the expense of other valuable options, then there is more ground to hypothesize that their drug use is disordered. Thus, introducing the possibility of choice during drug access appears to be a minimum experimental requirement for identifying and distinguishing drug addiction from non-disordered forms of drug use in laboratory animals. We recently used this choice-based screening method to study the neuronal correlates of cocaine choice and addiction in rats, with a special focus on the orbitofrontal cortex.

## COMPULSIVE COCAINE AND ALCOHOL SEEKING AND ITS LIMBIC CORTICOSTRIATAL SUBSTRATES

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Substance addiction is an enormous public health problem with major socio-economic consequences. Substance addiction afflicts more than 100 million people globally and accounts for more than 40% of the financial costs of all major neuropsychiatric disorders. Loss of control over substance intake, signified by e.g. continued use despite adverse consequences, is a key characteristic of addiction. Essential questions that remain largely unanswered, and that we address in our laboratory, are which mechanisms determine loss of control over substance use and contribute to an individual's risk to develop addiction. During the past decade, we and others have been developed animal models that explicitly capture loss of control over substance use. After extended cocaine self-administration experience, rodents show resistance to conditioned suppression of cocaine seeking. Recently, we have shown that rats also develop loss of control over alcohol seeking. Using an intermittent every-other-day two-bottle choice paradigm, we find marked individual differences in alcohol intake and preference, whereby subgroups of low and high alcohol consuming rats can be identified. High alcohol consuming rats displayed insensitivity to quinine modulation, indicative of loss of control over alcohol use. Moreover, similar to cocaine self-administration studies, after prolonged exposure to alcohol, rats develop resistance to conditioned suppression. We have started to employ these models to assess the neurobiological mechanisms involved in loss of control. Specifically, the involvement of the prelimbic cortex (PrL) and the orbitofrontal cortex (OFC) in control over cocaine seeking was determined by infusion of a baclofen/muscimol mixture in these regions prior to conditioned suppression tests. Inactivation of the PrL reduced conditioned suppression, while inactivation of the OFC did not alter suppression of cocaine seeking. Together, our findings show that rodents develop loss of control over cocaine and alcohol seeking and that activity of the PrL is essential to maintain control over cocaine seeking.

## **INDIVIDUAL-BASED APPROACH TO THE NEUROBIOLOGY OF TRANSITION TO COCAINE ADDICTION: FOCUS ON CORTICOSTRIATAL SYNAPTIC PLASTICITY**

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Cocaine addiction is a chronic relapsing disorder characterized by a loss of control over drug use and drug seeking that occurs in about 20% of users, after more or less protracted use. Cocaine use induces countless modifications in brain physiology. Which ones actually contribute to addiction is difficult to address without preparations specifically modeling uncontrolled drug use. We developed a model which uniquely allows observing transition to cocaine addiction in about 20% of rats, after protracted cocaine self-administration. This model allowed us identifying correlates of transition to cocaine addiction. In the nucleus accumbens, a form of synaptic plasticity, i.e. the NMDA receptor-dependent long-term depression (NMDAR-LTD), is suppressed in all subjects, after early drug use. Rats shifting to addiction maintain a permanent impairment of NMDAR-LTD, while rats keeping control on drug use recover it. In parallel, in the prelimbic cortex, mGluR2/3-dependent LTD is specifically abolished in rats showing addiction-like behavior; this form of plasticity being unaltered both after early drug use and in non-addicted rats. These data challenge the common conceptualization in which transition to addiction is seen as resulting from the development of brain alterations specifically in vulnerable subjects. Instead, transition to addiction is associated with the inability of vulnerable rats to engage active processes to counteract early cocaine-induced effects occurring in all users in drug primary sites of action. This default of counteradaptations in drug primary sites of action could underlie secondary specific adaptations in higher executive brain areas such as the one observed in the prelimbic cortex. Altogether, these results underline the importance of the behavioral preclinical models used in addiction research and more generally in experimental psychopathology.

## REPEATED STIMULATION OF THE INFRALIMBIC CORTEX REDUCES CUE-INDUCED RELAPSE TO COCAINE IN THE RAT CONFLICT MODEL

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A major hallmark of drug (and especially cocaine-) addiction is relapse to drug use following a period of abstinence. In humans and animal models, relapse can be reliably triggered by presenting cues previously associated with drug self-administration, a phenomenon termed cue-induced relapse (CIR). It has been suggested that changes in neuronal activity in the medial prefrontal cortex (mPFC) following repeated drug use may play a key role in CIR. Here we used a chronically-implanted bilateral linear microelectrode array (14 recording electrodes + 2 stimulating electrodes) to record local field potentials (LFPs) and induce subconvulsive electrical stimulations (SCES) in the mPFC of behaving rats. Following array implantation, rats were trained to self-administer cocaine by pressing a reinforcing lever, and each reinforcement was coupled with a light+tone cues. Thereafter, abstinence from drug-taking was induced using the 'conflict model'.

Next, mPFC-SCES (50 trains of 2.5 sec at 20 Hz every 15 sec, 0.2 ms pulse duration, 400  $\mu$ Amp) was applied daily (15min/day) for two weeks, simulating a typical human transcranial magnetic stimulation (TMS) -based treatment. The rats then entered a 30 min CIR-test, during which the cues were non-contingently presented. Cue-induced mPFC-LFP activity was recorded throughout the experiment. Whereas 80% of the rats that did not receive SCES 'relapsed' to lever-pressing, only 33% of the rats treated with SCES 'relapsed'. Additionally, during the CIR-tests, an increased power was observed in the alpha and beta frequency ranges in response to the cues, but this was attenuated in the ICES treated group. Finally, the electrophysiological response to stimulation was altered during treatment particularly in these bands. It therefore seems that repeated cocaine self-administration is associated with increased saliency of the drug-associated cues manifested, at least, as increased cue-induced alpha/beta mPFC activity, and that SCES treatment of the mPFC reduces relapse rates following abstinence.

## **CONTEXT-INDUCED RELAPSE TO ALCOHOL SEEKING AFTER PUNISHMENT- IMPOSED ABSTINENCE: BEHAVIOURAL AND NEURONAL MECHANISMS**

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In humans, places or contexts previously associated with alcohol use often provoke relapse during abstinence. This phenomenon has been modeled in laboratory animals using the context-induced reinstatement procedure where reinstatement of alcohol seeking is seen when the animal is tested in the original training context (A) after extinction of alcohol seeking in a different context (B). One limitation of this approach is that extinction training does not adequately capture the motivation for abstinence in human alcoholics who typically self-initiate abstinence, despite drug availability, due to the negative consequences of excessive use. We recently adapted the context-induced reinstatement procedure to study context-induced relapse in laboratory rats after abstinence is imposed by negative consequences (footshock punishment). Alcohol preferring P rats were first trained to lever press for alcohol in one context (A). We then continue alcohol self-administration in a physically distinct context (B) however lever pressing now causes response-contingent footshock. We increase the shock intensity until the rats have completely suppressed alcohol seeking. The relapse tests are conducted in extinction conditions, and the rats show increased alcohol seeking in context A compared to context B. We have taken a multi-disciplinary approach to study the neural circuits of this relapse. Using intracranial micro-injections, we have found that Lateral Hypothalamus (LH), Nucleus Accumbens shell (NAc shell), and Ventral Subiculum (vSub) are all critical neural substrates of this relapse. Using immunohistochemical detection of the neural activity marker Fos in combination with retrograde tracing, we have shown that context-induced relapse to alcohol seeking is associated with increased activity in NAc shell projections to LH, and vSub projections to NAc shell. Together, these data describe the critical neural circuitry by which alcohol-associated contexts promote relapse to alcohol seeking during abstinence.

***Ethanol and its metabolites: actions on  
dopamine, adenosine, opioid and  
endocannabinoid systems***

## ADENOSINE AS A POTENTIAL NEURAL MODULATOR OF SOME EFFECTS OF ETHANOL AND ACETATE ON BEHAVIOR.

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It has been postulated that a number of the central effects of ethanol are mediated via ethanol's metabolite acetate. Ethanol, mainly via acetate synthesis, increases the adenosinergic tone in the central nervous system. Increases in adenosine induce sedation and suppress motor activity. Ethanol at high doses also produces those effects. Although the role of acetate is much less known, we recently demonstrated in Sprague-Dawley rats, that intraventricular (ICV) as well as peripheral intraperitoneal (IP), administration of acetate suppressed locomotion in an open field. However, even higher acute IP acetate doses did not affect locomotion in CD1 mice, showing that rats seem more sensitive than mice to the sedative effects of acetate, as they also are with ethanol. In mice, chronic administration of acetate did not have an effect on open field spontaneous locomotion either, although it blocked ethanol-induced locomotion, supporting the hypothesis that acetate contributes to ethanol-induced motor suppression, and both drugs act similarly to adenosine on these behaviors. Chronic consumption of acetate did not have an effect on loss of righting reflex (LORR) induced by ethanol, but significantly increased the number of mice that did not achieve LORR, indicating that after chronic consumption, acetate induced some tolerance to ethanol's sedative effects. Adenosine and ethanol induced anxiolysis. However, acute administration of acetate did not affect anxiety, and ethanol induced anxiolysis was not affected by chronic administration of acetate. Caffeine, a non-selective adenosine receptor antagonist, decreased social interaction in rodents because of its anxiogenic effects, while ethanol increased social interactions, and was able to reverse the suppressant effect of caffeine on social exploration in mice. Thus, although acetate seems not to regulate anxiety, the anxiogenic effect on social interaction induced by blocking adenosine was reversed by a dose of ethanol that has demonstrated to be anxiolytic in different paradigms.

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## ROLE OF ACETALDEHYDE, DOPAMINE AND SALSOLINOL IN THE EFFECTS OF ETHANOL

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The role of acetaldehyde, the first metabolite of ethanol, in the effects of its parent compound has gone a profound re-evaluation in the last five decades since the description made in the early 50's that patients treated with the aldehyde dehydrogenase inhibitor, antabuse, could perceive more pleasurable effects when drinking small volumes of alcoholic beverages. However, the suggestion that acetaldehyde could be a mediator of some of the effects of ethanol did never setback the skepticism of the scientific community on the "acetaldehyde hypothesis" until the discovery of catalase, the enzyme that converts ethanol into acetaldehyde within the brain. The aim of our studies, within this scenario, was to further investigate the role of acetaldehyde and of the acetaldehyde-dopamine (DA) conjugate, salsolinol, on the ability of ethanol to elicit Extracellular Signal regulated Kinases (ERK), to exert motivational effects and to excite dopamine neurons in the posterior ventral tegmental area (pVTA). The results of our studies reveal that ethanol-derived acetaldehyde and salsolinol, similarly to ethanol, elicit ERK phosphorylation in the nucleus accumbens and conditioned place preference. In addition, our studies reveal that, similarly to ethanol, also acetaldehyde is orally self-administered under an opioid receptor-mediated control. Furthermore, the electrophysiological experiments disclose that, in order to excite DA neurons in the pVTA, a two-step sequential mechanism takes place: a) conversion of ethanol into acetaldehyde by the action of catalase and b) condensation of acetaldehyde with DA to produce salsolinol. Overall these results provide evidence in support of the view that the metabolism of ethanol plays a key role its motivational effects and strongly suggest that this also accounts for its ability to excite DA neurons.

**EVALUATION OF THE COMPLEX EFFECTS OF ETHANOL AND ACETALDEHYDE  
ON THE ACTIVITY OF DOPAMINE NEURONS IN THE POSTERIOR VTA:  
BEHAVIORAL IMPLICATIONS**

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How the administration of ethanol directly into the posterior ventral tegmental area (VTA) is able to modify the activity of the dopamine (DA) neurons located in this area involves a complex and not fully understood mechanism. Electrophysiological findings have shed light on this topic, suggesting that ethanol effects on VTA DA neurons could be the result of opposing ethanol effects on the activity of these neurons through two parallel mechanisms, one promoting and the other reducing the  $\gamma$ -aminobutyric acid (GABA) release onto VTA DA neurons. Behavioral experiments have shown that without any pharmacological intervention, the net effect of central ethanol administration is the activation of DA neurons. However, the use of adequate pharmacological strategies allowed uncovering the existence of the two opposite tendencies. In this line, data from our laboratory have shown that the activation of the VTA DA neurons observed after acute ethanol administration could be attributed to acetaldehyde (ACD) or its derivative, salsolinol, through interactions with mu-opioid receptors, whereas the non-metabolized fraction of ethanol could account for the reduction of this neuronal activity. Furthermore, GABA receptors, in particular the subtype A, would be implicated in the underlying mechanism through which the non-biotransformed fraction of ethanol exerts its depressant effects. Thus, the behavioral consequences observed after central ethanol administration would be dependent on the dose, concretely the balance between metabolized ethanol and the non-biotransformed fraction of ethanol, and their respective modulation in the GABAergic synaptic transmission onto VTA DA neurons.

## ROLE OF THE ENDOCANNABINOID SYSTEM IN THE REGULATION OF ACETALDEHYDE MOTIVATIONAL PROPERTIES

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Increasing evidence focuses on acetaldehyde (ACD), the first alcohol's metabolite, as the mediator of the rewarding and motivational properties of its parent compound. Indeed, ACD directly enhances dopamine neurotransmission (1) and has rewarding and motivational properties which involve D2 receptor signalling (2, 3). The endocannabinoid system modulates the primary rewarding effect of many drugs since it fine-tunes dopamine cell activity (4). In light of this, the present study aimed at investigating the contribution of the endocannabinoid system on oral ACD self-administration, by using an operant paradigm tailored for studying the motivational properties of drugs.

Rats were trained to lever press in order to get ACD solution (0,9%) along the experimental sessions: acquisition and maintenance; extinction; relapse, following ACD deprivation; conflict - response-contingent punishment- according to a modified Geller-Seifter procedure.

The effects of AM281, a selective CB1 antagonist (1mg/kg i.p.), on ACD motivational properties were evaluated during the extinction-, relapse- and conflict-sessions.

Our results show that oral ACD is able to induce and maintain an operant behaviour, and a higher number of responses during extinction, during the relapse phase, and a higher emission of punished responses, when compared with controls.

AM281 significantly decreased ACD-seeking behaviour during extinction ( $p < 0.001$ ), the number of lever presses during relapse ( $p < 0.001$ ) and to strongly decrease the punished responses during conflict ( $p < 0.001$ ).

We suggest that CB1 receptors are involved in the reinforcing and motivational properties of ACD. The evidence of ACD interactions with the endocannabinoid and the dopaminergic systems further strengthens the idea that it can contribute to the central effects of alcohol.

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*Ultrasonic vocalizations in rodents: a tool for  
the investigation of psychoactive drugs and  
neuropsychiatric conditions*

## **SIMILARITIES AND DIFFERENCES IN THE EFFECTS OF PSYCHOACTIVE DRUGS ON THE EMISSION OF 50-KHZ ULTRASONIC VOCALIZATIONS IN THE RAT AND ROLE OF NON-DOPAMINERGIC RECEPTORS**

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Rats emit 50-kHz ultrasonic vocalizations (USVs) in response to pleasurable stimuli, and these USVs are increasingly being used as a behavioral measure in the investigation of the motivational properties of drugs. Previous studies from our laboratory have demonstrated the existence of major differences in the acute and long-term effects of different drugs with rewarding properties on the emission of 50-kHz USVs. Moreover, earlier studies with amphetamine, which robustly stimulates 50-kHz USVs, have indicated that activation of the dopaminergic system is instrumental in the emission of these USVs. However, others have found that non-dopaminergic neurotransmitters also participate in this behavioral response.

The aim of this presentation is to provide an overview of the similarities and differences in the effects of various drugs on the emission of 50-kHz USVs, with a close eye on the relevance of these effects to the study of the motivational properties of drugs. Moreover, attention will be paid to the role of non-dopaminergic neurotransmitter systems in the emission of these USVs. In this regard, recent data on the role of the glutamate N-methyl-D-aspartate (NMDA) receptor and the adenosine A<sub>2A</sub> receptor in the acute and long-term effects of amphetamine and morphine on the emission of 50-kHz USVs will be discussed.

## ULTRASONIC VOCALIZATIONS: EVIDENCE FOR AN AFFECTIVE OPPONENT PROCESS DURING COCAINE SELF-ADMINISTRATION

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The motivational impetuses underlying the administration of psychomotor stimulants are complex. Indeed, both positive and negative affective states have been hypothesized to influence drug seeking and drug relapse. Recent work in our laboratory has used ultrasonic vocalizations in order to infer affective states within an animal model of drug addiction. Our results demonstrate that cocaine use produces an initial euphoric period which quickly declines as drug use continues. Moreover, we observed that animals' negative affective responses and motivational state were influenced by fluctuating levels of cocaine. Specifically, the greatest rates of responding and negative affect were observed when bodily levels of cocaine fell below animals' satiety threshold suggesting that repeated drug use is driven by negative reinforcement. Finally, our data demonstrate that responding at relapse is complex and that drug-seeking may persist in the absence of any overt affective response. Overall, these results provide novel support for the view that affect is one potential motivational factor influencing human drug use and suggest that ultrasonic vocalizations in the rat may provide a powerful tool for understanding the role of affect in addiction.

## **RAT 50-KHZ ULTRASONIC VOCALIZATIONS AS A TOOL FOR THE STUDY OF DRUG DEPENDENCE: THE EXAMPLE OF AMPHETAMINE**

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The instrumentation for studying the development of drug dependence in laboratory rodents has been greatly enriched with the techniques exploring their ultrasonic vocalization (USV). Since the beginning of the present decade, we were trying to clarify the relation between this technology and a variety of earlier behavioral approaches by studying, in adult male rats, the effects of repetitive amphetamine treatment on the rate of appetitive (so-called 50-kHz) USV and on performance in the various classical behavioral tests employed in studies on addictive substances, including tests of locomotor activity, conditioned place preference, etc. The subject rats were categorized into low and high responders/callers based on the sensitization of their 50-kHz USV response to the drug as assessed using the so-called two-injection protocol of sensitization (TIPS). Additionally, we were looking for possible predictors of the propensity for the sensitization and for neurochemical and biochemical correlates of the various behavioral effects of amphetamine. Our data demonstrated a considerable inter-individual diversity and intra-individual stability of repeated amphetamine treatment-induced sensitization of rat 50-kHz USV rate response to the drug. The TIPS-induced sensitization showed some correspondence with rats' sensitivity to pain and the latency of their USV response to the first amphetamine exposure, as well as with the conditioned place preference, but not with locomotor activity response. Amphetamine challenge resulted in clear increases both in the level of blood corticosterone and in the intensity of Fos expression in most studied brain regions of high callers but not of low callers. However, the TIPS-induced changes in total brain tissue levels of the studied monoamines, monoamine metabolites and aminoacids followed dissimilar patterns. These results indicate that appetitive USV, locomotor activity, conditioned place preference and changes in brain biochemistry and neurochemistry reflect different aspects of amphetamine sensitization in rats.

## RAT 50-KHZ ULTRASONIC VOCALIZATIONS AS A TOOL FOR THE STUDY OF ANXIETY AND DEPRESSION

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It is well known that rats emit 50-kHz ultrasonic vocalizations (USV) during or in anticipation of various appetitive situations, such as juvenile rough-and-tumble play, mating, food, or in response to drugs of abuse, especially amphetamine. On the other hand, 50-kHz USV emission is highly unlikely in anxiogenic and aversive situations. Therefore, it has been suggested that 50-kHz USV may provide a useful approach to study positive affective states in the sender. Besides, 50-kHz USV serve a communicative function, that is, they can elicit approach in a recipient rat, which can be measured by means of a playback approach<sup>1</sup>, and which can be impaired by prior social isolation during the play age, when 50-kHz calls play a prominent role during social play<sup>2</sup>. Furthermore, both emission and response to 50-kHz calls was found to be related to dopamine function in the nucleus accumbens (e.g.<sup>3</sup>), which indicates that studying these emotional and social phenomena may be helpful in models of neuropsychiatric diseases, such as depression or mania, where changes in dopamine function seem to play a prominent role. Evidence in favor of these arguments will be presented, including a novel psychopharmacological approach, where 50-kHz USV were used in an otherwise conventional model of mania, and where it was found that excessive 50-kHz USV, as elicited by systemic d-amphetamine, were normalized by established or potential antimanic drugs, like lithium or myricitrin<sup>4</sup>.

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## ULTRASONIC VOCALIZATION DEFICITS IN PINK1 KNOCKOUT MODEL OF PARKINSON'S DISEASE

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Parkinson disease (PD) is a progressive neurodegenerative disease that leads to a wide range of motor and non-motor deficits. Specifically, ENREF\_1 deficits manifest early, are devastating to quality of life, and are difficult to treat with standard medical therapies. The pathological hallmarks of PD include accumulation of the presynaptic protein alpha-synuclein and degeneration of substantia nigra dopaminergic neurons. However, there is no clear understanding of how or when this pathology contributes to voice dysfunction in PD. In the present study, we evaluated the effect of loss of function of the PTEN-induced putative kinase 1 gene in rats (*PINK1* KO), a model of autosomal recessive PD in humans, on ultrasonic vocalizations neurodegenerative pathologies. Behavioral measures included ultrasonic vocalizations and gross motor performance that were assayed at 2, 4, 6, and 8 months of age. Abnormal alpha-synuclein and tyrosine hydroxylase immunoreactivity were measured at 4 and 8 months. We show that compared to wildtype controls *PINK1* KO rats develop (1) early and progressive vocalization deficits; (2) decreased tyrosine hydroxylase immunoreactivity in the locus coeruleus that correlates with vocalization loudness; (3) decreased tyrosine hydroxylase immunoreactive density in the substantia nigra; and (4) alpha-synuclein neuropathology in brain regions important for cranial-sensorimotor control. This novel approach of using a *PINK1* KO model of PD to relate vocalization and swallowing dysfunction to pertinent underlying neurodegenerative pathology provides the foundational work necessary to define behavioral biomarkers for the development of disease-modifying therapeutics for PD.

*Neurobehavioral correlates  
of REM sleep disturbances*

## NEURO-CIRCUITS CONTROLLING MUSCLE ATONIA DURING PARADOXICAL (REM) SLEEP AND THEIR ROLE IN RBD

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Paradoxical sleep (PS) is characterized by muscle atonia induced by ponto-medullary-spinal pathways. It was first demonstrated that a pontine area recently named sublaterodorsal tegmental nucleus (SLD) contain the neurons inducing the muscle atonia of PS. Besides, it was shown that glycine induces the hyperpolarization of motoneurons during PS. We recently define in detail the network responsible of muscle atonia during PS combining Fos staining, retrograde tracing and immunohistochemistry or “in situ” hybridization of markers of cholinergic, glutamatergic, GABAergic and glycinergic neurons. We showed that glutamatergic neurons localized in the SLD triggered muscle atonia during PS by means of their descending projections to GABA/glycinergic neurons localized in the ventral medullary formation namely the ventral gigantocellular reticular nucleus (GiV). We further showed that these neurons project to the spinal cord and are activated during PS. To directly demonstrate the role of these glutamate and GABA/glycinergic neurons in PS atonia, we inactivated SLD glutamatergic or GiV GABA/glycinergic transmission using transfection with AAVs of short hairpin RNA specific of the mRNAs of the vesicular glutamate 2 (vGLUT2) or GABA/glycine vesicular (vGAT) transporters. These animals display absence of atonia and large movements during PS confirming the role of the SLD glutamatergic neurons and the GABA/glycinergic neurons in the induction of muscle atonia during PS. In line with these results, we propose that REM sleep behavior disorder (RBD) is due to a specific degeneration of PS-on glutamatergic neurons localized in the SLD or the glycinergic/GABAergic premotoneurons localized in the GiV.

## EFFECTS OF DOPAMINERGIC THERAPY ON REM SLEEP BEHAVIOUR DISORDER IN PARKINSON'S DISEASE PATIENTS

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The majority of Parkinson's disease (PD) patients exhibit a wide range of parasomnias, the most common of which is REM sleep behaviour disorder (RBD). Although the comorbidity of RBD and PD is well-established, little is currently known about the specific relation in between. Thus, this study was aimed at assessing the prevalence and clinical features of RBD in PD patients, and assessing potential correlations between the severity of the two illnesses, as well as the potential impact of PD therapy on the clinical course and pathophysiology of RBD.

The study included 261 PD patients (mean age:  $68.7 \pm 13.4$ ), divided in four treatment groups: 1) patients on non-dopaminergic therapy (n=86); 2) patients on levodopa treatment (n=87); 3) patients treated only with dopaminergic agonists (n=30); 4) patients treated with levodopa in combination with dopaminergic agonists (n=58). Clinical assessments for PD and RBD severity were performed via PD sleep score (PDSS), the RBD screening questionnaire, UPDRS as well as H&Y scores. Scores were compared across the four treatment groups using Kruskal-Wallis statistics. Correlations were performed by Spearman's rank, followed by Dunn's multiple-comparison post-hoc tests.

A total of 163 patients (62.45%) were screened positive for RBD. No significant correlation was found between sleep disturbance severity and either UPDRS or H&Y score across any of the treatment groups. Interestingly, RBD scores were significantly correlated with LEDD ( $P=0.02$ ) in the overall population of patients; post-hoc analyses revealed this effect was brought about by the scores of patients treated exclusively with levodopa.

These results suggest that levodopa may have an inducing/enhancing effect on RBD; these effects, however, are apparently countered by concomitant therapy with dopaminergic agonists (which activate D2 and D3, but not D1 dopamine receptors), potentially indicating a differential role of dopamine receptor subtypes in the modulation of RBD symptoms.

## NEUROPSYCHIATRIC FEATURES ASSOCIATED WITH REM BEHAVIOR DISORDER IN PARKINSON'S DISEASE

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REM behavior disorder (RBD) is a parasomnia characterized by complex and violent motor behaviors emerging from REM sleep, due to a lack of normal muscle atonia and/or an increased phasic muscle activity during this sleep stage. Up to 60% of patients with Parkinson's Disease (PD) have RBD and they tend to be more severely impaired in both motor and non-motor domains compared to PD-noRBD.<sup>1</sup> Therefore RBD in PD would be a marker of a more pervasive neurodegenerative process. Impulse Control Disorders occur in a subset of PD patients taking dopaminergic replacement therapy and include pathological gambling, compulsive eating, compulsive sexual behaviors and compulsive buying. We first aimed to assess the frequency of ICDs in 216 PD patients with and without RBD assessed by questionnaires. After adjusting for gender, age of onset, PD duration, PD severity, depression score and total and dopaminergic agonist-LEDD, we found that probable RBD was associated to a relative risk of 2.59 for any ICD symptoms ( $p=0.001$ ), and of 4.87 for symptoms of pathological gambling ( $p=0.049$ ) compared to PD-without pRBD.<sup>2</sup> Then we assessed the frequency of PSG-confirmed RBD in  $n=29$  PD patients with clinical diagnosis of ICDs vs.  $n=29$  PD without, finding that 86% of PD ICDs+ have RBD vs. 46% of PD ICDs- ( $p=0.001$ ). These results support the notion that RBD would represent a predisposing factor for ICDs. Finally, when excluding ICDs, we found that PD patients with RBD (PD RBD+,  $n=20$ ) were more apathetic (Lille Apathy Rating Scale score:  $-17.6\pm 14.0$  vs  $-26.8\pm 17.7$ ,  $p=0.04$ ) and more depressed (Hospital and Anxiety depression subscore:  $6.4\pm 4.1$  vs.  $3.9\pm 2.8$ ;  $p=0.02$ ) compared to sex- and age-matched PD RBD-. The two groups do not differ in LEDD or in measures of cognitive functions. Taken together these results suggest that RBD in PD would be associated to a more severe impairment of the mesocorticolimbic system compared to PD-noRBD.

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## NEUROSTEROIDS MEDIATE THE BEHAVIORAL CHANGES INDUCED BY REM SLEEP DEPRIVATION

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In humans and animal models, sleep deprivation (SD) leads to a number of emotional and cognitive phenotypes related to psychosis. In particular, we and others found that patients and rats subjected to SD displayed robust sensorimotor gating deficits, as tested via the prepulse inhibition (PPI) of the acoustic startle. Although we documented that these alterations are underpinned by dopaminergic alterations, the molecular substrates for these changes remain poorly understood. We recently reported that 5-alpha reductase (5AR), the enzyme converting progesterone into the neurosteroid allopregnanolone, is involved in the dopaminergic modulation of sensorimotor gating; thus, we hypothesized that 5AR and its neurosteroid substrates may play a role in the behavioral outcomes of SD.

Rats were deprived of REM sleep for 72h using the single platform method. Startle response and PPI were measured immediately after the SD period, in comparison with controls maintained in standard conditions. At the end of test, animals were euthanized and brain regions were harvested to assess 5AR expression and neurosteroid levels.

As expected, SD rats exhibited profound PPI deficits and changes in startle parameters, which were accompanied by a significant up-regulation of 5AR type 1 and 2 in the prefrontal cortex and ventral striatum. Moreover, PPI was inversely correlated with 5AR levels. The 5AR inhibitor finasteride (25-100mg/kg, IP) dose-dependently reduced PPI deficits and normalized neurosteroid imbalances induced by REM SD. Notably, progesterone (25 mg/kg, IP) and allopregnanolone (10 mg/kg, IP), respectively ameliorated and worsened SD-induced PPI disruption.

These results suggest that 5AR mediates the change in sensorimotor gating observed in sleep-deprived subjects, through alterations of neurosteroid homeostasis in the dopaminergic system. Furthermore, these findings highlight allopregnanolone as a core substrate for the behavioral impairments induced by SD.

***Basal Ganglia microcircuits in action:  
from synapses to behavior***

## PARALLEL INFORMATION PROCESSING IN STRIATAL NETWORKS

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Information processing in behaving animals has been the target of many studies in the striatum; however, its dynamics and complexity remain to a large extent unknown. Sensorimotor, associative and limbic functions are typically attributed to distinct striatal regions based on their reentrant corticostriatal connections. Such a network suggests the occurrence of segregated parallel processing of information between, but not within, these striatal regions. Chronic recordings of neuronal populations in dorsal striatum of mice during novel environment exposure reveal otherwise; non-overlapping populations of striatal projection neurons—the medium spiny neurons—reliably encode locomotion and environmental familiarity, whereas two subpopulations of short-spike interneurons encode distinct information: the fast spiking interneurons preferentially encode locomotion whereas the second type of interneurons preferentially encodes environmental familiarity. The three neuronal subgroups use cell-type specific coding schemes. The hypothesis of parallel processing within dorsal striatum is further supported by data collected from R6/2 Huntington's disease model mice and their control littermates during exposure to a novel environment. First, substantial alterations occur in the firing properties of the different subpopulations. Second, the encoding of locomotion by the medium spiny neurons is substantially impaired whereas the environmental familiarity encoding remains similar to that of controls suggesting differential sensitivity to disease induced impairment. Our results support the hypothesis that separate groups of striatal projection neurons convey different types of information and provide evidence for the existence of parallel processing circuits within the sensorimotor region of the striatum.

## GENERATING AND SHAPING NOVEL ACTION REPERTOIRES

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Some actions are innate or prewired (such as swallowing or breathing). Others are learned anew throughout life, likely through a process of trial and feedback. We used electrophysiology, imaging and optogenetics in behaving animals to understand how novel self-paced actions are generated, and how specific actions that lead to particular outcomes are then selected. We uncovered that dopamine is critical for the generation of novel actions, and that plasticity in cortico-basal ganglia circuits is necessary for action selection. Furthermore, as actions are shaped they become organized into chunks, and neural substrates of parsing and concatenation of motor chunks emerge in basal ganglia circuits.

## STRIATAL CHOLINERGIC INTERNEURONS IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disorder associated with a drop in cholinergic markers in the striatum. Giant, aspiny cholinergic interneurons are key elements in the striatal circuitry controlling movement. As the source of tonic cholinergic tone, they must be implicated in the hypo-cholinergic state. However, it is unknown whether their physiological properties are altered in the HD striatum. To address this issue, the intrinsic and synaptic properties of cholinergic interneurons were examined in presymptomatic Q175 and BACHD mouse models of HD. In *ex vivo* brain slices, the autonomous spiking of cholinergic interneurons was reduced in both HD models. Additionally, optogenetic activation of thalamic axons arising from the parafascicular nucleus of the thalamus evoked significantly smaller currents in cholinergic interneurons from HD models. The attenuation of thalamic responses was paralleled by an increased response to optogenetic stimulation of cortical axons, enabling these inputs to more readily induce burst-pause patterns of activity in cholinergic interneurons. This adaptation was dependent upon amplification of cortically evoked responses by post-synaptic voltage-dependent sodium channels. The amplification appeared to be due to a decline in inhibitory metabotropic glutamate receptor activation of protein kinase C, which is known to enhance Nav1 channel slow inactivation. Thus, there is a functional 're-wiring' of the striatal networks in HD models, which includes: i) reduced tonic activity of cholinergic interneurons and reduced phasic thalamic drive to cholinergic interneurons, both of which presumably contribute to the hypo-cholinergic state in HD; and ii) greater cortical control of phasic cholinergic interneuron activity, which is widely thought to shape the impact of salient stimuli on striatal action selection.

## **THE STRIATUM CONSTRAINS THE EXECUTION OF MOTOR HABITS THROUGH CONTINUOUS INTEGRATION OF CONTEXTUAL AND KINEMATIC INFORMATION**

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The striatum is required for the acquisition of procedural memories but its contribution to motor control once learning has occurred is unclear. I will present results obtained using a task in which rats learned a difficult motor sequence characterized by fine-tuned changes in running speed adjusted to spatial and temporal constraints. Specifically, tetrode recordings of spiking activity in the dorsolateral striatum (DLS) of well-trained animals revealed continuous integrative representations of running speed, position and time. These representations were weak in naive rats hand-guided to perform the same sequence and developed slowly after learning. Finally, DLS inactivation in well-trained animals preserved the structure of the sequence while increasing its trial-by-trial variability and impaired the animals capacity to make corrections after incorrect trial. We conclude that after learning the DLS continuously integrates task-relevant information to constrain the execution of motor habits. Our work provides a straightforward mechanism by which the basal ganglia may contribute to habit formation and motor control.

## MICROCIRCUITS UNDERLYING SENSORY INTEGRATION IN THE STRIATUM

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The basal ganglia are traditionally studied with focus on their motor functions, however they receive sensory inputs from the entire neocortical sheet, including primary sensory areas. Our aim is to elucidate the functional properties of cortical and striatal networks underlying sensory-motor processing. The striatal microcircuitry consists of the major projecting population, the medium spiny neurons (MSNs), and a diverse population of interneurons. Fast spiking (FS) interneurons provide robust and reliable feed-forward inhibition, targeting both direct and indirect pathway MSNs with high connection probability, and are considered important for the synchronization of their postsynaptic targets. Striatal cholinergic interneurons provide disynaptic inhibition to MSNs and under in vivo conditions display tonic and synchronized discharge. Whether and how GABAergic interneurons control cholinergic interneurons and affect their synchronicity or regulate other striatal interneuron types remains unknown. We have here combined multiple whole-cell recordings with optogenetics in order to directly characterize the target selectivity of intrastriatal inhibition provided by striatal GABAergic interneurons. I will also present in-vivo work demonstrating bilateral and multimodal sensory integration by individual striatal neurons in the healthy and dopamine-depleted striatum.

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*From circadian clock to human health –  
PART II. Circadian clocks: keys for health*

## **BRAIN AREAS AND PATHWAYS IN THE REGULATION OF GLUCOSE METABOLISM**

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Accumulating evidence indicates an association between the development of obesity and type 2 diabetes at the one hand and disturbances in circadian control at the other hand. Disruption of circadian rhythms can be caused by external factors such as shift work and jet lag, but also by pathophysiological factors including aging, depression and sleep disorders. It remains unclear, however, what the precise contribution of the suprachiasmatic nucleus (SCN), i.e., the central circadian pacemaker, is in the regulation of glucose and energy homeostasis.

The SCN plays an essential role in maintaining daily blood glucose concentrations. Indeed both glucose production and glucose uptake show a pronounced daily rhythm, with increased glucose uptake as well as glucose production at the time of awakening. We previously demonstrated how changes in autonomic nervous system activity contribute to the daily control of plasma glucose and insulin concentrations. More recent studies evidenced an important role for VIP, but not vasopressin, as an SCN output in the control of hepatic glucose production. In addition, hypothalamic orexin and oxytocin neurons turned out to be important targets for the SCN to transmit its glucoregulatory effects onto the autonomic nervous system.

Finally, using localized infusions of the sodium channel blocker tetrodotoxin (TTX) in the rat SCN, to silence SCN neuronal activity, combined with euglycemic hyperinsulinemic clamp studies we found that an acute reduction of SCN output resulted in hepatic insulin resistance as well as increased peripheral glucose uptake. These results indicate that a withdrawal of SCN neuronal activity at the end of the light period increases activity of orexin neurons, resulting in an increased hepatic glucose production as well as an increased peripheral glucose tolerance.

## MELATONIN AND CIRCADIAN RHYTHM DISRUPTION IN DISEASE

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The circadian rhythm of melatonin provides a robust biomarker of circadian clock timing in humans. The melatonin rhythm is driven by the central circadian pacemaker located in the hypothalamic suprachiasmatic nuclei (SCN). Melatonin timing is primarily determined by the light/dark environment that depends on a person's sleep/wake pattern and work/home environment. Assessment of circadian phase allows the diagnosis of circadian rhythm disorders (such as time zone travel, shift work, delayed sleep phase insomnia (DSPS), non-24 h sleep/wake disorder) as well as optimising the timing of chronobiotic treatment (light and/or exogenous melatonin) to reset disordered timing. Fewer studies have addressed changes in melatonin amplitude in health and disease. Melatonin amplitude/production is consistent within an individual but is highly variable between individuals, it declines with age and is acutely suppressed by light at night and some medication (e.g.  $\beta$ -blockers). Our recent research in controlled laboratory conditions has shown reduced melatonin amplitude in Type 2 diabetes compared with age and weight matched controls<sup>1</sup>. A night of total sleep deprivation has been shown to increase melatonin production compared to a night of sleep in healthy controls<sup>2</sup>. Whilst the association between disrupted sleep (restriction or deprivation) and metabolic disorders such as obesity, cardiovascular disease and diabetes is well established, the mechanisms underlying this link remain unclear. To this end we are doing metabolic profiling, or 'metabolomics', using liquid chromatography mass spectrometry (LC/MS) to characterise 24 h metabolite rhythms and the effect of sleep and sleep deprivation on the human metabolome<sup>3</sup>. This, along with assessment of melatonin production and timing, will help to better understand circadian and sleep-wake regulation and the associated metabolic pathways.

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## THE HUMAN SCN IN HEALTH AND NEUROPSYCHIATRIC DISORDERS: POSTMORTEM OBSERVATIONS

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Lesions in the human suprachiasmatic nucleus (SCN) region due to tumors result in a decreased expression of one of the major SCN peptides, arginine vasopressin (AVP), and in disturbed circadian rhythms. The human SCN contains also vasoactive intestinal polypeptide (VIP) and neurotensin and GABA is co-localized with one or more peptides. In postmortem human SCN, until approximately the age of 50 years, distinct day-night and seasonal fluctuations were found for the AVP- and VIP-expressing neurons. Moreover, structural and functional differences in relation to gender and sexual orientation are present in the SCN, as well as sex differences in aging. The SCN is driving pineal gland melatonin that influences many brain functions via the melatonin receptors (MT1, MT2). The pineal system, too, shows strong changes with aging. The SCN of Alzheimer (AD) patients - who suffer from circadian rhythm disturbances - exhibits a loss of AVP-, neurotensin- and MT1-expressing neurons. AVP-mRNA levels in the SCN and day-night fluctuations in pineal melatonin decrease already in preclinical AD stages. A combination of light and melatonin treatment was found to increase sleep efficiency and improve nocturnal restlessness, mood, performance, daytime energy and quality of life.

In Huntington's disease patients the SCN contained less VIP- and AVP expressing neurons. In depression an increased number of AVP-expressing neurons was observed, together with a decreased amount and a diminished circadian fluctuation of AVP-mRNA in the SCN. MT1 was also increased in the SCN in depression. In primary hypertension the number of AVP-, VIP- and neurotensin-containing neurons was reduced. The amount of AVP-mRNA expression in the SCN in glucocorticoid-treated patients was found to be two times lower. In addition, there was a strong decrease in the total number of profiles in the SCN that expressed AVP-mRNA.

## CIRCADIAN RHYTHMS: A KEY FOR INNOVATIVE THERAPEUTICS

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The role of circadian rhythms on the human physiological processes has been known for years. In the 1990's melatonin emerged as synchronizer of disorganized rhythms in the CNS disorders such as depression. These observations led us to propose Agomelatine as the first resynchronizing agent that can afford therapeutic benefit particularly in major depression. Recent advances have led to the discovery that all the organs have their own circadian clocks which orchestrate 24h oscillations, dependently or not from the master clock, the SCN. The fundamental unit of the clock is a cell-autonomous oscillator consisting of transcriptional-translational feedback loops. Heterodimeric transcription factors CLOCK/BMAL1 and NPAS/BMAL1 activate expression of the *period1/2* and *cryptochrome 1/2* genes. The protein products PER1/2 and CRY1/2 translocate to the nucleus. They inhibit CLOCK/BMAL and NPAS2/BMAL1 and repress their own expression. Alteration of the core loop can occur in several situations (external signals, diet, pathological situation, aging, mutation, etc.) leading to impaired functionality of the organ. For example *BMAL1* knockout leads to impaired pancreatic insulin secretion and glucose intolerance in mice. Kruppel-like transcription factor15 (KLF15) is activated by CLOCK/BMAL1 and KLF15 regulates the transcription of the genes encoding KvCHIP2 a cardiac ion channel, an important component required for myocardial repolarization. The circadian clocks control also inflammatory processes since LPS-induced inflammatory response is dependent on BMAL1 activity in macrophages. Recent advances will be discussed. Clocks may be considered as key-partners for innovative therapeutics.

*Personal genomics in psychiatric disorders*

## DEVELOPMENT OF AN ELECTRONIC PHARMACOGENOMICS ASSISTANT WITH EMPHASIS TO NEUROPSYCHIATRIC DISORDERS

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The rapid evolution of high-throughput genotyping technologies and the increased pace of production of genetic research data, are continually prompting the development of appropriate informatics tools, systems and databases as we attempt to cope with the flood of incoming genetic information. This dictates the development of information systems that would contribute to the creation of a powerful knowledge environment for genotype-to-phenotype information in the context of translational medicine. In the area of pharmacogenomics, and personalized medicine, it is evident that database applications providing important information on the occurrence and consequences of gene variants involved in pharmacokinetics, pharmacodynamics, drug efficacy and drug toxicity, will become an integral tool for researchers and medical practitioners. To this end, psychiatry and neurology are among the medical specialties that pharmacogenomics significantly contribute to the rationalization of drug treatment modalities. We present advances and challenges in the field of pharmacogenomics information systems, in particular the electronic molecular diagnostics assistant (eMoDiA), designed to provide personalized drug recommendations based on linked genotype-to-phenotype pharmacogenomics data, as well as to support biomedical researchers in the identification of pharmacogenomic related gene variants. At first, several external data sources are leveraged to extract and integrate pharmacogenomic information, by adopting the notion of a data warehouse, as the basic data model and the most appropriate to encompass the different requirements for database technology. The data warehouse is centered around the gene/Variant-drug-phenotype-recommendation concept, embodied in the fact table which references the dimensional tables around it, corresponding to the entities of: i) gene ii) drug, iii) diplotype, iv) phenotype and iv) clinical-annotations, guidelines and recommendations, which are utilized in order to fetch and transform data from the various heterogeneous data sources (PharmGKB, dbSNP, Affymetrix annotations, PubMed etc.) into the central data warehouse. The provisioned services are tuned in the framework of a single-access pharmacogenomics portal.

## AUTOPHAGY PLAYS A KEY ROLE IN SCHIZOPHRENIA AND BEYOND

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Autophagy, a highly regulated process, has a crucial impact on cellular homeostasis. Autophagy is particularly important in the highly sensitive cells of the nervous system—the neurons. By preserving the balance between synthesis/degradation and recycling of cellular components, autophagy is essential for neuronal survival. Blockade of autophagy in neurons led to cell death and neurodegeneration in rodents. Abnormality in autophagy may lead to activation of apoptosis as in the most prevalent neurodegenerative disease, Alzheimer's disease (AD) as well as the second prevalent neurodegeneration, Parkinson's disease. We have recently shown that beclin1, a key regulator of autophagy is decreased in the postmortem hippocampus of schizophrenia subjects compared to controls, indicating that autophagy plays a role in mental diseases as well as in neurodegenerative disorders. We have further shown a direct interaction between the microtubule associated protein 1 light chain 3 (LC3B, a main autophagy marker) and activity-dependent neuroprotective protein (ADNP), a protein essential for brain formation, which is deregulated in schizophrenia. ADNP haploinsufficiency in mice exhibits age-related neuronal death and tauopathy (with AD being the major tauopathy), cognitive and social autism-like dysfunction coupled to reduced hippocampal beclin1 mimicking schizophrenia. Interestingly, our most recent findings showed sexual dichotomy in ADNP hippocampal expression in mice and men, impacting on differential behavioral outcomes. NAP (NAPVSIPQ), represents the microtubule end binding site of ADNP that increases microtubule dynamics/stability and protects the autophagy process in cell culture and in microtubule-deficient animal models of schizophrenia. In clinical trials, based on NAP efficacy in animal models, NAP (davunetide) protected functional capacity (activities of daily living) in schizophrenia patients and provided neuroprotection. Recently, ADNP has been estimated to undergo *de novo* mutations in at least 0.17% of ASD cases paving the path to future investigations.

## A MICROARRAY AND PROTEOMICS STUDY OF LITHIUM-TREATED MICE AND KNOCKOUT MICE WITH LITHIUM-LIKE BEHAVIOR REVEALS A COMMON EFFECT ON MITOCHONDRIAL FUNCTION AND AUTOPHAGY – TWO POTENTIAL TARGETS FOR PERSONALIZED MEDICINE

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The inositol-depletion hypothesis proposes that lithium attenuates phosphatidylinositol signaling. Knockout-mice of two genes (*IMPA1*, *Slc5a3*) encoding for proteins related to inositol metabolism exhibit a lithium-like behavioral phenotype<sup>1</sup>.

We performed a DNA-microarray study searching for pathways commonly affected by chronic lithium-treatment and by the knockout of each of the genes. Data was analyzed using three different bioinformatics approaches. Commonly differentially expressed genes were verified by real-time PCR. A parallel proteomics study was also carried out, confirmed by Western blotting. We sought a potential behavioral correlate of the bioinformatics results using a pharmacological intervention examined in the bipolar related forced-swim test and amphetamine-induced-hyperlocomotion paradigm.

All bioinformatics analyses revealed up-regulation of mitochondrial function. Three of seven genes commonly differentially expressed in the three paradigms, *Cox5a*, *Ndufs7* and *Ndufab*, all members of the mitochondrial electron-transfer-chain, have been reported as associated with bipolar-disorder and/or lithium treatment. Their differential expression was verified by real-time PCR analysis. The proteomics study indicated that the mitochondrial function-related process, autophagy, is enhanced. The result of the bioinformatics analysis was consistent with an observed interrelationship between treatment with lithium and rotenone, an inhibitor of mitochondrial-function. Lithium and rotenone counteracted each other's effects in the forced-swim test and the amphetamine-induced hyperlocomotion paradigm.

The results (1) support reports of mitochondrial dysfunction in bipolar-disorder and its amelioration by lithium as well as reports of lithium-induced autophagy upregulation, all mediated via lithium's effect on inositol metabolism; (2) provide two candidate pathways the profile of which may possibly be developed to assist personalized medicine.

## **MECHANISM OF ACTION OF LITHIUM: WHAT HAVE WE LEARNED FROM IN-VITRO STUDIES IN HUMAN-DERIVED CELL LIENS?**

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Among mood stabilizers, lithium is the treatment of first choice in the management of bipolar disorders. The mood stabilizing and anti-suicidal properties of lithium have been known for decades, but its mechanism of action is still not completely understood. Studies in animals and human cells have shown that this ion influences the expression of hundreds of genes and interferes with the activity of many proteins and enzymes. Through this regulation, lithium interacts with a large number of cellular functions, including inositol metabolism, circadian rhythms, apoptosis, and neuroprotection pathways suggested to play a role in the pathophysiology of bipolar disorder and suicide. The most compelling and robust findings have been so far provided by studies performed on cell lines derived from patients characterized for clinical variables of interest, such as lithium response and suicidal behavior. These studies have shown that lithium significantly regulates transcription factors, microRNAs, and epigenetic factors. Most of these studies have focus on lymphoblastoid cell lines, as these cells can be easily set from lymphocytes. However, while lymphoblasts represent a valuable cell line to identify peripheral biomarkers of diseases and drug response, they do not allow exploring neuronal processes. This limitation is being overcome by the use of neurons derived from induced pluripotent stem cells, that can be set from fibroblasts or other peripheral tissues derived from patients. Findings from these studies are on their way and will provide highly-valuable information that will expand and integrate our knowledge on the mechanism of action of lithium.

*Neurolinguistics of Semitic Languages*

## ASPECTS OF ARABIC REPRESENTATION IN THE BRAIN OF NATIVE SPEAKERS: BOLD-fMRI STUDY

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The adult's cognitive functions are based on activation of specialized neuronal networks. Studies showed a set of well determined brain cortical tissue involved in the control of language function. In addition, Arabic language is shown to be very complex and might involve a complicated network of brain cells during language performance. The study goal is to establish the Arabic language verbal fluency functional map using simple model of paradigm and making use of BOLD-fMRI approach.

12 healthy-adult Arabic-speaking volunteers were recruited. They were non-smoking, right-handed, and without any neurological, psychiatric disorder. We used BOLD-fMRI to map the neuronal network involved in the control of verbal fluency of Arabic. The functional paradigm consisted of silent words generation alternating with counting during 30 seconds in each cycle. The Arabic characters were chosen according to the most important prevalence of use in the vocabulary of volunteers of this study.

The Arabic letters generating most words were used (أ, ح, م, ق, س, ر)

The fMRI results showed that the network of the Arabic verbal fluency is structured in equivalent way compared to already studied languages, in other words the right hemisphere is less stimulated than the left one, and this has considered volume and intensity of activations. However, different aspects were essentially found in cerebral lateralization in women. Similarities indicate the continuity in the processes and the neuronal structures underwent the functional control of different languages. While differences suggest that Arabic language have appropriate functional control characteristics.

This study of Arabic language opens new perspectives of exploration which would contribute to better information about the involved neuronal network in functional control of Arabic language.

## **DIFFERENT LEVELS OF DEFICITS IN CHILDREN WITH DYSLEXIA AS REVEALED BY LABBEL**

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Developmental dyslexia is a specific reading disorder characterized by difficulty in identifying written words; its prevalence is 10% among school children.

As part of a doctoral thesis, an Arabic dyslexia screening battery was administered initially to 70 Egyptian students, out of the 70 students, 22 were diagnosed dyslexics. The 70 students also completed the tests of the Arabic language assessment battery LABBEL, the results show that dyslexics have very low scores in word and letter discrimination tasks, suggesting a link between phonetic perception and phonological awareness. This result confirms the phonological deficit theory. Dyslexics also have low scores on the word repetition test, indicating a deficit in the short-term memory and an attention disorder. We also observed weak results in lexical decision test suggesting a deficiency in morphological treatment and phonological awareness, which prevents students to construct their mental lexicon.

For further study, 6 dyslexics and 5 normal readers participated in functional magnetic resonance imaging sessions. The results are being analyzed.

## THE EFFECTS OF MULTIPLE SCLEROSIS ON MORPHOLOGICAL PRIMING

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Multiple Sclerosis (MS) is a neurological disease characterized by extensive damage affecting large bundles of white matter fibers connecting different cortical areas. Morphological segmentation of complex words (e.g., *walked*) into stems (*walk*) and suffixes (*~ed*) has been argued to depend on intact white matter fibers. We therefore hypothesized that patients with early MS would lose the ability to segment auditorily presented words into their component morphemes even in a language like Arabic where morphological segmentation is *obligatory*, and would instead revert to storing and accessing words as whole forms.

Eight early MS patients and 35 normal controls performed an auditory-auditory priming experiment using the same set of Arabic words. The relationship between primes and targets was varied to form four conditions. In the first condition the prime and target shared a root and a transparent semantic relationship (+R+S) (e.g., [inzaalun]-[nuzuulun] *lowering-landing*). In the second, the relationship was purely morphological with primes and targets sharing a root, but no semantics (+R-S) (e.g., [rataabatun]-[tartiibun] *monotony-tidying up*). Conditions 3 and 4 were semantic (+S) and phonological (+Phon) controls respectively with semantically related pairs like [xayrun]-[niEmatun] *good-grace*, and phonological related pairs [maEdanun]-[muEAnidun] *mineral-stubborn*.

The normal controls showed the typical Arabic morphological priming with equivalent facilitation in the +R+S and the +R-S conditions, which was significantly different from the facilitation in the +S condition and the inhibition in the +Phon condition. In contrast, the MS patients showed significant priming in the +R+S and the +S conditions with no effects in the +R-S condition and no signs of inhibition in the +Phon condition. These findings support the view that morphological decomposition depends on the integrity of cortical parsing processes, which involve long-distance white matter projections, and are impaired due to diffuse demyelination in patients with early MS. Consequently these patients revert to full-form storage and access.

## VOWELLING AND SEMANTIC PRIMING EFFECTS IN ARABIC

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Both visually and auditorily presented words are processed faster when preceded by semantically related than unrelated words. This effect, known as semantic priming, has been studied in many languages as it provides important information on how words are accessed and stored in the mental lexicon. The first aim of this study was to examine the semantic priming effect in the Arabic language using both behavioral and electrophysiological methods. Results showed clear semantic priming effects at both levels and the characteristics of the N400, the brain signature of semantic processing, was very similar to previous results with other non-semitic languages<sup>1</sup>.

The second aim was to take advantage of one of the specificities of Arabic that is, words exist both under shallow (vowelled) and deep (unvowelled) orthographies. Results revealed larger N1 and N2 components of the Event-Related brain Potentials to vowelled words than unvowelled words suggesting that visual-orthographic complexity taxes the early word processing stages. Finally, the effects of semantic priming and vowellation did not interact indicating that both influence different stages of word comprehension. The implications of these results for understanding the processing of orthographic, semantic, and morphological structures in Modern Standard Arabic will be discussed as well as new perspectives for the understanding of language deficits.

*I. Mountaj, N., El Yagoubi, R. Himmi, M. Laghdar-Gazal, F., Besson, M. & Boudelaa, S., in press. Semantic priming and vowellation effects in Arabic. International Journal of Psychophysiology*

***Monoamines in neuropsychiatric disorders***

## SEROTONIN AND SIDE EFFECTS OF ANTIPSYCHOTICS

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Schizophrenia is a serious chronic psychiatric disorder, diverse in its clinical presentation, course of the disease and response to therapy. Lifelong treatment of schizophrenia is necessary to maintain social functioning and prevent chronic irreversible personality damage as well as relapse of symptoms. The neurobiological basis of schizophrenia is still insufficiently known, resulting in only partially successful treatment. This complex disease is treated with antipsychotic drugs, which are usually divided into first-generation antipsychotics (FGA), especially effective in the treatment of positive symptoms, and second-generation antipsychotics (SGA), which can reduce both the positive and negative symptoms of schizophrenia. Some patients do not respond satisfactorily to antipsychotics, while others develop side effects that substantially compromise the treatment, leading to discontinuation of therapy and frequent relapse of the disease. Acute or chronic extrapyramidal symptoms (EPS) appear frequently in patients treated with FGA, while side effects such as metabolic syndrome (MetSy) are often associated with the treatment with SGA. Various side effects of antipsychotics could be related to their different molecular targets, namely dopaminergic D2 (for FGA and SGA), or serotonergic 5-HT<sub>2A</sub> (for SGA) receptors. FGA and SGA are both antagonists of D2 receptors, but in contrast to the tight blockade of D2 receptors by FGA, the binding of SGA to D2 receptors is not long-lasting. In addition to the dopaminergic system, pharmacological studies also suggest the connection between antipsychotics, side effects and serotonergic system, probably via serotonergic receptors or by the effects of serotonin on dopamine release in the dopaminergic pathways. In order to improve schizophrenia therapy, recent studies are focusing on the genetic background of individual differences in the response to antipsychotic treatment as well as in the development of side effects.

## **ROLE OF SEROTONERGIC NEUROTRANSMISSION IN THE ONTOGENY OF REACTIVE AGGRESSION: FOCUS ON GENE X ENVIRONMENT INTERACTIONS**

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Gene-environment interactions have been shown to play a critical role in the development of aggression and other neuropsychiatric disorders. Several independent studies have highlighted that pathological aggression in males is often linked to the interaction of early-life abuse and/or neglect with allelic variants associated with low activity of monoamine oxidase (MAO) A, the key enzyme for the degradation of brain serotonin and norepinephrine. To explore the neural underpinnings of the interaction between early stress and low MAO A, we tested the impact of early maternal separation (MS) in MAO A<sup>Neo</sup> mice, a newly generated line of MAO A hypomorphic mutants. While MS did not significantly affect the aggressive behavior in either MAO A KO or wild-type (WT) mice, the same manipulation resulted in a robust enhancement of fighting responses in MAO A<sup>Neo</sup> mice, to a level comparable with that of MAO A KO counterparts. In addition, we found that the changes in MS-subjected MAO A<sup>Neo</sup> mice were supported by the activation of 5-HT<sub>2A</sub> serotonin receptors in the prefrontal cortex during the first week of postnatal life. These data parallel epidemiological findings on the interaction of low-MAO A allelic variants and early stress in males with respect to the development of antisocial behavior; furthermore, our findings provide a powerful translational platform to investigate the role of serotonin in the pathophysiology of aggression. Further studies in our laboratory are beginning to elucidate the neurodevelopmental mechanisms supporting the interaction of early stress and MAOA genetic variants in reactive aggression and related emotional disturbances.

## UNRAVELING THE CONTRIBUTION OF DOPAMINE RECEPTOR SUBTYPES TO THE ANTIPSYCHOTIC-LIKE EFFECTS OF 5 $\alpha$ -REDUCTASE INHIBITORS

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Our group has previously shown that the pharmacological inhibition of 5 $\alpha$ -reductase (5 $\alpha$ R), the key rate-limiting enzyme in neurosteroidogenesis, normalized several behavioral effects of non-selective DA receptor agonists in rats, including stereotyped behaviors, locomotor hyperactivity and prepulse inhibition (PPI) deficits. In addition, we recently found that in C57BL/6 mice the 5 $\alpha$ R blockade attenuated the behavioral responses ensuing D<sub>1</sub>-, but not D<sub>2</sub>-like receptor activations. Here, to further delineate the specific influences of 5 $\alpha$ R in the DAergic regulation of PPI, we tested the impact of the 5 $\alpha$ R inhibitor finasteride (FIN) on the PPI-disruptive effects of D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> DA agonists in Sprague-Dawley (SD) and Long-Evans (LE) rats. We found that the mixed D<sub>1</sub>/D<sub>2</sub> agonist apomorphine elicited PPI disruptions in both strain, and these deficits were reversed by FIN in SD, but not in LE rats. On the other hand, the D<sub>1</sub> receptor agonists SKF-38,393, SKF-82,958 and SKF-83,959 had no effects on PPI in SD rats. Nevertheless, the full D<sub>1</sub> agonist SKF-82,958 was able to reduce PPI parameters on LE rats, and these impairments were normalized by FIN. The D<sub>2</sub>/D<sub>3</sub> agonist quinpirole and the selective D<sub>2</sub> agonist sumanirole disrupted PPI in both strain, but FIN did not revert these effects. Of note, the pretreatment with FIN significantly restored the PPI deficits induced by the selective D<sub>3</sub> agonist PD-128907 in SD rats, whereas this latter compound failed to disrupt PPI in LE rats. Finally, to ascertain whether FIN may regulate D<sub>3</sub> receptors by pre- or -post synaptic mechanisms, microdialysis studies performed in the nucleus accumbens of SD rats revealed that the pretreatment with FIN counteracted the reductions of DA release produced by PD. These results indicate that 5 $\alpha$ R plays a crucial role in the dopaminergic regulation of gating through multiple mechanisms, based on pre- and post- synaptic actions via D<sub>3</sub> and D<sub>1</sub> receptors, respectively.

## Role of 5-HT1A receptor palmitoylation in development and maintaining of depression

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The 5-HT<sub>1A</sub> serotonin receptor belongs to the G-protein coupled receptor superfamily and is involved in a number of physiological effects, such as regulation of mood, neurogenesis and respiratory activity. The 5-HT<sub>1A</sub> receptor also plays an important role in several psychiatric disorders, including depression and anxiety. We have previously shown that the 5-HT<sub>1A</sub> receptor is modified by covalently attached palmitate and that its palmitoylation is essential for receptor-mediated activation of inhibitory G<sub>i</sub>-protein and downstream effector signalling<sup>1</sup>. We have also demonstrated that a significant fraction of the 5-HT<sub>1A</sub> receptor resides in membrane rafts, while the yield of the palmitoylation-deficient receptor in these membrane microdomains is considerably reduced. These results demonstrate that receptor palmitoylation serves as a targeting signal responsible for the retention of the 5-HT<sub>1A</sub> receptor in membrane rafts. More importantly, the raft localization of the 5-HT<sub>1A</sub> receptor appears to be involved in receptor-mediated signaling<sup>2</sup>. Although critically involved in regulation of receptor-mediated signalling and lipid rafts localization, palmitoylation doesn't influence homo-oligomerization of 5-HT<sub>1A</sub> receptors<sup>3,4</sup>. In the present study, using classical radioactive labeling in combination with a novel acyl-biotinyl-exchange (ABE) assay, we identified 4 different palmitoylation enzymes (DHHCs) involved in 5-HT<sub>1A</sub> receptor palmitoylation. Depletion of these DHHCs reduces palmitoylation of 5-HT<sub>1A</sub> receptor and impairs receptor functions in heterologous system as well as in hippocampal neurons. In addition, ABE analysis of intact brain tissues from mice revealed that 5-HT<sub>1A</sub> receptor is palmitoylated *in vivo*, and that the level of receptor palmitoylation is significantly reduced in mice subjected to the fear conditions. Functional importance of receptor palmitoylation was further confirmed by observation that knocking-down of relevant DHHCs in forebrain of mice resulted in spontaneous development of depressive behaviour. These results suggest that regulated activity of 5-HT<sub>1A</sub> receptor-specific palmitoylation enzymes can represent a novel molecular mechanism specifically regulating receptor functions.

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## ROLE OF 5-HT<sub>7</sub> RECEPTOR IN PSYCHIATRIC DISORDERS

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The serotonin subtype 7 (5-HT<sub>7</sub>) receptor has been cloned from the rat, mouse, human and guinea pig and it exhibits a high degree of interspecies homology (~95%), but a low-sequence homology with other 5-HT receptors (~ 40%). The 5-HT<sub>7</sub> receptor is coupled to the stimulatory Gs-protein, and receptor stimulation results in activation of adenylyl cyclase leading to a rise of cAMP concentration<sup>1</sup>. In addition, this receptor is coupled to the G12-protein to activate small GTPases of the Rho family<sup>2</sup>. The highest densities of 5-HT<sub>7</sub> receptors have been observed in the hypothalamus, thalamus, hippocampus, cortex and brain stem. In peripheral tissue, 5-HT<sub>7</sub> receptors are present in intestinal and vascular smooth muscle<sup>3</sup>. The widespread distribution of the 5-HT<sub>7</sub> receptor in the brain is suggestive of multiple central roles. Due to the availability of selective agonists, antagonists and knockout mice, a better understanding of 5-HT<sub>7</sub> receptor function has been obtained and many important roles have been identified in circadian rhythm, thermoregulation, sleep, endocrine regulation, cognition, anxiety, depression, schizophrenia, epilepsy, migraine and pain<sup>1</sup>. The present communication will give a survey of the most recent advances related to the role of 5-HT<sub>7</sub> receptor in psychiatric disorders, with major emphasis on prospective therapeutic interventions.

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*The juvenile brain: vulnerable period  
to the effects of stress and diet on neural  
plasticity and memory*

## **PERINATAL EMOTIONAL EXPERIENCE INTERFERES WITH THE FUNCTIONAL MATURATION OF PREFRONTAL-LIMBIC CIRCUITS: RISK OR BENEFIT?**

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While the negative consequences of stress, i.e. the elevated risk to develop mood disorders after perinatal exposure to stressors, are well-recognized in mental health research, the potential of early life stressors to mediate the development of stress resilience (the concept of stress “inoculation”) is less acknowledged. We study the immediate, long-term and transgenerational impact of perinatal adverse experience on the functional maturation of prefronto-limbic circuits using functional imaging, structural synaptic analysis, which is complemented by the assessment of epigenetic (histone modifications, DNA methylation) changes, which are assumed to mediate experience-induced neuronal plasticity. Based on clinical findings we also analyze individual and gender-specific vulnerability and resilience.

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## **STRESS DIFFERENTIALLY REGULATES PREFRONTAL CORTEX PLASTICITY AND EXTINCTION IN THE JUVENILE AND ADULT ANIMALS**

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The neural circuit linking the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) has crucial roles in both the acquisition and the extinction of fear conditioning. Exposure to behavioral stress has been shown to enhance fear responses, impair extinction and to severely affect memory processes. We and others previously showed that the function, plasticity and the morphology of the BLA and the mPFC is severely affected by stress. Specifically, we showed that exposure to brief episode of stress results in impaired extinction and attenuated plasticity in the form of long-term potentiation in the mPFC of the adult animal (Maroun and Richter-Levin, 2003; Akirav et al., 2009; Richter-Levin and Maroun, 2010).

Knowing that juvenility is a critical period during which the mPFC still undergoes changes and susceptible to the adverse effects of stress, we recently addressed whether exposure to same stressor at juvenility will similarly affect extinction and mPFC plasticity as was observed in the adult animal.

Our results show that exposure to stress is associated with enhanced ability to extinguish fear responses in the post-weanling pup. Similarly, stress was associated with enhanced LTP (Schayek and Maroun, 2014).

These results show that stress differentially modulates extinction and plasticity in the post-weanling pup as compared to the adult animal and point that the mechanisms of extinction and stress-induced changes in extinction and plasticity are not similar across development.

## **High-fats diets and behavioural disorders in adolescent mice: focusing on the role of hippocampus and prefrontal cortex**

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High-fat diets (HFD) have a deleterious impact on hippocampal and prefrontal areas, leading to impaired learning deficits as well as to an altered perception of reward. It has been suggested that hyperglycemia and insulin resistance triggered by energy-dense diets account for behavioural deficits, although the influence of diet composition itself cannot be discarded. Our team is devoted to investigate the influence of HFD on brain processes related to memory and reward in adolescent mice by means of behavioural, morphological, genomic, neurochemical and electrophysiological approaches. Our data show that HFD initially induced changes in brain areas involved in the perception of food as a rewarding stimulus, accounting for initial hyperphagia. Nevertheless, chronic HFD treatment evoked hypophagia that could be related with the inhibition of reward-processes observed in conditioned place-preference and food self-administration tests. Moreover, our data show that short-term HFD triggered responses coherent with the activation of sensory inputs that concern cortical areas. In contrast, chronic HFD consumption caused behavioural deficits, characterized by the impairment of hippocampus-dependent learning and memory tasks (eight-arm radial maze and novel object recognition tests). Under these conditions, HFD reduced basal synaptic transmission and induced relevant changes in synaptic plasticity, such as long-term depression, together with a down-regulation of glutamatergic transmission within the hippocampus. Memory impairment in adolescent mice was accompanied by an increase of dendritic spine density in CA1 pyramidal neurons that correlated with the up-regulation of neural cell adhesion molecule (NCAM) in this area whereas no effects were found in adult mice receiving a similar dietary treatment. Our results provide further evidence of the susceptibility of the hippocampus and prefrontal cortex to HFD in adolescent individuals.

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**DIFFERENTIAL EFFECTS OF JUVENILE AND ADULT EXPOSURE TO OBESOGENIC DIET ON AMYGDALA- DEPENDENT EMOTIONAL MEMORY: SPECIAL EMPHASIS ON GLUCOCORTICOIDS**

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In addition to metabolic and cardiovascular disorders, obesity is associated with adverse cognitive and emotional outcomes. Its growing prevalence during adolescence is particularly alarming since it is a decisive period for maturation of the amygdala and the hypothalamic-pituitary-adrenal (HPA) stress axis, both required for life-long cognitive and emotional processing. Here, we evaluate whether juvenile diet-induced obesity alters amygdala-dependent emotional memory and whether it is dependent on HPA axis deregulation. Exposure to high-fat diet (HFD) from weaning to adulthood, i.e. covering adolescence, exacerbates long-term emotional memories as assessed by odor-malaise or tone-shock association. Juvenile HFD also enhances malaise-induced neuronal activation of the basolateral complex of the amygdala (BLA), which correlates with protracted plasma corticosterone release. HFD exposure restricted to adulthood does not modify all these parameters indicating adolescence is a vulnerable period to the effects of HFD-induced obesity. Finally, exaggerated emotional memory and BLA synaptic plasticity after juvenile HFD are alleviated by a glucocorticoid receptor antagonist. Altogether our results demonstrate that juvenile HFD alters HPA axis function leading to an exacerbation of amygdala-dependent synaptic and memory processes. Adolescence represents a period of increased susceptibility to the effects of diet-induced obesity on amygdala that may promote maladaptive emotional processing later in life.

## EFFECTS OF DIFFERENT DOSES DICLOFENAC SODIUM ON DEVELOPMENTAL FEATURES OF HIPPOCAMPUS IN RATS A HISTOPATHOLOGICAL AND STEREOLOGICAL ANALYSIS

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In the present study, it was aimed to examine the toxic effects of exposed to different doses of diclofenac sodium on the rat hippocampus by stereological and histopathological methods. For this purpose, fifteen adult, female *Wistar albino* rats were randomly placed into five groups as follows. These are pure control, saline, low dose (3.9 mg/kg), medium dose (9 mg/kg) and high dose (18mg/kg) diclofenac sodium (DS) groups (n=3 pregnant female). After mating, saline and DS groups (low-medium-high dose groups) were exposed to saline or DS injection during the second trimester of the pregnancy. On the 7<sup>th</sup> day of *postnatal* life, all animals were euthanized with an overdose of anesthetic. Then, the brain tissue samples were analyzed by histological and stereological techniques. In the diclofenac sodium administered groups were observed a significant decrease in terms of the average neuron number in the CA1, CA2 and CA3 regions and whole hippocampus compared to pure control and saline groups. Light microscopic investigation of the drug administered groups indicated significant decrease in the number of healthy neurons. Additionally; in the high dose group, it was identified more necrotic neurons compared to the other groups. In this study it was shown by means of stereological techniques that using of diclofenac sodium caused to decrease in the number of the neurons occupying the CA1, CA2, and CA3 regions and whole hippocampus.

*Neurobiology of pain*

## ROLE OF THE TREK POTASSIUM CHANNELS IN COLD AND WARM THERMOSENSATION AND IN PAIN PERCEPTION

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The appropriate perception of noxious stimuli is essential for the response to noxious and potentially harmful situations. The detection of thermal, mechanical or chemical stimuli relies on the activity of dedicated nociceptive nerve fibers in the skin and body tissues. The nociceptive nervous system is characterized by a high degree of plasticity, which is exacerbated in pathophysiological conditions.

We have now studied the role of the K<sup>+</sup> channels of the TREK channels (TWIK-Related K<sup>+</sup> channels) family in temperature perception and pain. TREK1, TREK2 and TRAAK are hyperpolarizing inhibitory background K<sup>+</sup> channels with two-pore domains (K2p) that play a major role in membrane polarization and cell excitability. TREK channels are mechano-gated and thermosensitive channels with multiple regulations by polyunsaturated fatty acids, lysophospholipids, and G protein-coupled receptors.

We will present evidence that TREK channels controls the appropriate perception of both warm and cold temperatures. TREK2, TREK1 and TRAAK channels appear to have complementary roles in thermosensation. We observed that TREK channels are involved in hyperalgesia in inflammation. We also observed that down-regulation of TREK channels is involved in cold allodynia often associated with the chemo-induced peripheral neuropathy induced by the treatment with the anticancer drug oxaliplatin.

Our observations suggest that positive modulation of TREK channels expressed in sensory neurons may have beneficial analgesic effects in painful conditions and peripheral neuropathic conditions.

## SEZ6 IS A NEUROPATHIC PAIN MODULATOR AND NOVEL BINDING PARTNER OF A2A

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Pain of neuropathic origin afflicts ~8% of the population and affected individuals suffer from exaggerated pain responses including allodynia and hyperalgesia. The drugs gabapentin and pregabalin are used to treat neuropathic pain and they exert their effects via the  $\alpha 2\delta$  receptor, an accessory subunit of voltage-sensitive calcium channels. Emerging evidence implicates  $\alpha 2\delta$  in excitatory synaptogenesis and our recent data reveal that Seizure-related gene 6 (Sez6) protein binding to  $\alpha 2\delta$  can promote this effect. Thus, we hypothesized that Sez6 contributes to the synaptic gain-of-function in spinal cord dorsal horn neurons in neuropathic pain.

We tested Sez6<sup>-/-</sup> mice and wild-type (WT) controls for mechanical (von Frey), cold and heat-induced sensitivity before and after partial sciatic nerve chronic constriction (CCI) or sham surgery (nerve exposed but not ligated). Spinal cord and brain tissue was processed for neuron tracing after Golgi-Cox staining. Patch-clamp recordings were made from superficial dorsal horn neurons in acute slices from lumbar spinal cord.

While similar levels of mechanical and cold allodynia were observed in control and Sez6<sup>-/-</sup> mice from 10 days after surgery, Sez6<sup>-/-</sup> mice showed attenuated heat hyperalgesia and this resolved more rapidly over time compared to WT controls. In the medial pre-frontal cortex, pyramidal neurons showed a Sez6- and CCI-dependent increase in dendritic spine density and CCI-induced changes in spine density were seen on spinal cord L3/L4 putative wide dynamic range neurons. Preliminary data indicate that, in contrast to controls, TrpV1 agonist-induced spontaneous excitatory post-synaptic potential frequency is not significantly increased in Sez6<sup>-/-</sup> superficial dorsal horn neurons after CCI.

Sez6 is required for the development of heat hyperalgesia and implicated in central sensitization of this pathway after chronic constriction injury in a neuropathic model. Sez6-dependent effects are observed along the central “pain” neuraxis.

## UNDERSTANDING MICRORNAS IN PAIN MECHANISMS

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Chronic pain can arise from many origins: neuropathy, inflammation or cancer. It most often results from a combination of factors, which makes understanding of its mechanism complex and therefore its treatment difficult. Long-term pain sensitization involves gene-expression changes in the nociception pathways. Thus, significant efforts have been made to study the role of transcriptional regulation and post-translational in pain mechanisms. MicroRNAs (miRNAs) are a class of non-coding RNAs that have the ability to inhibit mRNA translation. A fundamental concept of miRNAs' mode of action is that they do not fully hybridize to their target mRNA to exercise their regulatory effect. Thus, a given miRNA will be able to inhibit the translation of several mRNAs simultaneously. This feature could be very relevant in the context of multi-factorial chronic pain. Our team focuses on the role of miRNAs in the mechanisms of neuropathic pain and cancer pain. We have demonstrated that in neuropathic pain, microRNA miR-103 is able to exert an integrative regulation of the three subunits composing the calcium channel Cav1.2. We previously showed that Cav1.2 in the dorsal horn of the spinal cord plays a critical role in the mechanism of chronic pain. In addition, we show that intrathecal injection of miR-103 in neuropathic animals restores a basal level of expression of Cav1.2 and thus normal pain perception. Recently, we investigated the involvement of miRNAs in cancer-associated pain. Our first results indicate that in bone-cancer pain, miR-124 could act as a macro-control by modulating the expression of several structural proteins of the synapse. As well, in chemotherapy-related pain, a specific miRNA may simultaneously regulate pathfinding proteins in the nociceptors of dorsal root ganglia. Thus, modulating pain perception by altering pain pathways functional morphology. Our objective is to analyze the relevance of these multi-targeting miRNAs and evaluate the associated therapeutic potential.

## **INTERPLAY BETWEEN PAIN AND INHIBITORY CONTROL: RELEVANCE FOR IMPULSIVE / COMPULSIVE NEUROPSYCHIATRIC DISORDERS**

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There is growing evidence that pain is a cognitive process stemming from the integration of nociceptive information leading to the subjective representation of a highly aversive state. But altered cognitive functions, such as in depression or impulsive / compulsive behaviors, can also lead to an aberrant representation of an aversive state, so called psychic pain. There is to date limited interest in the way pain and cognition interplay, at different levels of integration in the brain to promote either sensory or psychic chronic pain, nor is there much about the potential neurobiological overlap between these two categories of pain. These appealing questions have not been investigated perhaps because of the limitations of the current preclinical models that do not allow to measure sophisticated behaviors motivated by pain or pain relief.

This presentation will provide a state of the art of the literature and a psychobiological framework whereby new avenues for future research can be identified alongside the design of new animal models.

## OPIOID RECEPTORS ROLES IN PAIN: ADVANCES FROM GENETIC AND PHARMACOLOGY APPROACHES

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Opiates are powerful drugs to treat severe pain. The clinically used opiates activate mainly mu opioid receptors while both mu and delta receptors constitute active targets for the development of novel analgesics. Opioid receptors are expressed in neural circuits that underlie pain processing and mood. Targeted mutagenesis in mice has allowed demonstrating the distinct implication of each receptor in these behavioral responses. A major challenge today is the identification of key opioid receptor populations, which control physiological and pathological pain as well as associated emotional disorders. Recently the knowledge on opioid receptors roles in pain has improved with findings from conditional knockout of mu and delta receptors approaches. Particularly, we deleted peripheral receptors in Nav1.8-expressing neurons and highlighted their importance in both opioid endogenous tone-mediated and exogenous mu and delta opioid-induced analgesic responses. Also, we characterized novel delta opioid selective agonists and showed their potent analgesic effects in both inflammatory and neuropathic pain models. Recently, ligand-biased agonism at opioid receptors was discovered and the field is under development. The combined use of opioid ligands together with distinct opioid receptor mutant mice has also allowed demonstrating this functional selectivity *in vivo*. Genetic approaches on opioid receptors have been instrumental in understanding opioid receptor biology. They will contribute to open novel perspectives toward targeting therapeutic goals in pain and psychiatric diseases.

*Joint Symposium with COST Action FA1301–  
CephsInAction meets MNS2015*

## WHAT DOES THE FUTURE HOLD FOR INVERTEBRATE NEUROSCIENCE?

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The invertebrate organisms have provided neuroscience with hugely informative experimental platforms that have shaped our understanding of fundamental mechanisms governing the workings of neural systems. In this talk I will provide a perspective on the past contribution of invertebrate systems to neuroscience and consider what future directions might be in the face of a changing landscape of funding priorities.

## CONSERVATION AND CONVERGENCE IN THE EVOLUTION OF THE OCTOPUS NEURAL SYSTEM MEDIATING LEARNING AND MEMORY

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Octopuses accomplished advanced, vertebrate-like behavior with a relatively simple invertebrate brain. Therefore, studying the octopus neurophysiological basis of learning and memory (L&M), provide high potential for generating new ideas and better understanding of the evolution, development and functional organization of those mechanisms regarding complex learning behavior. Do the octopuses utilize conservative molluscan L&M mechanisms or have they evolved novel mechanisms, possibly through convergent evolution with vertebrates? Answering this question may reveal evolutionary and biological principles of L&M systems. We are focusing on the octopus vertical lobe (VL), a brain region highly involved in L&M which resemble *the mammalian hippocampus and the insect mushroom bodies*. The VL has the typical connectivity of feed-forward ‘fan-out fan-in’ association networks, with non-NMDA activity-dependent LTP at the fan-out synapses. We recently discovered that this LTP is most likely mediated by NO. We have found that 5-HT has conserved its facilitative effect on molluscan synaptic transmission in the VL but has ‘lost’ its long-term modulatory effects. However, it indirectly enhances the activity-dependent LTP induction. Octopamine (OA) has a similar short-term facilitatory effect but, unlike 5-HT, it attenuates LTP induction and depotentiates consolidated LTP. Thus, 5HT and OA may serve, respectively, as specific positive and negative reinforcement signals during learning, allowing the octopus to associate between a situation and its positive or negative consequences. In summary, the octopus VL shares global properties with complex L&M systems of vertebrates and insects, yet its synaptic plasticity is based on extensive adaptations of conserved molluscan mechanisms. This suggests that convergence to complex L&M systems can be achieved in multiple independent ways.

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**IDENTIFYING MOLECULAR AND CONNECTIVITY ARCHITECTURE  
SHARED IN MAMMALIAN AND OCTOPUS BRAINS**

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Classic neuropsychological studies of the cephalopod brains have identified specific centers that are involved in tactile and visual cognitive tasks. The systems circuitry of how sensory inputs are relayed to these centers has, however, remained almost unexplored with modern methodologies. We chose to ask a simple question, to what extent are sensory maps preserved in octopus brain organization and what developmental genes are related to shape the cognitive centers. Our findings demonstrate that in octopus brain, sensory maps and molecular expression code are a characteristic feature of CNS organization compared to those of vertebrate brains.

**OCTOPAMINE IN OCTOPUS BRAIN:  
A LONG HISTORY FOR MAPPING A ‘NEGLECTED’ NEUROMODULATOR**

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Octopamine has been originally identified in the 1948 in the salivary glands of the cephalopod mollusk *Octopus vulgaris*. It is a monoamine found in plants, invertebrates and vertebrates. While it only occurs as a trace amine in vertebrates, octopamine (OA) is one of the most abundant biogenic amines occurring in the nervous system of invertebrates. It is considered the invertebrate counterpart of noradrenaline.

OA and/or its receptor have been identified in a large number of invertebrate species, and play a role in several physiological and behavioral processes acting as neuromodulator, neurotransmitter, or neurohormone by prompting the whole organism to a “dynamic action”.

Interestingly, while scientists largely explored OA presence and functioning in many taxa, the study of its role in octopus, where it has been originally discovered, and in other cephalopods has been neglected, and a detailed account of its distribution has been missing.

After more than 60 years, we explored the octopus brain to understand the significance of the exceptionally elevated concentrations of octopamine found in this cephalopod. We characterized, for the first time, octopaminergic neuronal populations in the brain lobes of *O. vulgaris* by immunofluorescence labeling. A possible role of OA for the visual and chemo-tactile sensory-motor processing is also provided by functional comparison with other invertebrates .

## WHY MOTOR CONTROL IN THE OCTOPUS IS ‘FULL OF SURPRISES’

Benny Hochner

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Previous and recent findings on the unique motor control system of the octopus led us to conceptualized the idea that the octopus, because of its soft body with eight long and flexible arms and unusual morphology, is an exceptionally good instructive system to show that ‘embodied-organization’ is not only an important concept for designing autonomous robots<sup>1</sup>, but it is likely also an important constraint in the evolution of highly adaptive and efficient motor control mechanisms. This is especially apparent in animals like the octopus that have evolved from a soft bodied, shell protected, hardly moving mollusks into highly active maneuverable predators. Indeed, we find that the research of octopus motor behavior is a vivid biological demonstration of concepts like: embodied organization; embodied intelligence; emergence; self-organization; morphological computation; and reconfiguration.

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***5-HT receptors and neuropsychiatric disorders:  
new pharmacological targets for old diseases***

## NEUROCHEMICAL AND BEHAVIORAL IMPACT OF A SEROTONERGIC LESION IN THE NON-HUMAN PRIMATE

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It is noteworthy that serotonergic neurons degenerate in Parkinson's disease. To determine the role of this 5-HT injury besides the dopaminergic one in the L-DOPA-induced dyskinesia and neuropsychiatric-like behaviors, we developed a new monkey model exhibiting a double DA/5-HT lesion by sequentially using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 3,4-methylenedioxy-N-methamphetamine (MDMA, better known as ecstasy). By longitudinal positron emission tomography imaging and post-mortem immunohistochemistry, we demonstrated that MDMA injured serotonergic nerve terminals in the brain of MPTP monkeys. By post-mortem autoradiography, we further characterized the impact of both MPTP and MDMA lesions, as well as of L-DOPA treatment, on the expression of 5-HT<sub>1A</sub> and 5-HT<sub>6</sub> receptors. We next investigated the behavioral impact of the MDMA-driven serotonergic lesion. Remarkably, it abolished the L-DOPA-induced dyskinesia and neuropsychiatric-like behaviors, without altering the anti-parkinsonian response. These data demonstrate that the serotonergic presynaptic fibers play a critical role in the expression of both motor and non-motor symptoms in PD, and highlight that an alteration of the 5-HT and DA systems is involved in specific basal ganglia territories for different symptoms.

**5-HT<sub>1A</sub> RECEPTOR AGONISTS FOR THE TREATMENT OF L-DOPA-INDUCED  
DYSKINESIA IN PARKINSON'S DISEASE: BIASED AND MIXED AGONIST ACTIONS,  
A TRANSLATIONAL PERSPECTIVE**

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Parkinson's disease is characterised by the emergence of motor and non-motor symptoms. The primary nigrostriatal dopaminergic deficit can be managed by treatment with dopamine receptor agonists in the initial disease phases. However, the need for intervention with the gold-standard therapy and dopamine precursor levodopa (L-dopa) is inevitable as the disease course progresses. However, L-dopa treatment is often associated with the emergence of debilitating dyskinesia caused by aberrant uncontrollable "false neurotransmitter" release of dopamine following conversion L-dopa within serotonergic neuronal terminals. Reducing the serotonergic tone by the application of (5-hydroxytryptamine; 5-HT)<sub>1A</sub>, 5-HT<sub>1B</sub> receptor agonists or other serotonergic strategies may help in the alleviation of these effects. Particularly, 5-HT<sub>1A</sub> receptor agonists have been shown to reduce the expression of established dyskinesia in the non-clinical and clinical setting, but can often be associated with a reduction in the antiparkinsonian efficacy of L-dopa. Here we review the role of 5-HT<sub>1A</sub> receptor agonists in reducing dyskinesia. Firstly, we explore non-clinical and, where available, clinical outcomes with compounds showing either a lack of selectivity or potency at the 5-HT<sub>1A</sub> receptor, such as sarizotan, eltoprazine and pardoprunox (SLV308). Finally, we address exciting new developments with compounds possessing much greater potency and selectivity at 5-HT<sub>1A</sub> receptors, for example Befiradol (NLX-112).

## SEROTONIN<sub>2B</sub> RECEPTOR-DOPAMINE INTERACTION: NEW OPPORTUNITIES FOR IMPROVED TREATMENTS OF SCHIZOPHRENIA

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Recent studies suggested that the central serotonin<sub>2B</sub> receptor (5-HT<sub>2B</sub>R) might represent an interesting pharmacological target for pathological conditions depending upon mesolimbic dopamine (DA) dysfunction, such as schizophrenia, Parkinson's disease and drug addiction. However, the role of 5-HT<sub>2B</sub>Rs in the control of DA ascending pathways remains weakly investigated to date, especially as regard the mesocortical DA pathway. This study was therefore aimed at evaluating the influence of selective 5-HT<sub>2B</sub>R antagonists (LY 266097, RS 127445) on DA outflow, measured, using *in vivo* microdialysis, in the rat striatum, nucleus accumbens (NAc) shell and core subregions, and medial prefrontal cortex (mPFC).

LY 266097 (0.63 mg/kg, i.p.) and RS 127445 (0.16 mg/kg, i.p.) had no effect on striatal and NAc core DA outflow, but significantly reduced and increased basal DA outflow in the NAc shell and the mPFC, respectively. LY 266097 reduced significantly the increase in NAc shell DA outflow induced by haloperidol (0.01mg/kg, s.c.) administration, but failed to alter haloperidol-induced DA outflow in the striatum. Conversely, the effect of haloperidol on mPFC DA release was significantly potentiated by RS 127445 administration.

Altogether, these findings demonstrate that 5-HT<sub>2B</sub>Rs exert a region-dependent modulation of DA ascending pathways, by providing, specifically, no effect in the striatum, and opposite facilitatory and inhibitory controls on NAc and mPFC DA outflow. Also, they highlight the therapeutic potential of 5-HT<sub>2B</sub>Rs for pathological conditions requiring an independent modulation of DA pathways, such as schizophrenia or Parkinson's disease.

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## 5-HT<sub>2C</sub> RECEPTOR CONTROL OVER SUBSTANCE USE DISORDERS

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In the search for attractive pharmacotherapeutic approaches for the treatment of substance use disorder, central 5-HT<sub>2C</sub> receptors have been attributed a particular role. In fact, they exert tonic and phasic inhibitory controls over the dopaminergic pathways and modulate the activity of GABAergic and glutamatergic system pathways<sup>1</sup>. Preclinical data consistently show that pharmacological activation of 5-HT<sub>2C</sub> receptors can efficiently alter drug-taking and drug-seeking behavior. As 5-HT<sub>2C</sub> receptor agonists reduce the reinstatement mechanisms, this observation suggests that these drugs may have therapeutic potential for preventing cue-controlled craving and relapse in human addicts<sup>2</sup>. Moreover, 5-HT<sub>2C</sub> receptors are engaged in feeding behavior and their agonists have also prominent effects on consummatory behaviors in general. Recent studies indicate also significance of 5-HT<sub>2C</sub> receptors to mood disorders, including depression. As depression and substance use (e.g., cocaine use) disorders are common concurrent diagnoses, by using preclinical models (bilateral olfactory bulbectomy with a variety of self-administration and extinction/reinstatement procedures) we studied the effects of 5-HT<sub>2C</sub> receptor agonists in cocaine-treated rats. Our data show the efficacy of 5-HT<sub>2C</sub> receptor agonists to reduce the co-occurrence of cocaine reward and depression as well as the cocaine-seeking behavior observed in both phenotypes. The last finding demonstrates the potential clinical utility of using 5-HT<sub>2C</sub> receptor agonists in the pharmacotherapy of cocaine seeking enhanced by co-existing depression.

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## SEROTONIN<sub>7</sub> RECEPTOR ANTAGONISM AS A FAST ACTING ANTIDEPRESSANT STRATEGY

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In term of efficacy and onset of action, current antidepressant drugs still suffer from important drawbacks. Hence, we recently found that the pharmacological blockade of serotonin 7 receptors [5-hydroxytryptamine (5-HT<sub>7</sub>) receptors] produces a faster and safer antidepressant response than fluoxetine (Prozac®), the most prescribed antidepressant.

In rats, we showed that SB-269970, a selective 5-HT<sub>7</sub> receptor antagonist, exerts an effective antidepressant-like effect as assessed in the forced swim test. Moreover, we revealed *in vivo* that 5-HT<sub>7</sub> receptors negatively regulate the firing activity of dorsal raphe 5-HT neurons and become desensitized after a chronic treatment with fluoxetine. In contrast to fluoxetine, a one-week treatment with SB-269970 did not decrease 5-HT neuronal firing activity but desensitized 5-HT autoreceptors, enhanced the hippocampal cellular proliferation rate (an effect prevented by a 5-HT depletion) and counteracted the anxious/depressive-like behaviour that present the olfactory bulbectomized rat model. Finally, early life treatment with fluoxetine, but not with SB-269970, induced anxious/depressive-like behaviours in adulthood.

These results indicated that the 5-HT<sub>7</sub> receptor participates in both mood regulation and the antidepressant effect of SSRIs, and that 5-HT<sub>7</sub> receptor antagonists may represent a new class of antidepressant drugs with safer and faster therapeutic action.

***Targeting phosphodiesterases  
for the treatment of brain disorders***

## **IMPACT OF PHOSPHODIESTERASE 9A INHIBITION ON ABERRANT CORTICALLY-EVOKED SPIKE ACTIVITY IN THE STRIATUM OF RODENT MODELS OF HUNTINGTON'S DISEASE**

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Huntington's disease (HD) is a progressive neurodegenerative disorder associated with abnormal expansion in CAG trinucleotide repeats within the HD gene and degeneration of striatal medium-sized spiny projection neurons (MSNs). Recent studies indicate that the metabolism of cyclic nucleotides by phosphodiesterases (PDEs) is likely to be dysfunctional in MSNs in the HD striatum. Studies providing evidence for both decreased cAMP/cGMP synthesis and deficits in corticostriatal transmission in advanced HD suggest a link between cyclic nucleotide signaling and disrupted MSN network activity. The present study assessed spontaneous and cortically-evoked firing in wild-type (WT) animals, full-length BAC transgenic line 5 HD rats (TG5), and Q175 heterozygous knock-in mice (5-7 months old) treated with vehicle or the PDE9A inhibitor PF-4447943 (3.2 mg/kg, s.c.). All animals were anesthetized with urethane and single-unit spike activity was isolated during low frequency electrical stimulation (0.5 Hz, 0.5 msec, 0.4-1.0 mA) of the ipsilateral motor cortex. Compared to WT controls, both TG5 rats and Q175 mice exhibited decreased spike probability during cortical stimulation. Systemic administration of PF-4447943 significantly decreased the onset latency of cortically-evoked spikes at all stimulation intensities in WT rats as compared to vehicle-treated controls. Systemic administration of PF-4447943 also decreased the onset latency of cortically-evoked spikes in TG5 rats and reversed deficits in spike probability observed in these animals. Parallel studies are ongoing in the Q175 mice. The effectiveness of PF-4447943 for increasing cortically-driven activity in the tgHD rat striatum supports the targeting of PDE9A for therapeutic intervention in HD. Indeed, drugs which act to facilitate cGMP signaling could be useful therapeutic agents for restoring corticostriatal transmission in HD, and potentially, alleviating motor and cognitive symptoms of this disease.

## CHARACTERIZATION OF MUTATIONS IN PDE10A WHICH LEAD TO A HYPERKINETIC MOVEMENT DISORDER IN HUMANS

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Inhibition of PDE10A has been shown to be of benefit in preclinical models of Schizophrenia and Huntingtons disease (HD). Unfortunately the clinical data so far in schizophrenia has not been positive. At the level of human genetics PDE10A has not been linked to either of these diseases/disorders. Intriguingly, in collaboration we have now identified 2 unrelated families with mutations in the PDE10A gene, who present with a hyperkinetic movement disorder and abnormal skin pigmentation. This presentation will focus on a family with a mutation Y107C. *In vitro* experiments show that this PDE10A mutation leads to instability in both PDE10A mRNA and protein, and that the residual protein is not targeted properly to the cell membrane. I will also describe the characterization of a Pde10a Y107A knock-in mouse model. At the molecular level it recapitulates the decreases in protein and mRNA seen *in vitro* while at the behavioral level it has a range of motor phenotypes, which we can relate to the human carriers of the mutation. Overall the talk will highlight a clear role for PDE10A in regulating movement and a previously unknown role in the control of skin pigmentation.

## PHOSPHODIESTERASE INHIBITORS AS A THERAPEUTICAL APPROACH TO HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an autosomal-dominant inherited neurodegenerative disorder characterized by motor dysfunction, cognitive decline and emotional and psychiatric disturbance. The genetic mutation is characterized by a CAG expansion, resulting in formation of a mutant huntingtin protein with an expanded polyglutamine repeat region. Mutant huntingtin interacts with and impairs the function of a number of factors, in particular the cAMP response element-binding protein (CREB) and the Brain-derived neurotrophic factor (BDNF). In this view, drugs targeted at counteracting CREB loss of function and BDNF decrease have been considered as powerful tools to treat HD. Recently, cyclic nucleotide phosphodiesterase (PDE) inhibitors have been used successfully to increase levels of CREB and BDNF in HD models. Indeed, PDE IV, V and X inhibitors have been shown to afford neuroprotection through the modulation of CREB and BDNF. In this review, we will summarize the data supporting the use of PDE inhibitors as therapeutical approach to fight HD and we will discuss the possible mechanisms of action underlying these effects.

## PDE4 AS A TARGET FOR COGNITION ENHANCEMENT

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Phosphodiesterases (PDEs) are enzymes that differ in their substrate, i.e. cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP), being hydrolyzed. cAMP-specific PDE4 and cGMP-specific PDE5 are both involved in short-term as well as long-term memory processes. However, the exact timing of elevation of central cGMP and/or cAMP levels is critical to influence a particular memory process. For instance, cGMP influences only early consolidation of information into long-term memory, while cAMP affects late consolidation processes only. On the other hand, elevations of central cGMP levels or cAMP levels after treatment with a specific PDE5 or PDE4 inhibitor, respectively, both improve short-term memory. However, in contrast to studies including rodents and monkeys, it was found that PDE5 inhibition had no effect at all on cognition including memory processes in healthy humans. It is clear that the transition of a drug from preclinical to clinical creates translational hurdles. Nevertheless, based on the expression patterns of its isoforms in the brain, PDE4 appears more interesting for CNS targeting than PDE5. Pharmacological therapies aimed at reducing PDE4 and in particular the isoform PDE4D are known to produce severe emetic side effects. Yet, rodent data indicated that particularly this isoform PDE4D may be specific for cognition. Thus the future for disease-specific PDE4 enzyme inhibition lies in the development of PDE4 isoform-specific inhibitors without emetic effects. Within this context, data will be shown and discussed whether PDE4 can be considered as a candidate to improve human memory.

## PRECLINICAL PROFILE OF PDE1 INHIBITORS FOR COGNITION ENHANCEMENT

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Cyclic nucleotide (both cAMP- and cGMP-dependent) signaling pathways are implicated in the modulation of memory processes. Eleven phosphodiesterase (PDE) enzyme families, encoded by at least 21 distinct genes, have been identified. The PDE1 enzymes, dual-substrate enzymes that hydrolyze and inactivate both cGMP and cAMP, are uniquely regulated by through calcium/calmodulin-dependent pathways. The novel object recognition (NOR) task is a useful paradigm for studying memory performance in rats under naturalistic conditions, measuring the innate behavioral tendency of rodents to explore novel objects in their environment. Performance in NOR responds to cAMP and cGMP availability, under control of several PDE families. Here, we characterize the preclinical profile of ITI-214, a potent inhibitor of phosphodiesterase 1 (PDE1), as an agent to improve memory performance.

ITI-214 was assayed in *in vitro* for potency and selectivity as a PDE1 inhibitor using recombinant human enzymes and for off-target binding (NovaScreen SEP). Effects of ITI-214 (0.1 or 10 mg/kg) or vehicle on NOR were assayed in rats. ITI-214 was assayed (1mg/kg) in the rat conditioned avoidance response (CAR) paradigm in combination with risperidone (0.8mg/kg).

ITI-214 inhibited PDE1A ( $IC_{50}=33\text{pmol}$ ) with >1000-fold selectivity over other PDE families (PDE4D) and minimal off-target binding interactions. Oral ITI-214 (2h prior to T1) significantly increased discrimination index (d2) 24h later, indicating enhanced memory acquisition in the absence of effects on exploratory behavior, and improved early and late memory consolidation and retrieval, when dosed before or after T2. ITI-214 did not disrupt risperidone effects in CAR. The robust memory-enhancing effects of ITI-214 were shared by other potent PDE1 inhibitors representing distinct chemical scaffolds.

In conclusion, ITI-214 improved multiple memory domains across a broad dose range (0.1-10mg/kg) without disrupting activity of a clinical antipsychotic medication. The data support ITI-214 as an enhancer of memory performance suitable for use as an adjunct with antipsychotic medications.

*Multi-target compounds  
in neurodegenerative diseases*

**DESIGNED MULTI TARGET DRUGS TARGETING NEUROPROTECTION,  
NEURORESTORATION AND MITOCHONDRIAL BIOGENESIS VIA ACTIVATION OF  
HIF1 $\alpha$ , SIRT1,PGC-1 $\alpha$  AND TFAM**

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Novel therapeutic approaches for the treatment of Alzheimer's disease (AD) comprise drug candidates designed specifically to act on multiple CNS targets, rather than a single "receptor" as has been done with cholinesterase inhibitors. Major pathology of AD is the accumulation of iron in nucleus basalis, dentate gyrus, amyloid plaques, and tangles and increase in monoamine oxidase (MAO). The iron contributes to the onset of oxidative stress and glutaminergic excitotoxicity via interaction with hydrogen peroxide generated by the reaction of MAO. We have synthesized several multi target non-toxic, brain permeable iron chelator drugs, M-30 and HLA-20, possessing propargyl MAO inhibitory moiety, with neuroprotective and neurorestorative activities. These drugs possess antiapoptotic, pro-survival neurorescue effects, induction of neuronal differentiation and regulation of amyloid precursor protein (APP) and  $\beta$ -amyloid ( $A\beta$ ) levels. They induce the outgrowth of neurites in neuronal cell cultures, trigger cell cycle arrest in G0/G1 phase and enhance the expression of growth associated protein-43, HIF (Hypoxia Inducing Factor), and increase brain levels BDNF, GDNF, VEGF and erythropoietin. This has been shown to be associated with the inhibition of iron dependent prolyl-4-hydroxylase that regulates HIF. Both M30 and HLA-20 process APP via activation of alpha secretase. They possess neurorestorative activity in in vivo models of Parkinson's disease and restore the cognitive deficit in APP/PSI double transgenic mice and the streptozotocin (STZ) models of AD. The dual control of mitochondrial biogenesis and energy metabolism is regulated by silent information regulator-1 and -3 (SIRT1 and SIRT3). The peroxisome proliferator activated receptor  $\gamma$  co-activator 1 $\alpha$  (PGC-1 $\alpha$ ) is a transcriptional co-activator that is a central inducer of mitochondrial biogenesis in cells. SIRT1 is necessary for HIF-1 $\alpha$  protein accumulation and activation of HIF-1 target genes and activates PGC-1 $\alpha$ -mediated transcription of nuclear factor (Tfam) and mitochondrial genes encoding for proteins promoting mitochondria proliferation, We have shown that M30 and HLA-20 activate SIRT1, PGC-1 $\alpha$ , and Tfam in cell cultures and consider them as a novel therapeutic approach for neurodegenerative disorders.

## MULTIPOTENT DRUGS BASED ON DONEPEZIL FOR THE POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE

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In this communication we will update our recent results on the synthesis and biological evaluation of new multipotent molecules derived from *N*-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1*H*-indol-2-yl)methyl)-*N*-methylprop-2-yn-1-amine (**ASS234**),<sup>1-5</sup> able to bind ChE and MAO enzymes, and based on donepezil as reference molecule, for the potential treatment of Alzheimer's disease.

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## NEW MULTIFUNCTIONAL METAL-CHELATORS MOLECULES BASED ON DONEPZILO FOR ITS POTENTIAL USE IN ALZHEIMER'S DISEASE THERAPY

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Due to the multifactorial nature of AD, it has prompted the search of new Multi-Target-Directed Ligands (MTDL), based on the “one molecule, multiple target” paradigm. Thus, in this context, multifunctional molecules able to simultaneously bind both cholinesterases and monoamine oxidases have been investigated<sup>1,2</sup>. Besides this, new multifunctional molecules with metal chelating properties have been studied<sup>3,4</sup>. Herein we report the biological evaluation of a new series of Donepezil+Propargylamine+8-Hydroxyquinoline (DPH 1-7) hybrids. All them showed to be potent and selective inhibitors of both MAO isoforms and AChE / BuChE with metal chelating properties. The most promising compound DPH6 was tested on mice with experimentally induced amnesia. DPH6 was capable to significantly decrease scopolamine-induced learning deficits in healthy adult mice. To sum up, the well-balanced anti-cholinesterase and MAO inhibitory profile, in addition to the other attractive pharmacological properties described here, **DPH6** is a new lead compound of a new **WMY** series of analogues that deserves further investigation for the potential therapeutic treatment of Alzheimer's disease.

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## MULTIPLE TARGETING WITH HISTAMINE H<sub>3</sub> RECEPTOR ANTAGONISTS

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The neuronal human histamine H<sub>3</sub> receptor (H<sub>3</sub>R) is a highly topical target for numerous neurological diseases<sup>1</sup>. Pitolisant is a H<sub>3</sub>R antagonist in late stage of clinical development for the treatment of excessive daytime sleepiness with Parkinson patients. Since we and others have developed a non-imidazole H<sub>3</sub>R pharmacophore, it is possible to combine high affinity H<sub>3</sub>R properties to other pharmacophore elements or to functional moieties with designed physicochemical properties.

The H<sub>3</sub>R pharmacophore has successfully been combined with different pharmacophore elements like histamine H<sub>1</sub> receptor, histamine H<sub>4</sub> receptor, muscarinic M<sub>2</sub> receptors, dopamine receptor subtypes and also lipid signaling. NO-releasing elements, neurotransmitter transporters as well as different enzymes have been added as target on H<sub>3</sub>R ligands. For example, we have combined properties for inhibition of histamine *N*-methyltransferase, the main central histamine metabolizing enzyme<sup>2</sup>. The inhibition of the autoreceptor stimulates the histamine secretion and the inhibition of the catabolic enzyme prolong the histaminergic effects. This should stimulate the acetylcholine (ACh) release and improve cognitive impairments. On the way to boost these cognition enhancing effects we have additionally added properties for inhibition of ACh esterase and butyrylcholine esterase to these molecules combining the designed effects on four targets simultaneously<sup>3,4,5</sup>.

These combination principles on functional properties of H<sub>3</sub>R antagonists can be applied to different physicochemical properties<sup>6</sup> or designed pharmacological properties of novel potential therapeutics.

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## **Oral Free Communications**

*Regulation of reward, motivation,  
feeding and sexual behavior*

## **CB1 AND MU OPIOID RECEPTORS INTERPLAY IN THE NUCLEUS ACCUMBENS CORE UNDERLIES SOCIAL REWARD IN ADOLESCENT RATS**

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Social play is a highly rewarding activity and it is modulated by interactions between the endocannabinoid anandamide and endogenous opioids. The principal endocannabinoid 2-arachidonoylglycerol (2-AG) regulates emotional behaviors in rodents. However, its contribution in social play and the neural substrates underlying opioid-cannabinoid interactions in the modulation of this behavior remain unknown. Our previous studies demonstrated that anandamide and endogenous opioids act within the nucleus accumbens core (NAcC) to modulate social play behavior and that 2-AG mediates long-term depression in the NAcC. Thus, we hypothesized that this brain region also underlies the 2-AG modulation of social play behavior in adolescent rats and the interplay between cannabinoid and opioid receptors in social reward.

Systemic administration of JZL184, a compound which prolongs the effects of locally released 2-AG, increased social play behavior in adolescent rats. These effects were blocked by intra-NAcC infusion with either the CB1 antagonist SR141617A or the  $\mu$ -opioid antagonist CTAP. Thus, stimulation of CB1 and  $\mu$ -opioid receptors within the NAcC is necessary for 2-AG to modulate social play. Likewise, social play enhancement by systemic treatment with the opioid agonist morphine was prevented by intra-NAcC infusion of SR141716A. These behavioral findings were supported by our electrophysiological results showing that CB1 antagonism blocked the inhibitory effect of the  $\mu$ -opioid agonist DAMGO on excitatory field synaptic responses (fEPSP) in rat adolescent NAcC slices; likewise, the inhibitory effect of the CB1 agonist CP55940 on fEPSP was significantly reduced by  $\mu$ -opioid antagonist naloxone. Interestingly, these effects were completely abolished in CB1 and  $\mu$ -opioid receptors knockout mice suggesting a functional interaction between CB1 and  $\mu$ -opioid receptors in the NAcC.

Altogether, these data show that CB1 and  $\mu$ -opioid receptor crosstalk in the NAcC underlies cannabinoid and opioid stimulation of social play behavior in adolescent rats, extending previous findings indicating complex functional interactions between these two receptors.

## INFLAMMATORY PAIN DESENSITIZES MU OPIOID RECEPTOR IN VTA: IMPACT ON OPIOID SELF-ADMINISTRATION IN RATS

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The use of opioid therapies in patients with chronic pain and previous history of opioid abuse is a challenge in the medical practice because of drug abuse liability. Surprisingly, few studies have investigated how inflammatory pain could alter opioid intake patterns and none of them have focused in an opioid dependent population. In the present study, we investigated the effect of inflammatory pain on mu opioid receptor (MOR) response to either DAMGO or heroin by conducting microdialysis studies in NAc and recordings of miniature IPSCs in VTA slices. Moreover, we studied the changes induced by inflammatory pain on heroin (50, 100 and 200 µg/kg/infusion) self-administration under fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement. Our results indicate that inflammatory pain partially desensitizes MORs in the VTA. Animals injected with CFA showed a significant reduction in the DA release in NAc induced by either heroin iv administration or DAMGO intra-VTA microinjection vs control animals. In fact, inflammation showed to alter DAMGO inhibition of GABA release in VTA slices. Accordingly, animals injected with CFA showed a clear deviation from the expected linear response in the dose response curve for heroin under FR2, increasing their intake when tested with high doses (100 and 200 µg/kg/infusion). Finally, very high doses of heroin (200 µg/kg/infusion) were needed to maintain the breakpoint under PR schedule of reinforcement. In conclusion, these results suggest that inflammatory pain can alter the motivational properties of heroin by affecting MORs sensitivity. Therefore, these data suggest that inflammatory pain induces changes in the VTA-NAc pathway which in turn may facilitate opioid dose escalation in order to maintain the rewarding properties of the drug.

## FEMALE SEX INCREASES REINSTATEMENT OF METHAMPHETAMINE SEEKING BEHAVIOUR AFTER A FORCED ABSTINENCE IN RATS

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Clinical evidence suggests differential characteristics of drug addiction in men and women which is in line with preclinical findings. However, the relapse is not readily assessed in animal models to provide a tool for innovative drug development. Therefore, the aim of this study was to assess differences in relapse-like behaviour after a period of forced abstinence. This paradigm simulates of the real clinical situation and can be used to evaluate potential drugs that are able to suppress the drug-seeking behaviour.

Adult male and female Sprague-Dawley rats were deeply anesthetized and a permanent intracardiac catheter was implanted to the right atrium. IV self-administration procedure was conducted in operant boxes using nosepoke operanda (Coulbourn Instruments, USA). Active nosepoke activated the infusion pump delivering one infusion of methamphetamine (0.08 mg/kg). When the drug intake was stabilized (maintenance phase) a period of forced abstinence was initiated and rats were kept in their home-cages for 14 days. Finally, one reinstatement session in operant boxes was conducted.

Methamphetamine seeking behaviour was assessed during the maintenance as number of infusions (n.s.) and methamphetamine dose in mg/kg where females self-administered significantly lower dose of drug ( $p=0.038$ ). After 14 days of forced abstinence the relapse rate was assessed as a number of active nosepokes during the reinstatement session expressed as a percentage of active nosepoking during the maintenance. Females displayed approximately 300 % active nosepokes compared to 48 % in males ( $p\leq 0.001$ ).

This indicates higher vulnerability to relapse of methamphetamine addiction in female rats. Therefore, this paradigm using operant drug self-administration and reinstatement of drug-seeking after forced abstinence model can be used for preclinical screening for potential new medications specific for women.

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## CHARACTERIZATION OF THE NEUROPSYCHOPHARMACOLOGICAL PROFILE OF THE NEW PSYCHOACTIVE SUBSTANCE METOXETAMINE IN RATS

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Methoxetamine (MXE) is a novel psychoactive drug structurally with ketamine (KET)-like properties that users perceived as safe despite its severe adverse consequences. Here we provide the first evaluation of its effects on reward, mood and behavior. We initially tested the effect of an acute intraperitoneal (i.p.) administration of MXE (1-5 mg/kg) on motor activity and emotional states in male rats. MXE significantly affected motor activity by inducing hypomotility during the first 20-min but hypermotility from 40 up to 80-min after administration. The elevated plus maze (EPM) test did not reveal statistically significant differences between MXE- (1 and 0.5 mg/kg) and VEH-treated groups. Conversely, when evaluated in the marble burying test (MBT) MXE (1 mg/kg)-treated rats buried a significantly higher number of marbles than controls. In the forced swim test (FST), MXE (0.5 and 1 mg/kg) did not affect immobility, swimming or climbing. Moreover, when tested in a self-administration substitution protocol, the intravenous (i.v.) dose of MXE 0.25 mg/kg fully substituted for KET 0.5 mg/kg (i.v.), with significant differences from saline but not from KET. Conversely, MXE 0.125 mg/kg (i.v.) showed a partial substitution only, while MXE 0.5 mg/kg (i.v.) did not substitute for KET. In addition, MXE (0.5 and 0.25 mg/kg, i.v.) induced a significant and time-dependent enhancement of dopamine extracellular levels in the nucleus accumbens shell. Consistently, MXE (0.031-0.5 mg/kg, i.v. cumulative doses) stimulated dopamine neurons activity in the ventral tegmental area. Altogether, data indicate that MXE did not affect coping behavior (FST), but at high doses it alters spontaneous motor activity while at low doses may induce anxiogenic effects, such as neophobia (MBT) but not spatial anxiety (EPM). Finding that at appropriate doses MXE is able to substitute for KET in the SA paradigm suggests that it possesses rewarding effects, a notion supported by both neurochemical and electrophysiological data.

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## **A TWO-NEURON SYSTEM FOR GOAL-DIRECTED DECISION-MAKING IN THE DEFINED FEEDING NETWORK OF *Lymnaea stagnalis***

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Food searching is an example of a goal-directed behaviour that is essential for survival. During this behaviour an animal must be able to accurately judge the presence or absence of potential food and make adaptive decisions to maximise food intake with minimal energetic cost. In order to locate a potential food source the pond snail *Lymnaea* performs active sampling of its environment. The expression of this goal-directed behaviour is dependent on the animal's level of satiety making *Lymnaea* an ideal system to study the interaction between sensory inputs, internal states (hunger and satiety) and decision making at single cell resolution in a well-defined circuit. We used both whole animal behaviour and circuit analysis in reduced preparations from *Lymnaea* to elucidate the neural mechanisms of the motivational state dependent decision-making process operating during food-searching behaviour. We found that the core circuit is comprised of just two neurons, one encoding the presence or absence of food-related sensory input and the second acting as a gain controller for the animal's motivational state and we characterize these two pathways and their interactions at the point that a decision is made. Our results provide a detailed cellular understanding of the mechanisms involved in the two stages of the feeding-related decision-making process and a new theoretical framework for the identification of the core neuronal mechanisms underlying similar decision making processes in other model systems.

## DIFFERENTIAL ENGAGEMENT OF A PHARYNGEAL CIRCUIT TO SUPPRESS FEEDING DURING FOOD DEPRIVATION

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Animals must regulate their feeding rate as food availability changes. In the nematode *Caenorhabditis elegans*, which feeds on bacteria, feeding rate can be observed in the intact animal by visually scoring the movement of the pharyngeal grinder. We have used this paradigm to investigate the molecular and cellular basis of behavioural plasticity in the pharyngeal system in situations in which food is abundant or in which it has been absent for short to prolonged periods of time. This shows that the suppression of pharyngeal behaviour during food deprivation is not simply explained by the lack of the excitatory signals that drive feeding in the presence of food. Rather, pharyngeal activity is titrated to a lower level by a fine balance between an excitatory, largely peptidergic, and inhibitory, largely glutamatergic, pathway. Using laser ablation and optogenetics we have assigned a role to a specific pharyngeal neuron which signals in a circuit that is activated by food removal to suppress feeding. This basic framework in which feeding is controlled through the net effect of finely tuned excitatory and inhibitory drives resonates with mammalian hypothalamic control of feeding and suggests that fundamental organisation of this basic animal drive may be conserved through evolution from nematode to human.

**INVOLVEMENT OF DOPAMINE IN THE DIFFERENCES IN SEXUAL BEHAVIOUR  
BETWEEN ROMAN HIGH AND LOW AVOIDANCE RATS: BEHAVIORAL,  
PHARMACOLOGICAL AND NEUROCHEMICAL FINDINGS**

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Roman High (RHA) and Low Avoidance (RLA) rats display opposite behavioral traits: RHA rats are active copers, impulsive and prone to abuse drugs while RLA rats are reactive copers, hyperemotional and prone to develop depressive-like symptoms. These differences are linked to differences in brain monoamine (mainly dopamine) function and neuroendocrine responses to stress. RHA and RLA rats differ also in sexual behavior, with RHA rats displaying higher motivation and better copulatory performance than RLA rats, and their sexual behavior differentially influenced by dopamine agonists and antagonists [1,2]. Together with the well-known role of dopamine in sexual behavior, findings suggest that the sexual differences between RHA and RLA rats may be due to differences in dopamine neurotransmission.

In order to test this hypothesis, naive (never exposed to a receptive female) and sexually experienced (which underwent five copulation tests) RHA and RLA rats implanted with a microdialysis probe aimed at the shell of the nucleus accumbens (NAs), were used in a classical appetitive/consummatory test of sexual behavior, during which copulatory parameters were recorded and dialysate aliquots collected from the NAs for dopamine determination by HPLC-ECD. The results show that the higher sexual motivation and better performance of RHA vs. RLA rats occurred concomitantly with a higher dopamine release, as shown by the higher dopamine concentration found in the NAs dialysate of RHA vs. RLA rats. These differences between the two lines were greater in naive animals and persisted, although attenuated, in experienced animals. These findings confirm that a different mesolimbic dopaminergic tone exists in RHA and RLA rats, which may be responsible, at least in part, for their different copulatory patterns, and provide insights into the differences among individuals in the neural basis of motivated behaviours and their relationship with vulnerability to abuse natural and/or drug rewards or to develop depressive disorders.

*1. Sanna et al. (2014). *Physiol Behav* 127:27-36.*

*2. Sanna et al. (2014). *Pharmacol Biochem Behav* 124:211-219.*

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*Neurological and psychiatric diseases*

## EFFECTS OF MANGANESE NEUROTOXICITY ON THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN THE RAT

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Excessive accumulation of Manganese (Mn) in the brain causes serious central nervous system dysfunctions known as Manganism or Mn-induced Parkinsonism. In addition to motor disturbances, cognitive and neuropsychiatric deficits have been underlined. However, the sleep/wake disorders upon Mn exposure have not been evaluated. In this study, we undertook an evaluation of the rest-activity rhythms in rats daily treated with MnCl<sub>2</sub> (10mg/kg i.p). Circadian locomotor activity was assayed under a light:dark cycle, constant darkness, and under an re-entrainment to shifts to a new light dark cycle. Rats were first exposed to 12:12 LD for 5 weeks corresponding to the treatment period, at the end of treatment rats were maintained in the same lighting condition for 21 days, then the rats were divided into two groups. The first one was released into constant darkness for 21 days, and the other was released into 12:12 LD advanced by 6h for 21 days. The results show that under a daily 12:12 LD, during and post treatment, Mn treated rats exhibit a more fragmented and less stable rest-activity rhythm with a decrease in: amplitude, global activity and activity during the dark phase. The activity onsets were also significantly less precise. When the LD cycle was advanced by 6h, a stable re-entrainment was achieved after 4 days in control rats, whereas Mn-treated rats did not display stable re-entrainment at least during 21 days in our experiment conditions. Upon transfer from LD to DD, Mn treated rats showed a reduction in the subjective night activity, global activity, stability and increased fragmentation. Our results imply that, in addition to motor and non motor disturbances observed in Manganism, Mn leads to impairment of the rest-activity rhythms. These changes may be attributed to a downstream effect involving disconnection of the central circadian clock from its output target networks controlling locomotor function.

## NEUROANATOMICAL ALTERATIONS IN A TRANSGENIC RAT MODEL OF HUNTINGTON'S DISEASE DETECTABLE BY CONVENTIONAL HISTOLOGY BUT NOT WITH ULTRA-HIGH-FIELD MRI

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Aim of the study was to further characterize the process of neurodegeneration in transgenic Huntington's disease (tgHD) rats, we performed a longitudinal MRI and a histological study.

Volumetric changes in the striatum, lateral ventricle, and cerebral cortex of tgHD rats were investigated at 9, 12, and 18 months of age by means of *in vivo* ultra-high-field MR imaging (7-T) and stereology. Stereological analysis did not show any significant striatal atrophy or neuronal cell loss in the striatum at the early stage of the disease; however, it revealed a significant reduction in striatal cell volume. Moreover, we observed a substantial atrophy in the striatum, enlarged ventricular volume, and a thinning of the cerebral cortex in the brains of tgHD rats only at the late disease phase of the disease, when compared to the wild-type (WT) littermates. Surprisingly, MR imaging did not show any detectable volumetric change in the investigated structures between tgHD and WT rats at different time-points. Our findings show that neuronal shrinkage precedes the striatal atrophy in tgHD rats. Moreover, as in human HD, the manifestation of striatal atrophy appears prior to cortical thinning in tgHD rats. Contrary to our expectation, *in vivo* ultra-high-field MR imaging failed to detect the volumetric changes.

## MODELLING SLEEP ALTERATIONS IN PARKINSON'S DISEASE: TRANSLATIONAL INSIGHTS

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Parkinson's disease (PD) is a chronic neurodegenerative disease diagnosed by a constellation of motor and non-motor symptoms. Using a variety of animal models, significant advances have been made to alleviate the motor symptoms of the disease. Unfortunately, this translational success has been tempered by the recognition of the still challenging and debilitating effects of non-motor symptoms. Impairments of sleep and wakefulness are among these non-motor symptoms that may predate the motor aspects and inevitably worsen over disease progression. Within a translational perspective, We will discuss the construct, face and predictive validity of the available animal models of PD to mimic sleep and wakefulness disturbances experienced by PD patients. A new neural network perspective will be advanced that holds the promise of generating valid translational models and could lead to efficient therapies of sleep/wakefulness alterations of PD.

## **RETINAL FLUORESCENT ANGIOGRAPHY PREDICTS BLOOD-BRAIN BARRIER DAMAGE AND NEUROPSYCHIATRIC MORBIDITY IN DIABETIC PATIENTS**

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Diabetic retinopathy (DR) is a common vasculopathy associated with neurocognitive disorders and categorized into two clinical types: non-proliferative (NPDR) and proliferative (PDR). PDR is characterized by a severe dysfunctional blood-retinal barrier (BRB) and is reliably diagnosed using fluorescent angiography (FA). The BRB is similar in structure and function to the blood-brain barrier (BBB). Since BBB dysfunction plays a role in the pathogenesis of brain disorders, we hypothesized that PDR will be associated with worse neurocognitive outcome. The purpose of this study was to investigate the predictive value of angiography-proven PDR on neuropsychiatric morbidity. A retrospective study was conducted based on a diabetic patients database (n=2982) diagnosed with NPDR (n=2606) or PDR (n=376) following FA. Ten year follow-up based on medical records was analyzed and patients' probability to develop all-cause and specific brain pathologies was examined. We used Kaplan-Meier survival analysis, Cox proportional hazards and logistic regression modeling to examine association between DR severity and neurocognitive outcomes adjusting for confounders.

Patients with PDR had significantly higher rates of all-cause brain impairments ( $P < 0.001$ ) and specifically epilepsy ( $P = 0.006$ ), psychosis ( $P = 0.024$ ) and stroke ( $P = 0.005$ ) compared to NPDR. The estimated mean time to develop any neuropsychiatric event was significantly shorter for PDR (5.7 vs. 6.3 years,  $P < 0.001$ ). Cox adjusted hazard ratio for developing all-cause brain disease was significantly higher for PDR (HR = 1.37, 95% CI 1.16-1.61,  $P < 0.001$ ) and PDR was a significantly strong independent predictor for all-cause brain disorders (OR 1.30, 95% CI 1.04-1.64,  $P = 0.022$ ) and epilepsy (OR 2.16, 95% CI 1.05-4.41,  $P = 0.035$ ).

Retinal angiography is the most reliable diagnostic method to identify vessel alterations. Our results suggest that retinal vessel pathology reflects brain microvascular dysfunction and predicts the risk for specific neuropsychiatric disorders.

## ERK EXPRESSION IN THE CEREBRAL CORTEX AND AMYGDALA OF ROMAN HIGH- AND LOW-AVOIDANCE RATS DURING THE ACQUISITION OF ACTIVE AVOIDANCE BEHAVIOR

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The identification of the molecular mechanisms underlying learning and memory, especially those involved in fear- and instrumental-conditioning, is a major challenge in Neuroscience. Different brain areas are involved in fear- and instrumental conditioning: thus, it has been proposed that the conditioned stimulus (CS) is conveyed to the visual (V1) and auditory (A1) primary cortices whereas the unconditioned stimulus (US) is conveyed to the somatosensory cortex. These areas project to the lateral amygdala (LA), which in turn is interfaced with the central amygdala (CeA). Notably, extracellular signal-regulated kinase (ERK)-dependent neuronal plasticity has been observed in all the above mentioned areas. Roman high- (RHA) and low- (RLA) avoidance rats, which are psychogenetically selected for, respectively, rapid versus poor acquisition of active avoidance behavior, represent a valid approach to study the neurobiological mechanisms of instrumental learning; hence, we evaluated the performance of RHA and RLA rats over 40 min in a two-way active avoidance test and related their behaviors to the activation of ERK in V1, A1, and the amygdala (AMYG). To this aim, RHA and RLA rats were assigned to one of three groups: "shuttle box" (exposed only to the apparatus for 40 min); "CS" (exposed to CS), and "CS+shock" (exposed to both CS and US).

The results confirmed the significantly higher number of avoidances of RHA rats versus RLA rats in the test (CS+shock). Moreover, the high performance of RHA rats, but not the poor one of RLA rats, was positively associated with ERK activation in the A1 and V1 cortices of the CS and CS+shock groups, supporting the pivotal role of these areas in instrumental learning using auditory and visual cues. Conversely, no differences were found in the AMYG of either line, probably because this brain area is mainly involved in the consolidation rather than acquisition of active avoidance behavior.

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## EFFECTS OF PAROXETINE ON PTSD-LIKE SYMPTOMS IN MICE

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After exposure to a severe traumatic event, avoidance, fear sensitization, and increased anxiety are among features that can persist over time in people developing posttraumatic stress disorder (PTSD). Basic research on treatment interfering with these symptoms can provide insights to improve PTSD treatment. The purposes of the present study were to induce these behavioral changes in mice and examine whether paroxetine would interfere with their expression.

Mice were submitted to avoidance training with a low (0.4 mA) or high (1.5 mA) foot-shock intensity, as mild and severe stressors, respectively, and posttraining avoidance was evaluated 1 and 12 days later. Fear sensitization, measured as increased freezing to a neutral tone, and enhanced contextual fear, measured as increased freezing to a conditioned context (wherein all mice received a 0.4-mA footshock), were assessed during this time window. An elevated plus maze test was also used to assess mouse anxiety-like behavior. Persistent avoidance, persistent fear sensitization, and long-term enhancement of contextual fear and increased anxiety-like behavior were established only in mice that received the 1.5-mA foot-shock during avoidance training. Paroxetine (at 8 mg/kg/day), injected from day 5 to day 11 after avoidance training, suppressed all of these behavioral changes. These data provide additional evidence for the role of paroxetine against expression of PTSD-like behaviors in mice.

**A NOVEL NEUROPHYSIOLOGICAL MARKER FOR HIGH-RISK BEHAVIOUR: DOES THE FRONTAL N400 EVENT-RELATED BRAIN POTENTIAL DEMARCAT BEHAVIOURAL DISINHIBITION TOWARDS DANGEROUS SOCIAL ROLES IN DWI OFFENDERS?**

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Driving while intoxicated (DWI) remains a significant consequence of drug and alcohol misuse worldwide; resulting in over 1.24 deaths each year<sup>1</sup>. Despite punitive laws and an awareness of the dangers associated with DWI, some drivers are not deterred. It is possible then, that the decision to engage in dangerous, high-risk behaviours happens at the unconscious level, before conscious and reflective thought can discourage them from acting maladaptively. This project investigated whether the N400, a preconscious electrical potential in the brain, could be used as a neurophysiological marker for risk-taking. The N400 indexes inhibitory neurological processes<sup>2</sup>, therefore amplitude differences during a risk-taking task could explain why certain individuals engage in more high-risk behaviours, such as DWI. For example, if DWI offenders display an attenuated N400 response to risky social roles (e.g., stuntman), it would insufficiently repress their unconscious will to engage in the role. Therefore, two hypotheses (H) were tested: H1) DWI offenders accept significantly more risky social roles than non-offenders; and H2) DWI offenders demonstrate significantly smaller N400 amplitudes for risky roles than non-offenders.

Twenty-five drivers convicted of 1+ DWI events and twenty-five non-offenders were recruited. Participants decided whether they would engage in each of 465 social roles at any point in their life, while being subjected to electrophysiological recordings. DWI offenders accepted 5 times the amount of risky social roles of non-offenders, and demonstrated significantly *larger* N400 amplitudes for risky roles, compared to non-offenders.

While H1 was confirmed, H2 was rejected. The finding that a larger N400 (i.e., greater hypoactivation) correlated with an increase in risky role selection may suggest that offenders selectively repress negative thoughts regarding the consequences of engaging in high-risk behaviours. Our findings can be used to design more effective prevention programs specifically aimed at people with this cognitive impairment.

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*Neurotransmission and brain plasticity*

## ACTIVITY-DEPENDENT NEUROTRANSMITTER PLASTICITY AFFECTING BEHAVIOR

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Neurotransmitter identity has been thought to be fixed and immutable, but recent studies demonstrated that changes in electrical activity rapidly and reversibly reconfigure the transmitters and corresponding transmitter receptors that neurons express (Dulcis et al., 2008, 2013). Induction of transmitter expression can be achieved by selective activation of afferents recruited by a physiological range of sensory input. Strikingly, neurons acquiring an additional transmitter project to appropriate targets prior to transmitter respecification in some cases, indicating the presence of reserve pools of neurons that can boost circuit function. The potential clinical value of such circuit-specific NT switch for treatments of neurological disorders in the nervous system is what drives my research. The discovery that changes in photoperiod dynamically regulate the numbers of neurons expressing dopamine and somatostatin in the hypothalamus, affecting stress response in adult rats, was a first step toward the understanding of this form of plasticity. Neuronal networks are identifiable by their neuronal composition, anatomical circuit connectivity, physiological input and output, and molecular signatures of transcription factors, ion channels and neurotransmitters, both during development and in the adult. Sufficient information has been obtained to design experiments to test the existence of additional reserve pools in the brain. Since recruitment of reserve pool neurons is activity-dependent, I am currently testing this hypothesis on brain nuclei where this form of plasticity may be induced by activation of specific circuits. We have now identified another neuronal network in the mouse brain that responds to perinatal nicotine exposure by ectopically expressing dopamine in otherwise non-dopaminergic neurons of the ventral tegmental area. Such upregulation of dopamine expression leads to changes in nicotine consumption in the adult. The ability to manipulate this form of plasticity could provide the basis for novel non-invasive treatment of disorders of transmitter and receptor metabolism in the developing and mature nervous system.

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**EVOLUTIONARY CONSERVED MECHANISMS  
OF ASSOCIATIVE LEARNING IN *Lymnaea***

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*Lymnaea* provides highly valuable experimental models for top-down analyses of associative learning and memory. Using classical and operant conditioning paradigms, molecular mechanisms of consolidation, maintenance, retrieval, reconsolidation and forgetting of associative memory have been investigated. Long-term memory (LTM) forms after multi-trial reward and aversive conditioning but unusually, also after single-trial reward conditioning ('flash-bulb memory'). Molecular mechanisms of LTM involve highly conserved signaling pathways (NO/cGMP, cAMP/PKA, PKC, PKM, MAPK, NMDA receptors, CaMKII, GluA1 receptors), transcriptional regulation of gene expression by CREB and C/EBP and new protein synthesis. Cellular mechanisms of LTM involve synaptic or non-synaptic plasticity in key modulatory interneurons of the feeding network. Importantly, a number of conserved molecular processes involved in LTM have been traced from the behavioral level to single identified neurons.

## SENSORY INTEGRATION IN THE MOUSE STRIATUM

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The basal ganglia are involved in several motor, cognitive, and emotional functions. The striatum is the input layer of basal ganglia, receiving excitatory inputs from thalamus, and most cortical areas, including sensory, motor and association areas. The striatum has been mainly studied with regard to motor and reward related functions but its functional role in sensory processing is largely unknown. Our objective is to elucidate the role of the striatum in the sensory processing. To that end we obtained *in vivo* whole-cell patch-clamp recordings from neurons in the dorsal striatum and in cortical layer V of the barrel field (BF) in anesthetized mice. We recorded the evoked responses to ipsi- and contralateral whisker deflections, and responses to simultaneous whisker and visual stimulus. Recorded neurons were stained and identified by their morphological and electrophysiological properties, as well as by their biochemical expression of D1 and putative D2 dopamine receptors (direct and indirect MSNs respectively). Our results show that; **A.** all neurons recorded in dorsal striatum responded to both whisker stimulation. **B.** individual striatal neurons in the dorsomedial striatum integrate multimodal information, responding to both tactile and visual stimuli. **C.** the multimodal and bilateral integration was always sublinear, with maximal summation occurring after synchronization of the onset of tactile and visual responses. **D.** direct and indirect MSNs integrate bilateral whisker input differently. **E.** striatal interneurons (cholinergic and fast spiking) integrate bilateral tactile information, and at least fast spiking interneurons can also integrate visual, **F.** that the responses were mediated by excitatory and inhibitory inputs, with inhibition following the excitation by few milliseconds of delay. In summary, our results strongly suggest that the striatum acts as a multisensory integration structure.

## DIFFERENTIAL MODULATION OF NEUROTROPHIN RECEPTOR EXPRESSION AND SIGNALING BY TYPE I INTERFERONS

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Type I interferons (IFNs), including IFN- $\alpha$  and IFN- $\beta$ , are immunomodulatory cytokines that can act in the brain to induce either anti- or pro-inflammatory effects. For instance, IFN- $\beta$  is known to produce beneficial effects in multiple sclerosis, but also to cause several neuropsychiatric side-effects, including depression and cognitive deficits. Moreover, both IFN- $\alpha$  and IFN- $\beta$  have been shown to be involved in neurodegenerative processes in humans and animals. An important aspect of central IFN action is the interaction with neurotrophins, which are master regulators of neurogenesis, survival and differentiation of neuronal and non-neuronal cells. Although IFNs have been shown to affect neurotrophin synthesis and release in various cell types, relatively little is known on their effects on expression and functional activity of the Trk neurotrophin receptors. We have previously reported that in primary neurons and differentiated SH-SY5Y neuroblastoma cells long-term exposure to type I IFNs induces a down-regulation of the TrkB receptor and impairs the neurotrophic activity of BDNF, which is known to sustain synaptic plasticity, cognitive functions and mood control. Conversely, type I IFNs cause up-regulation of the p75NTR/TrkA receptor complex, which is associated with enhanced NGF signaling and attenuation of IFN- $\beta$  pro-apoptotic effects. Here, we show that IFN- $\beta$  also alters the expression and signaling of the TrkC receptor, which mediates the neurotrophic activity of NT3. We found that in differentiated SH-SY5Y cells prolonged exposure to IFN- $\beta$  inhibits NT3-induced activation of different signaling pathways, including PI3K/Akt, PLC $\gamma$ 1 and ERK1/2, and impairs NT3 anti-apoptotic activity. These effects are associated with an enhanced expression of the truncated TrkC-T1 isoform. TrkC-T1 knockdown in IFN- $\beta$ -treated cells potentiates TrkC pro-survival signaling and restores NT3 ability to inhibit IFN- $\beta$ -induced apoptosis. These data indicate that IFN- $\beta$  can impair NT3/TrkC function through a novel mechanisms involving the up-regulation of TrkC-T1, which acts as a dominant-negative receptor of TrkC.

## **DOPAMINE D3 RECEPTOR SIGNALING AND ITS DOWNSTREAM SIGNALING TARGETS ARE ALTERED AS A FUNCTION OF OPIATE EXPOSURE STATE IN THE BASOLATERAL AMYGDALA**

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The potent rewarding effects of opiate drugs facilitate the formation of associative memories linked to the drug experience and play a key role in triggering relapse. These memories are encoded in the basolateral amygdala (BLA). Intra-BLA processing of opiate-related reward memories is mediated by dopamine D1 receptors (D1R) and D2R-type signaling as a function of opiate exposure state. D1Rs are required for acute opiate memory formation in the previously drug-naïve state, but D2R signaling is necessary for memory formation during opiate dependence and withdrawal. The D3R is highly expressed in the mesocorticolimbic system, and is also critical for the learning and memory of emotional memory. There is evidence of changes to D3R availability in chronic opiate users, thus raising the question of the molecular mechanisms underlying alterations to D3R expression and function following chronic opiate use. D3 activation is linked to both Akt-GSK-3 and calcium/calmodulin kinase pathways, which both play important roles in synaptic plasticity, and are crucial for the formation of conditioned reward in limbic regions. Here, we identify opiate exposure-induced changes to intra-BLA D3Rs and their downstream molecular targets. Protein analysis revealed that opiate dependence and withdrawal results in a downregulation of intra-BLA D2R and D3R, but not D1R expression. Additionally, the expression of CaMKIV and calcineurin are profoundly affected, and preliminary evidence suggests pGSK-3 may be changed as well. We further use a place conditioning procedure paired with targeted microinfusions to probe the behavioural significance of these molecular alterations. We found that intra-BLA D3 activity is not necessary for the formation of opiate reward memories in a previously opiate-naïve state, but that opiate dependence and withdrawal renders these memories sensitive to manipulations of D3 signaling. Overall, this work demonstrates that opiate dependence and withdrawal affects the expression and function of intra-BLA D3DRs and its downstream molecular targets.

**SODIUM/CALCIUM EXCHANGER (NCX): A NEW DRUGGABLE TARGET  
IN NEURONAL DYSFUNCTIONS FOLLOWING HYPOXIC-ISCHEMIC INJURY  
IN NEONATAL MICE**

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Hypoxic-ischemic encephalopathy (HIE) accounts for the majority of developmental, motor and cognitive deficits in children, leading to life-long neurological impairments. Since transient brain ischemia followed by reoxygenation alters ionic homeostasis in adult brain by modulating the expression and the activity of the plasmamembrane sodium/calcium exchanger (NCX) we aim to demonstrate the involvement of NCX in the pathophysiology of HIE. Experimental HIE was induced in postnatal day 7 (P7) mice by unilateral ligation of the right common carotid artery and subsequent 60 min exposure of animals to 8% O<sub>2</sub>. Expression profiles of NCX protein from embryo stage to adulthood was carried out both in HI and in naïve hippocampus mice. To assess the effect of NCX pharmacological activation, brain infarct volume was evaluated in propidium iodide stained hippocampus sections, obtained at several time points after the administration of the newly synthesized NCX activator Neurounina. Moreover, the effect of NCX activation on learning and memory was evaluated in P60 mice subjected to neonatal hypoxia ischemia. An age-dependent NCX1 and NCX3 increased expression was evidenced in hippocampus of naïve mice, by contrast, NCX1 and NCX3 expression, in the hippocampus of mice subjected to neonatal hypoxia ischemia was significantly reduced starting from 7 days after injury. NCX2 hippocampal expression was not modulated in mice subjected to neonatal hypoxia ischemia. Interestingly, the activation of NCX induced by Neurounina administration 3 hours and 5 hours after injury reduced infarct volume and in p60 mice subjected to neonatal hypoxia ischemia improved the spatial and object memory. Together these findings strongly suggest the involvement of NCX in mediating brain damage and long term deficits induced by neonatal hypoxia ischemia.

# **POSTER SESSION I**

**NEUROGENESIS, PLASTICITY  
& CELL DEATH**

**P 01.****SOX-2 EXPRESSION IN SHEEP BRAIN: A POSSIBLE ROLE IN ADULT NEUROGENESIS**

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Neurogenesis was first defined as the creation of the nervous tissue-forming cells, known to be active only in the pre-natal stages. Later on, it was found that some species can perform regeneration of their nervous tissue even after birth. Recent studies on rodents had proved that some areas in the adult mammalian brain can also undergo adult neurogenesis, this includes the subventricular zone (SVZ), the subgranular zone (SGZ) of the dentate gyrus, the hypothalamus and the most recently discovered; the dorsal vagal complex (DVC).

In order to expand the phylogenic occurrence of adult neurogenesis in mammals, we worked on sheep, an interesting useful model due to: its similarities with the primate brain, its long life span which exceeds rodents in studying adult neurogenesis, since it is highly affected by age.

Sox-2 is one of the transcription factors ensuring the proper function of adult neural stem cells, as it is a major mediator of the Notch signaling pathway that has a role in maintaining the precursor pool in the adult SGZ. Sox-2 is one of the four pluripotency-inducing genes leading to iPS cells, whereas Sox-2 deletion in adult mice results in a loss of hippocampal neurogenesis. Here we examined the expression of Sox-2 in two brain areas capable of adult neurogenesis (SVZ and DVC) and in cell pellets obtained from cell cultures of these areas. We will subsequently examine the expression of Sox-2 in the brainstem of the urodele *Pleurodeles Waltlii*, one of the most efficient amphibian species capable of adult neurogenesis.

**A CORDAL, NOT GANGLIONIC, PATTERN OF CEPHALOPOD BRAIN NEUROGENESIS**

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From the large-brained cephalopods to the acephalic bivalves, molluscs show a vast range of nervous system centralization patterns. Despite this diversity, molluscan nervous systems are, when broadly considered, organized as medullary cords, as seen in chitons, or as ganglia, which are typical of gastropods and bivalves. The cephalopod brain is exceptional not just in terms of its size; its relationship to a molluscan cordal or ganglionic plan have not been resolved from study of its compacted adult structure. One approach to clarifying this puzzle is to investigate the patterns of early cephalopod brain neurogenesis, where molecular markers for cephalopod neural development may be informative. We report here on early brain pattern formation in the California two-spot octopus, *Octopus bimaculoides*. Employing gene expression for the pan-bilaterian neuronal marker ELAV and the atonal-related neuronal differentiation genes NEUROGENIN and NEUROD, as well as immunostaining with a Distaless-like homeoprotein antibody, we found that the octopus central brain forms from concentric cords rather than bilaterally distributed pairs of ganglia. We conclude that the cephalopod brain, despite its great size and elaborate specializations, retains in its development the hypothesized ancestral molluscan nervous system plan of medullary cords, as described for chitons and other aculiferan molluscs.

## P 03.

## OCTOPUS PALLIAL NERVE REGENERATION

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The cephalopod mollusk *Octopus vulgaris* is able to regenerate various tissues, including central and peripheral nervous system after injury. To better understand the mechanisms involved in nerve regeneration in the common octopus, we examined neural regeneration occurring after complete transection of one of the two pallial nerves (belonging to the peripheral nervous system) in living individuals under anesthesia. Animals were examined after three, seven and 14 days. Scar tissue was observed at the level of muscle and connective tissues lesioned to expose the nerve. A thick scar also appeared between the two nerve stumps, mainly formed by hemocytes which appear to actively proliferate, acting in debris removal and possibly fostering axon regrowth. Injured axons regenerate quickly from the proximal stump, initially projecting in many directions. The majority of fibers of the distal stump appear to degenerate. At a later stage axonal growth appear to be more organized along the connective tissue. Intriguingly, 14 days post lesion mitotic neural cells between the fibers of the distal stump have been identified.

Our results show the massive involvement of neural stem cells occurring exclusively inside the injured nerve tissue, before appearance of proliferating neural cells. We suggest the occurrence of a dedifferentiation process of the connective tissue cells enveloping nerve fibers to a stem cell-like state.

**CELLULAR PLASTICITY AND REVERSIBILITY IN WATER STRESSED RATS: DP71  
IMPLICATION**

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The magnocellular neurons cell bodies are compounds of supraoptic nucleus (SON) of the hypothalamus. They secrete two neurohormones, vasopressin (AVP) and oxytocin (OT) which are involved in the regulation of water and mineral balance. The SON as the hypothalamo-neurohypophysial axis (HNHA) have the feature undergoing a profound neuro-glial anatomical reorganization in response dehydration. These changes are strictly related to the structural and functional organization of the cytoskeleton. Among the proteins of the cytoskeleton are found subcortical dystrophins.

The aim of our job is to study in the Wistar rat NSO dystrophins involvement in the process of cell plasticity and its reversibility. In order to monitor the distribution of these proteins in water-stress conditions, we subjected the rats to 14 days of 2% NaCl loading, followed by 30 days rehydration. This study was discussed by immunohistochemical and Western blotting approaches and systemic parameters analyze in different conditions of stress or recovery.

The results indicate that 14 days of salt loading cause changes in osmolality, hematocrit and plasma sodium levels. These parameters are indicators of a state of significant osmotic shock. Dystrophins are expressed by neurons and glial cells. The marking is located at the glial expansions, at the ventral glial limitant and pericapillary levels. The intensity of this marking is increased in water stress condition and decrease with rehydration. By Western blot, the major protein that appears is a 71kDa dystrophin (Dp71). The Dp71 quantity decreased following dehydration and increased again after rehydration. The amount of this protein does not reach the control of the threshold.

Our results show the reversibility of cell plasticity phenomenon reported at the HNHA. In addition, dystrophins seem to play an important role both in the changes observed at the time of water stress and at the level of system recovery after rehydration.

P 05.

**JAK4D, A FIRST-IN-CLASS THYROTROPIN-RELEASING HORMONE (TRH)-BASED COMPOUND, PROTECTS AGAINST FREE RADICAL RELEASE AND CELL DEATH INDUCED BY INTRASTRIATAL KAINATE**

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Given the complex and multifaceted nature of neurodegenerative disorders, it is likely that pharmacological interventions modifying multiple pathogenic mechanisms may be more effective than those targeting a single injury process. While multifaceted intervention could conceivably be accomplished by a cocktail of targeted drugs, an attractive alternative would be a single multifunctional therapeutic agent. JAK4D, a thyrotropin-releasing hormone (TRH)-based compound targeting the central signalling system of TRH, offers such a multifunctional approach to treating neurodegeneration.

The purpose of our study was to determine the ability of JAK4D to function as a neuroprotective agent against free radical release, cell death and microglial activation induced by intrastriatal microdialysis of an excitotoxic concentration of kainate (KA) in conscious rats.

Systemic administration of JAK4D (10 mg/kg i.p.) 40 min prior to KA (1 mM) application significantly reduced the formation of hydroxylated terephthalic acid, OH-TA, which reflects the production of hROS in rat striatal extracellular space. Central KA administration reliably induced apoptotic cell death in striatal tissue. *Ex vivo* analysis of striatal tissue showed that JAK4D pre-treatment significantly reduced KA-induced apoptosis, as measured by TUNEL immunolabeling and caspase-3 activation. Qualitative immunohistochemical examination of striatal sections from KA-treated rats indicated the occurrence of microglial activation, identified by the presence of cells showing increased IBA1 immunoreactivity. In striatum of control rats, no activated microglial cells were observed. Tissue from rats pre-treated with JAK4D showed less intense immunolabelling, indicating that JAK4D treatment also decreased microglial activation, a recognized pathogenic factor in CNS disorders.

JAK4D treatment, therefore, was shown to protect against free radical production and neuronal damage in this model. Attenuation of KA-induced hROS by JAK4D suggests that reduction of oxidative stress is one means by which it may exert neuroprotective effects. These results provide a possible mechanistic rationale in support of the therapeutic potential of JAK4D in neurodegeneration.

# **NEUROPROTECTION & TOXICITY**

## P 06.

**NOVEL INDOLE AND PYRIDOINDOLE DERIVATIVES WITH MULTI-TARGET-DIRECTED  
NEUROPROTECTIVE ACTION**

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Alzheimer's disease and type 2 diabetes mellitus have been reported to show common features connected mainly with malfunctions in glucose metabolism. They result in elevated sorbitol levels in brain, mostly derived from glucose flux through the astrocyte polyol pathway and accompanied also by oxidative stress. After years of extensive study of aldose reductase (ALR2) inhibitors (1) and neuroprotective compounds with antioxidant effect (2,3), we have approached systematic search for other positive beneficial properties in profiling our compounds as multi-target drug leads (MTDLs). The aim of this study was to uncover new activities of several prospective compounds towards different amine oxidases (monoamine oxidase A – MAO-A, monoamine oxidase B – MAO-B and semicarbazide-sensitive amine oxidase – SSAO), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) involved in the neurological disorders. The set of 12 tested compounds was chosen mainly from the derivatives of indole-1-acetic acid and pyridoindole derivatives.

The *in vitro* activity of tested compounds against human MAO A/B was determined using fluorometric method with clorgyline and *R*-deprenyl as reference compounds. The most active compounds **1-5** were also evaluated as hAChE, hBuChE and bsSSAO inhibitors. Donepezil was used as a reference for hAChE and hBuChE, while phenelzine was used as a reference for bovine SSAO.

The most prospective compound (±)-cis,2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1Hpyrido[4,3-b]indole (1), already proved as an effective neuroprotectant (2,3), showed promising inhibition activity towards MAO-A, MAO-B, BuChE and SSAO.

1. Stefek M., et al.. (2008). *Bioorg Med Chem* 16, 4908

2. Stolic S. et al. (2006). *Cell Mol Neurobiol* 26, 1495

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## P 07.

**NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF MDG548, A NOVEL PPAR $\gamma$  AGONIST**

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Peroxisome proliferator activated receptor (PPAR) $\gamma$ , with its efficacy on modulation of neuroinflammatory response, is a potential pharmacological target in Parkinson's disease (PD). We tested a new compound PPAR $\gamma$  agonist MDG548, which shows an high binding affinity for this receptor. Neuroprotection mediated by this compound was evaluated in vitro and in vivo. Protection against H<sub>2</sub>O<sub>2</sub> neurotoxicity was tested in vitro: MDG548 dose-dependently increased cell viability of rat cortical neurons co-treated with H<sub>2</sub>O<sub>2</sub> or pre-exposed to MDG548 prior to H<sub>2</sub>O<sub>2</sub> and the effect was fully inhibited by the PPAR $\gamma$  antagonist GW9662. NF-kB activation was investigated to assess anti-inflammatory activity; MDG548 dose-dependently decreased NF-kB activation induced by LPS. For in vivo testing, C57BL/6J mice received either subacute MPTP (20 mg/kg i.p. once a day for 4 days) in association with saline or escalating doses of MDG548 (2, 5, 10 mg/kg i.p.), or chronic MPTP (25 mg/kg i.p) plus probenecid (100 mg/kg i.p) twice a week for 5 weeks, in association with saline or MDG548 (2 mg/kg i.p.). Stereological counting showed that MDG548 prevented the MPTP-induced reduction in TH-positive cells in the substantia nigra pars compacta (SNc) at all doses tested and in both MPTP models. Moreover, evaluation of the inflammatory response after subacute MPTP treatment showed that CD11b(+) microglia and iNOS-IR were increased by the neurotoxin as compared to vehicle in the SNc while they were reduced by MDG548 co-treatment, which attenuated microglia proliferation and morphological changes, as well as iNOS-IR. MDG548, considering also its potential anti-inflammatory effect, might be an alternative in the search for potent PPAR $\gamma$  agonists to be tested as disease-modifying drugs in PD.

*Lecca D, Nevin DK, Mulas G, Casu MA, Diana A, Rossi D, Sacchetti G, Carta AR. 2015. Neuroprotective and anti-inflammatory properties of a novel non-thiazolidinedione PPAR $\gamma$  agonist in vitro and in MPTP-treated mice. Neuroscience. 2015 Apr 20. [Epub ahead of print]*

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## P 08.

**CEMTIRESTAT, A NOVEL ALDOSE REDUCTASE AND MONOAMINE OXIDASE INHIBITOR,  
IN MULTITARGET DIRECTED NEUROPROTECTION**

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Neuropathies of the peripheral and autonomic nervous systems affect up to half of all people with diabetes, and are major risk factors for foot ulceration and amputation. The etiology is multifactorial: metabolic changes in diabetes may directly affect neural tissue. These include elevated polyol pathway activity, oxidative stress, the formation of advanced glycation and lipoxidation end products, and various pro-inflammatory changes. The polyol pathway mechanism may induce additional metabolic changes. Thus aldose reductase (ALR2), the first enzyme of polyol pathway, represents a potential therapeutic target. Also inhibition of monoaminooxidases was observed to have neuroprotective effect due to its enhancing of monoaminergic transmission and could contribute to the overall therapeutic potential.

Cemtiresat (3-mercapto-5H-1,2,4-triazino[5,6-b]indole-5-acetic acid) was identified as a new promising inhibitor of ALR2 with IC<sub>50</sub> in submicromolar region and selectivity factor relative to ALR1 (aldehyde reductase) and AKR1B10 around 400 and 375, respectively (Stefek et al. 2015). Analysis of the enzyme kinetics showed uncompetitive inhibition with non-covalent type of binding. Molecular "docking" identified key interactions with specific amino acid residues of the ALR2, ALR1 and AKR1B10 binding sites. In ALR2 inhibition, cemtiresat only mildly differentiated between glyceraldehyde, methylglyoxal, 4-hydroxynonenal and GS-4-hydroxynonenal used as substrates. The compound was readily taken up by isolated red blood cells and efficiently protected the erythrocytes against hemolysis induced by t-BuOOH. Ability to scavenge free radical species was proved in a DPPH test. In the experimental model of diabetes induced in rats by streptozotocin, cemtiresat (50 mg/kg/day, administered i.g. for 5 consecutive days) significantly inhibited accumulation of sorbitol in sciatic nerves. In addition, an efficient inhibition of hMAOA (human monoamine oxidase A) and hMAOB (human monoamine oxidase B) enzymes by cemtiresat was recorded with IC<sub>50</sub> in micromolar range.

On balance, cemtiresat, originally projected as aldose reductase inhibitor, owing to its multiple inhibitory activities, appears a promising multitarget directed neuroprotective agent.

*Stefek, M. et al. (2015). Identification of Novel Aldose Reductase Inhibitors Based on Carboxymethylated mercaptotriazinoindole Scaffold. J Med Chem DOI: 10.1021/jm5015814.*

**P 09.****NEUROCHEMICAL MECHANISMS INVOLVED IN MDMA-INDUCED  
NEUROTOXICITY IN MICE**

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Several preclinical reports describe the amphetamine-related drug 3,4-methylenedioxymethamphetamine (MDMA) as a dopaminergic neurotoxin in mice. The aim of this study was to determine the effects of a chronic-intermittent MDMA treatment on the levels of two different markers of dopaminergic function, the dopamine transporter (DAT) and the enzyme tyrosine hydroxylase (TH), in striatum, medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) of mice during and after MDMA treatment. Moreover, this study explored the possible involvement of the GABAergic and serotonergic systems in MDMA-induced neurotoxicity, by evaluating glutamic acid decarboxylase-67 (GAD-67) and serotonin transporter (SERT). Mice received either MDMA (10 mg/kg i.p.), twice a day/twice a week, from post natal day (PND) 56 to PND 81 (group 1) or MDMA from PND 56 to PND 106 (group 2). Group 1 and group 2 were both sacrificed 3 days after the last drug administration, whereas a third group of mice, which received MDMA from PND 56 to PND 117, was sacrificed 2 weeks after the last drug administration (group 3). Mice in groups 2 and 3 showed a reduction of DAT-positive fibers in striatum and mPFC, and a reduction of GAD-67-positive fibers in striatum, mPFC and hippocampus. Conversely, only mice in group 3 showed a reduction of TH-positive fibers in striatum, mPFC and hippocampus. SERT-positive fibers in striatum and NAc, as well as DAT-, GAD-67- and TH-positive fibers in NAc were not affected by MDMA treatment. Results from DAT and GAD-67 immunofluorescence in striatum and mPFC suggest that dopaminergic degeneration is associated with degeneration of striatal GABAergic transmission, and that DAT may represent an earlier marker of dopaminergic degeneration than TH.

**P 10.****DIFFERENT NEUROTOXIC EFFECTS INDUCED BY COMBINED ADMINISTRATION OF MDMA PLUS CAFFEINE, IN ADULT AND ADOLESCENT MICE**

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The use of amphetamine-related drugs and their association with caffeine-containing beverage is very common among adolescents and young adults. On this base we studied the neurotoxic properties of MDMA and caffeine association in adolescent (28 days) or adult (3 months) mice. Previous results showed that acute repeated MDMA treatment induced a decrease in dopaminergic neurons in substantia nigra pars compacta (SNc) of adult and adolescent mice, whereas TH-positive fibers in striatum were decreased in adult mice only. However MDMA were associated with caffeine, a more pronounced degeneration in adolescent compared with adult mice was observed. To better clarify the molecular mechanism at the base of the different neurotoxic effect of this drug association at different ages, we evaluated the nNOS expression, which plays a critical role in the integration of dopaminergic and glutamatergic transmissions, in the striatum of adolescent or adult mice treated with MDMA (4X20 mg/kg), alone or in combination with caffeine (2x10 mg/kg). To confirm the neurodegeneration induced by MDMA+caffeine, we evaluated the dopamine transporter (DAT) expression in the striatum of adolescent and adult mice. nNOS immunohistochemistry revealed that MDMA induced an increase in striatal nNOS-positive neurons only in adult mice. Notably, MDMA+caffeine association induced nNOS expression in adolescents. The DAT immunohistochemical analysis showed a decrease of DAT-positive fibers in both adult and adolescent mice treated with MDMA alone or MDMA+caffeine. These data confirm the previous results concerning neurodegeneration and suggest that the use of caffeine in association with MDMA during adolescence may worsen the toxicity elicited by MDMA.

**P 11.****THE CHANGES IN EXTRACELLULAR LEVEL AND TISSUE CONCENTRATION OF DA AND 5-HT INDUCED BY MDMA AND CAFFEINE GIVEN CHRONICALLY IN A “BINGE” MODEL IN THE MOUSE BRAIN**

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3,4-methylenedioksymethamphetamine (MDMA), psychostimulant with addictive potential is used occasionally or more often repeatedly in short time intervals in combination with caffeine (CAF) to gain stronger stimulatory effect. CAF is frequently ingested as "energy drink" or coffee during abstinent period to alleviate decrease in mood and fatigue. The aim of our research was to investigate the effect of combined chronic treatment of MDMA (4 x 10 mg/kg) and CAF (2 x 5 mg/kg) in a “binge” model on changes in DA and 5-HT release as well as DA and 5-HT tissue content in the mouse brain. The extracellular level and tissue content of DA and 5-HT was determined by HPLC with electrochemical detection. CAF potentiated release of 5-HT induced by MDMA having a weaker effect on release of DA enhanced by MDMA. MDMA alone increased DA content in the mouse striatum, frontal cortex and hippocampus and decreased DOPAC and HVA in the striatum, frontal cortex and hypothalamus. CAF alone increased DA, DOPAC and HVA content in the striatum but not in the other brain regions. The effect of MDMA on DA content was diminished by CAF only in the hippocampus and frontal cortex but not in the mouse striatum. 5-HIAA but not 5-HT content was decreased by MDMA alone and CAF had no influence on these changes. Our findings indicate increased stimulation of 5-HT neurons under conditions of intermittent administration of MDMA alone or in combination with CAF as compared to acute treatment. On the other hand, DA neurons seem to respond weaker to both psychostimulants in comparison to effect of their single doses. The changes in tissue content of both monoamines reflect rather the effect of MDMA and CAF on DA and 5-HT release from nerve terminals but not a neuronal damage.

## P 12.

**TOXICOLOGICAL RESEARCH 1,2,3,4-TETRAHYDROISOQUINOLINE AN  
EXO/ENDOGENOUS AMINE WITH ANTIDEPRESSANT-LIKE ACTIVITY – *IN VIVO*,  
*IN VITRO* AND *IN SILICO* STUDIES**

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1,2,3,4-Tetrahydroisoquinoline (TIQ), an endogenous amine naturally occurring in mammalian brain can easily penetrate through the blood-brain barrier. It possess significant neuroprotective properties (1) observed in various neurotoxicity models. It has also affinity to the monoamine oxidase (MAO) and in low micromolar concentration reversibly inhibits MAO A and B activity. That more, TIQ shows antidepressant-like activity in the variety animal models of depression (2,3). In that light, this compound might be effective for the depression therapy in a clinical setting but the success of this drug is determined not only by good efficacy but also by an acceptable ADMET profile. Although a large variety of medium- and high-throughput *in vitro* ADMET screens are available, being able to predict some of these properties *in silico* is valuable. Today, it is recognized that employing computational ADMET, in combination with *in vivo* and *in vitro* predictions as early as possible in the drug discovery process, helps to reduce the number of safety issues. The aim of the study was to assess the degree of histopathological changes in rats tissues (liver, kidney, lung) after acute and chronic administration of TIQ. Additionally, prediction of its properties in terms of absorption, distribution, metabolism, elimination and toxicity in the human body was performed. The obtained data did not demonstrate toxic effects of tested substance in *in vivo* and *in vitro* studies in rats, and *in silico* ADMET prediction in the human body. These results can help to discover or model a new effective antidepressant drug and have important clinical significance in the treatment of depression. Additionally, the use in the treatment of depression substances occurring endogenously, having neuroprotective and antidepressant-like effects in the CNS would also be beneficial in controlling the adverse CNS inflammatory processes accompanying depression. Acknowledgements: Grant No. 2013/11/N/NZ7/00358

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**MEMORY, COGNITION & BEHAVIOR**

## P 13.

**NEURAL MECHANISMS INVOLVED IN PUNISHMENT-RELATED MEMORY-GUIDED ATTENTION**

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Previous studies have demonstrated the ability of spatial contextual long-term memories (LTMs) to drive attention and enhance perception of relevant objects within natural scenes. Orienting attention from LTM involves activity in brain regions participating in retrieval of memories for spatial context (i.e. hippocampus) as well as in the frontal-parietal network for visual-spatial attention<sup>[1]</sup>. In real-world situations, however, both memory and attention are strongly influenced by motivational factors. Remembering the outcomes of past experiences, such as successful avoidance of an aversive consequence, is crucial to adaptively generate expectations and allocate attention to perceptual events within our complex environment. Nevertheless, whether and how the neural circuits engaged by attention, memory and punishment-related motivational processes might work together to optimize perception is unknown. We used functional magnetic resonance imaging (fMRI) to examine the neural mechanisms involved in punishment-related memory-guided orienting. By employing an experimental paradigm that integrates contextual LTMs associated or not to successful avoidance of punishment (i.e. potential monetary loss) and orienting of attention within natural scenes, we observed that past avoidance-punishment outcomes of memories potentiate the impact of memory-based orienting on perception, as revealed by faster responses to discriminate target objects that appeared at memorized locations associated with avoiding punishment (avoidance trials) relative to those appearing at memorized locations without punishment-related associations (safe trials). fMRI data showed that brain regions known to be involved in memory-guided attention (1) were commonly activated both in the avoidance and safe trials; importantly, avoidance-related associations specifically modulated activity in frontal-parietal regions engaged by attentional control (2). These results provide new evidence about how memory, attention and motivational processes may interact in the human brain.

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## P 14.

**TIME COURSE OF THE EFFECTS OF PUNISHMENT AVOIDANCE-ASSOCIATED  
MEMORY-GUIDED ORIENTING ON PERCEPTION**

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Past experiences, stored as long-term memories (LTM), can bias attention to enhance identification of relevant objects within natural scenes. Previous research has shown that these effects are further potentiated by the rewarding properties of the remembered experience (1). However, it is still an open question whether this memory bias extends to punishment-related associations. Some studies suggest that the brain encodes avoidance of an aversive outcome in a similar way as receipt of a reward (2). Here we combined behavioral measures with recordings of brain activity with high temporal resolution to examine whether spatial contextual memories associated to successful avoidance of punishment could modulate memory-based visual search processes within natural scenes and the level(s) at which visual neural processing is biased by avoidance-associated contextual LTMs. We recorded event-related potentials (ERPs) while participants (N=27) performed a memory-cued orienting task in which recent LTMs for specific locations of target objects within natural scenes that could be associated with avoidance of an aversive outcome (i.e. potential monetary loss) or without punishment-related associations were used as attentional cues predicting where the upcoming target would appear within the scene. Results showed that avoidance-related associations enhanced the speed of responses to identify target objects in the memory-cued orienting task. The moment-by-moment record of neurophysiological activity elicited by target objects, enabled us to observe that this behavioral benefit by avoidance punishment associations was accompanied by modulations of ERP markers of early visual processing (P1 component, 80-110 ms post-target onset) and target selection processes (N2pc, 200-270 ms). These findings demonstrate a role of punishment avoidance in regulating how efficiently spatial memories drive visual search in cluttered scenes and dynamically impact different stages of ongoing visual neural processing.

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**P 15.****ENDOCANNABINOID SYSTEM AND CONSOLIDATION OF AVERSIVE MEMORY:  
BEYOND CANNABINOID RECEPTOR SUBTYPE 1**

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Emotional events have a strong impact on memory processes. It is well established that the endocannabinoid system is crucially involved in regulating memory processes and emotional states. However, contrasting findings have been reported concerning the effects induced by exogenous manipulation of the endocannabinoid signaling on emotional memory modulation. Endogenous cannabinoids, and their analogues, show binding affinity for other receptor families beyond the cannabinoid receptors subtype 1 (CB1) and 2 (CB2), among them the PPAR $\alpha$  and the TRPV1 receptors.

The aim of this study was to investigate the receptor subtypes involved in mediating cannabinoid effects on memory consolidation for aversive experiences. To this purpose, different groups of male Sprague Dawley rats were treated (i.p.) immediately after training of an inhibitory avoidance task with the following compounds: the cannabinoid receptor agonist WIN55,212-2 (0.3-3 mg/kg); the anandamide hydrolysis inhibitor URB597 (0.1-0.3 mg/kg) or the 2-AG hydrolysis inhibitor JZL184 (0.25-1 mg/kg); the CB1 receptor antagonist SR141716 (0.3-3 mg/kg); the CB2 receptor antagonist SR144528 (0.03-0.3 mg/kg); the PPAR- $\alpha$  receptor antagonist GW6471 (1-4 mg/kg); the TRPV1 receptor antagonist capsazepine (5mg/kg). An enhancing effect on memory consolidation was observed by potentiating cannabinoid signaling through systemic administrations of WIN55,212-2, URB597 or JZL184 but no effects were found by blocking cannabinoid, TRPV1 or PPAR- $\alpha$  receptor activity. WIN55,212-22 effects on memory consolidation predominantly depended on CB1 receptor activation. However, the enhancing effects of URB597 on memory consolidation were only partially blocked by co-administration with a cannabinoid or PPAR-alpha or TRPV1 receptor antagonists, suggesting a concomitant activation of all these receptors. Interestingly, JZL184 enhanced memory retention via a selective CB2-mediated signaling. Our results suggest that memory consolidation for aversive events is a process finely modulated by multiple neurotransmission pathways simultaneously activated.

This evidence drives beyond the CB1-centered classical hypothesis, and opens up new possible area of research in the understanding of the neural underpinnings of emotional memory.

## P 16.

**THE EFFECT OF CO-TREATMENT WITH ANTIDEPRESSANTS AND RISPERIDONE ON THE MK-801-INDUCED CHANGE IN OBJECT RECOGNITION MEMORY IN MICE**

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Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning and affects approximately 1% of the world's population. Although the currently available atypical antipsychotics have some efficacy in alleviating social and cognitive dysfunction, this effect is small and mechanisms of this action is still unknown. Several clinical reports have suggested that the antidepressant-induced augmentation of risperidone (an atypical antipsychotic) activity may efficiently improve treatment of the negative and certain cognitive symptoms of schizophrenia. Studies on the effect of MK-801 (a NMDA receptor antagonist) on object recognition memory suggest a decreased memory retention when MK-801 is given before the first, introductory session.

Thus, the aim of the present study was to evaluate the effect of antidepressants mirtazapine or escitalopram and risperidone on the effect of MK-801, given prior to the first introductory session, on performance in the object recognition memory test. The experiments were carried out on male Albino-Swiss mice. The mice were tested for the ability to discriminate between an old, familiar and a novel object. Mirtazapine or escitalopram and risperidone were given 30 min before MK-801, and MK-801 was administered 30 min before the first introductory session. Memory retention was evaluated 1.5 h after the introductory session.

The present results showed that MK-801 (0.2 mg/kg) decreased memory retention when given before the introductory session. Risperidone at a higher dose (0.1 mg/kg) reversed that effect. Co-treatment with an ineffective dose of risperidone (0.01 mg/kg) and mirtazapine (2.5 and 5 mg/kg) or escitalopram (5 and 10 mg/kg) abolished the deficit of object recognition memory induced by MK-801.

The obtained results suggest that mirtazapine and escitalopram may enhance the antipsychotic-like effect of risperidone in the animal test modeling some cognitive symptoms of schizophrenia.

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P 17.

**IMPROVING AGE-RELATED MEMORY IMPAIRMENTS WITH DONEPEZIL:  
WHY YOU CAN'T HAVE IT ALL**

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We examine one of potential explanations for inconsistent efficacy of cholinesterase inhibitors like donepezil on cognitive aging. Based on a previous experiment in mice showing that donepezil had no effect on the aging-associated impairment of long-term memory at a dose restoring the impairment of short-term memory and *vice versa*, we hypothesized that restoring these memory impairments rely on different and partially conflicting neurobiological mechanisms. We first translated previous observation to aged humans by using virtual analogs of the two radial-maze tasks used in mice, and showing differential efficacy of donepezil between the two tasks. One task evaluates flexibility of memory relative to invariant food locations, as a model of declarative long-term memory (DLTM), the remembering of facts and events in the long-term. The other task taxes short-term memory of always changing food locations (varying memory) as a model of everyday memory relative to highly repetitive events with only temporary value (e.g. “where did I park my car”). Aged participants displayed lower performance levels than young subjects in both tasks but only the age-associated impairment in DLTM was improved by a currently used dose of donepezil. To explain this dissociation, we then investigated brain effects of donepezil in aged mice, using Fos protein expression as marker of neuronal activation induced by memory testing. By studying varying memory under different levels of interference, we demonstrated (i) that *organization/update* was the critical component improved by donepezil, (ii) this improvement was associated with reduction of age-related Fos over-activation in the hippocampus, (iii) and contradictory to restoring DLTM impairment that requires enhancing hippocampal activity. Thus, improving aging-related defects in DLTM formation and organization/update of varying memory requires opposite activity modulation of the same brain systems, which cannot be achieved by the same donepezil dose. Present findings highlight important constraints for treating aging-related memory disorders.

## DEEP BRAIN STIMULATION TO RESTORE MEMORY LOSS: TARGETS AND MECHANISMS

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Deep brain stimulation (DBS) has gained interest as a potential therapy for advanced treatment-resistant dementia. However, possible targets for DBS, optimal stimulation parameters, and mechanisms of action are not yet clear. Here, we compared the effects of DBS of the fornix, CA1 subregion of the hippocampus, mammillothalamic tract, anterior thalamic nucleus and entorhinal cortex in an experimental rat model of dementia. Rats with scopolamine-induced amnesia were assessed in a spatial memory task with different DBS parameters. Potential mechanisms of action were assessed by means of c-Fos immunohistochemistry, microdialysis experiments as well as BrdU and retroviral GFP labeling in the hippocampus to evaluate neurogenesis. When comparing all structures, DBS of the fornix resulted in the most potent memory restoring effect. This effect was dependent on high current densities, and not on the frequency of stimulation. The behavioural improvement was accompanied with a selective activation of cells in the CA1 subfield of the hippocampus and a substantial increase in the levels of hippocampal acetylcholine. Particularly, acetylcholine levels increase in the first 20 min of DBS, but then decline again with continued stimulation. The results on neurogenic changes are still being analyzed at this moment. Our findings suggest that within the circuit of Papez only fornix DBS was able to restore memory functions in an experimental model of dementia, when a specific set of stimulation parameters was applied. We conclude that intermittent or closed-loop stimulation might be necessary to maintain beneficial effects on memory functions when applying chronic DBS of the fornix.

P 19.

**A NOVEL, SELF-ADJUSTING DELAY DISCOUNTING PROCEDURE FOR THE STUDY OF CHOICE IMPULSIVITY IN RATS**

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Cognitive traits such as impulsivity are believed to play a role in psychiatric disorders such as addiction and schizophrenia. Here, we developed a novel delay discounting procedure in which delays are determined by rats' choices. Rats have to choose between a lever associated with the immediate delivery of a small reward or a lever associated with the delivery of a bigger reward after an "adjustable" delay that increases or decreases in 5-sec steps. After the establishment of a stable baseline, we investigated the effects of stimulation or inhibition of the dopamine system using amphetamine, the D1 antagonist SCH212390 and the D2 antagonist raclopride. Rats' behavior stabilized after approximately 20 sessions. As expected rats showed a clear preference for bigger rewards at short delays and as delays increased progressively switched to immediate rewards. Average delays under baseline conditions or after saline were 15 sec. Amphetamine increased the choice of delayed rewards whereas D1 antagonist decreased the choice of delayed rewards. On the other hand, both dopamine D2 and D1 antagonists blocked amphetamine-induced increases in impulsivity. Our procedure allows a precise measure of impulsivity of choice. Stable baseline responding is achieved rapidly and is maintained over several weeks. As in other delay discounting procedures, behavior was sensitive to manipulations of the dopamine system confirming a major role of this system in impulsivity. This procedure is a new behavioral tool to investigate the neurobiological basis of individual differences in impulsivity.

## P 20.

**INFLAMMATORY PAIN DESENSITIZES MU OPIOID RECEPTOR IN VTA: IMPACT ON OPIOID SELF-ADMINISTRATION IN RATS**

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The use of opioid therapies in patients with chronic pain and previous history of opioid abuse is a challenge in the medical practice because of drug abuse liability. Surprisingly, few studies have investigated how inflammatory pain could alter opioid intake patterns and none of them have focused in an opioid dependent population. In the present study, we investigated the effect of inflammatory pain on mu opioid receptor (MOR) response to either DAMGO or heroin by conducting microdialysis studies in NAc and recordings of miniature IPSCs in VTA slices. Moreover, we studied the changes induced by inflammatory pain on heroin (50, 100 and 200 µg/kg/infusion) self-administration under fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement. Our results indicate that inflammatory pain partially desensitizes MORs in the VTA. Animals injected with CFA showed a significant reduction in the DA release in NAc induced by either heroin iv administration or DAMGO intra-VTA microinjection vs control animals. In fact, inflammation showed to alter DAMGO inhibition of GABA release in VTA slices. Accordingly, animals injected with CFA showed a clear deviation from the expected linear response in the dose response curve for heroin under FR2, increasing their intake when tested with high doses (100 and 200 µg/kg/infusion). Finally, very high doses of heroin (200 µg/kg/infusion) were needed to maintain the breakpoint under PR schedule of reinforcement. In conclusion, these results suggest that inflammatory pain can alter the motivational properties of heroin by affecting MORs sensitivity. Therefore, these data suggest that inflammatory pain induces changes in the VTA-NAc pathway which in turn may facilitate opioid dose escalation in order to maintain the rewarding properties of the drug.

P 21.

**IN VIVO ACTIVATION OF SK CHANNELS REDUCES THE DOSE OF NMDA RECEPTOR ANTAGONIST NEEDED TO PRODUCE ANTINOCICEPTION**

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N-methyl-D-aspartate receptor (NMDAR) antagonists have been shown to reduce mechanical hypersensitivity in animal models of chronic inflammatory pain. In the majority of clinical studies NMDAR antagonists have shown efficacy in the treatment of chronic pain, however their clinical use has been limited by a very narrow therapeutic window and the inability to adequately manage the adverse effects that can occur at therapeutic doses. Small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels (SK) have been shown to modulate NMDAR activity in the brain. Here, we demonstrated that *in vivo* activation of SK channels in the spinal cord can alleviate mechanical hypersensitivity in a rat model of inflammatory pain. Intrathecal (i.t.) administration of the SK channel activator, 6,7-Dichloro-1H-indole-2,3-dione 3-oxime (NS309), dose-dependently reverses complete Freund adjuvant (CFA)-induced mechanical hypersensitivity, as well as the reduced apamin-sensitive SK channel-mediated currents recorded from superficial laminae of the spinal cord. Postsynaptic expression of the SK channel subunit, SK3, is significantly reduced in the dorsal horn (DH) after CFA. Double-immunostaining for SK3 subunit and NMDAR subunit, NR1, shows co-expression of these proteins, indicating that SK3-containing channels and NMDAR could be localized in close proximity compatible with functional interaction. Moreover, we demonstrate that i.t. co-administration of sub-effective dose of NS309 with an NMDAR antagonist, reduces the dose of NMDAR antagonist, DL-2-Amino-5-phosphonopentanoic acid (DL-AP5), required to produce antinociceptive effects in the CFA model. This reduction could attenuate the unwanted side effects associated with NMDAR antagonists, giving this combination potential clinical implication.

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## P 22.

EXPLORING NOCICEPTION IN *Octopus vulgaris*

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Directive 2010/63/EU includes cephalopods as the sole representatives among invertebrate animals in the list of species regulated for experiments. This is based on the precautionary assumption that cephalopods are species capable of perceiving pain, distress and lasting harm. The Working Party of the Institute of Medical Ethics (WPIME) identified the criteria that might provide evidence for pain experience in animals: *i.* the possession of appropriate nervous receptors (nociceptors) and of pathways that connect those receptors with the central nervous system; *ii.* the involvement of brain centres that could have the capacity to generate pain sensation; *iii.* the presence of receptors for endogenous and/or exogenous opioids in the nervous system; *iv.* the existence of behavioural responses to noxious stimuli and the evidence of learning in relation to these stimuli.

The existence of a “pain” circuitry in octopus and their allies has been questioned for decades. Nevertheless, the inclusion of cephalopods in article 1 of Directive 2010/63 is based on evidences supporting only two of the criteria stated by WPIME, i.e. the presence of a suitable nervous system, and the presence of a behavioural response to a noxious stimulation.

Our aim is to investigate some of the WPIME criteria, starting from exploring the presence of nociceptors in the peripheral nervous system of the cephalopod mollusc *Octopus vulgaris*.

We focus our attention on: Substance P and CGRP (Calcitonin Gene Related Peptide), two neurotransmitters involved in nociceptive signals transduction and conduction; the Vanilloid Receptor (TRPV1), a transmembrane ionotropic receptor which encodes noxious chemical and mechanical stimuli; isolectin IB4, from *Griffonia simplicifolia*, a commonly used marker for non-peptidergic nociceptors.

We present our results following immunohistochemical techniques mapping on the suckers and the arm of the animal the presence of ‘noci’-receptors positive to neuropeptides as Substance P and CGRP, receptors as TRPV1, or binding with isolectin IB4.

**NEURODEGENERATIVE & NEUROPSYCHIATRIC DISORDERS**

**P 23.****ATTENTION DEFICIT HYPERACTIVITY DISORDER AMONG SCHOOL CHILDREN OF KENITRA CITY (NORTH WEST OF MOROCCO)**

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Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood brain disorders who can continue through adolescence and adulthood. A previous study of our research unit, conducted in the Gharb-Chrarda-Bni Hssen, has shown that 33% of rural school children suffer from this disorder (Azzaoui et al., 2011).

The aim of this study is to evaluate the Attention Deficit Hyperactivity Disorder in urban schooled children living in Kenitra city (Capital town of the Gharb-Chrarda-Bni Hssen region, North West of Morocco).

The study was realized among 239 school children, aged between 6 to 16 years, and living in Kenitra city. Two questionnaires are used for evaluating the ADHD status of children; Conners questionnaires and The Diagnostic and Statistical Manual of Mental Disorders (DSM IV) questionnaire.

Using the DSM IV and the Conners questionnaire (Parents' version), 33.8% and 30.1% of children suffer from ADHD, respectively. However, using the Conners questionnaire (teacher's version) and Conners short version, 21.8% and 18.4% of children suffer from ADHD, respectively.

This primary study reveals, regardless of the test used to evaluate the ADHD, that the prevalence of this disorder is important. Deeper studies that predict the major cause of this disorder and its remediation are undergoing.

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**P 24.****FORETHOUGHT IN YOUTH WITH ATTENTION DEFICIT/HYPERACTIVITY DISORDER: AN FMRI STUDY OF SEX-SPECIFIC DIFFERENCES**

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Majority of studies investigating neurocognitive processing in attention deficit/hyperactivity disorder (ADHD) have been conducted on male participants; very few studies have looked specifically at females or examined sex differences in ADHD. Among various cognitive anomalies observed in ADHD, deficit in forethought seems particularly important as children with ADHD often fail to adequately use previous information in order to prepare for upcoming responses. Thus, the main goal of the present study was to assess sex-specific differences in behavioral and neural correlates of forethought in youth with ADHD relative to typically developing (TD) individuals.

21 TD youth and 24 youth with ADHD were asked, in a series of trials, to judge whether two pictures told a congruent story or not. Reaction time, performance accuracy, and cerebral activations using functional magnetic resonance imaging (fMRI) were recorded during all sessions.

Although no differences in performance were found between males and females when controlling for age, significant sex-specific differences in the patterns of cerebral activations were apparent. Thus, relative to the same-sex TD participants, boys with ADHD had extensive bilateral frontal and parietal hypo-activations, while girls with ADHD demonstrated more scattered and circumscribed hypo-activations in the right temporal, parietal, frontal and cerebellar regions.

Present results revealed that both boys and girls with ADHD show diminished cerebral activations during performance of a forethought task. Most regions of under-activations observed in the study were consistent with previous reports in individuals with ADHD during performance of executive tasks. Nevertheless, the pattern of deficits was different in boys and girls, suggesting a different neurocognitive strategy utilized by the two sexes, as well as emphasizing the importance of including both males and females in the cognitive and neuroimaging investigations of ADHD.

## P 25.

## ASSOCIATION OF THE TNF $\alpha$ , TNFB AND TNFR2 GENETIC POLYMORPHISMS WITH PARANOID SCHIZOPHRENIA

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Schizophrenia is a severe mental disorder characterized by a complex symptomatology, affecting most aspects of cognition, emotion and behavior. This disease affects 1% of the population. It was postulated that "schizophrenia is probably neither a single disease entity and nor is it a circumscribed syndrome, it is likely to be a conglomeration of phenotypically similar disease entities and syndromes". The determinism of this disease is multifactorial and according to the neurodevelopmental hypothesis, perinatal inflammation associated with a genetic predisposition could alter brain development responsible for its failure in adults. Clinical Diagnosis proves neuro-inflammation signs in schizophrenic's brain. In addition, the pro-inflammatory cytokines (IFN $\alpha$ , TNF $\beta$ , IL1, IL6) modulate the expression of IDO enzymes involved in the catabolism of tryptophan and serotonin metabolism and glutamate, mechanisms that control the behavior. Based on these data, we hypothesized that functional genetic variants of TNF-TNFR signaling may predispose to the development of schizophrenia.

To test our hypothesis, we conducted a case-control study to find an association between schizophrenia and several selected polymorphisms (-238G/A TNF $\alpha$ , +252A/G TNF $\beta$  and 676T/G TNFR2 genes). This study was conducted by the RFLP-PCR genotyping technique on a population composed of 200 patients and 200 controls, all recruited in Monastir University Hospital, Tunisia. Our results show that the frequency of mutated alleles of the studied polymorphisms was higher in patients than in controls with a statistically significant difference. When we divided patients according to the clinical classification, our results show that the paranoid type is very strongly associated with all these polymorphisms (p=0.0029, p=0.00003, p=0.012 respectively), comparatively with the undifferentiated and disorganized types of schizophrenia.

In conclusion, given the important role of TNF  $\alpha/\beta$  cytokine signaling in the immune and inflammatory response and the susceptibility of these polymorphisms to paranoid schizophrenia showed by our results, we suggest that these functional polymorphisms may contribute to the development of this disease by the dysfunction of the TNF  $\alpha/\beta$  system. Using a larger sample size and extend the study to other populations could be of particular importance to valid these findings.

## P 26.

**ASSOCIATION OF THE IFN $\gamma$  AND IFNGR1 GENETIC POLYMORPHISMS WITH  
PARANOID SCHIZOPHRENIA**

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Schizophrenia is a severe mental disorder characterized by a deficiency of all functions of cognition, which affects 1% of the population. The determinism of this disease is multifactorial and according to the neurodevelopmental hypothesis, perinatal inflammation associated with a genetic predisposition could alter brain development responsible for its failure in adults.

Since the pro-inflammatory cytokines (IFN $\gamma$ , TNF $\alpha$ , IL6, IL1) modulate the expression ofIDO enzymes involved in the catabolism of tryptophan and serotonin metabolism and glutamate, mechanisms that control the behavior, we hypothesized that functional genetic variants of the signaling of IFN $\gamma$ -IFNGR may predispose to the development of schizophrenia.

To test our hypothesis, we conducted a case-control study to find an association between schizophrenia and several selected polymorphisms (874A/T of IFN $\gamma$  and Gln64Arg of IFN $\gamma$ R2). This study was conducted by the RFLP-PCR genotyping technique on a population composed of 220 patients and 150 controls for polymorphism 874A/T and 205 patients and 100 controls for polymorphism Gln64Arg, all recruited in Monastir University Hospital, Tunisia. Our results show that the frequency of mutated alleles of both polymorphisms was higher in patients than in controls with a statistically significant difference. When we divided patients according to the clinical classification, our results show that these polymorphisms are very strongly associated with the paranoid type ( $p=0.000006$  for IFN $\gamma$  and  $p=0.005$  for IFN $\gamma$ R2), but not with the undifferentiated and disorganized types of schizophrenia.

In conclusion, our results show an association between polymorphisms (874A/T of IFN $\gamma$  and Gln64Arg of IFN $\gamma$ R2) and schizophrenia, suggesting that these polymorphisms may play an important role in the development of these diseases. Knowing that the polymorphism 874A/T has an influence on the expression of IFN gamma gene and the polymorphism Gln64Arg IFN $\gamma$ R2 gene could alter receptor function of IFN $\gamma$ , it would be interesting to clarify the role of these polymorphisms in the pathogenesis of schizophrenia.

## P 27.

**VGF PEPTIDES: A POSSIBLE ROLE IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)  
NEURODEGENERATIVE MECHANISMS**

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The neuronal VGF precursor yields a number of cleaved peptides, some of which proved active on synaptic strengthening, or against neuronal apoptosis. The 4.8kD VGF fragment was reduced in CSfluid from ALS patients, and decreased in both CSfluid and plasma from G93A-SOD1 mice in parallel with progression of muscle weakness. In spinal motoneurons, adenoviral expression of VGF attenuated excitotoxic injury. We assessed VGF peptide profiles in G93A-SOD1 mice (ALS model: pre-symptomatic, early and advanced stages, vs. wild-type / WT) and in plasma from ALS patients (vs. age matched controls, N=50+50, 45-70 yrs), while the motoneuronal NSC-34 cell line was used to address possible bioactivity of certain VGF peptide/s (VGFp). Antisera were raised against short sequences at the VGF precursor C-terminus, or at either end of known cleaved VGFp, used for immunohistochemistry and ELISA.

In WT mice, lumbar and cervical motoneurons showed selective profiles, with TLQP, NERP-2, and 4-8kDa VGF peptides mostly present in perikaria, with distinctly decrease in the advanced stage of mutant SOD1 mice. VGF C-terminus peptides, instead, also found in many axons and terminals, were reduced early from presymptomatic stage animals.

In ELISA, plasma TLQP immunoreactivity showed significant reduction in mutant SOD1 mice (20% of WT mice,  $p < 0.05$ ), as well as in ALS patients (12% of controls,  $p < 0.05$ ). Conversely the VGF C-terminus containing peptides were reduced in advanced stage SOD1 mice (35% of WT mice,  $p < 0.05$ ). Preliminary data revealed that, upon treatment with the VGF peptide TLQP-21 (1nmol/ml), the Na-arsenite stressed NSC-34 neuronal cells seemed to show increased viability (113%).

In conclusion, VGFp appeared to be involved in the ALS neurodegenerative mechanisms in human, as well as in mouse models with a possibly trophic effect for TLQP-21 only, that needs future investigation.

## P 28.

**THE EFFECT OF CO-TREATMENT WITH RISPERIDONE AND ANTIDEPRESSANTS IN AN ANIMAL MODEL OF THE NEGATIVE SYMPTOMS OF SCHIZOPHRENIA IN RATS**

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Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning and affects approximately 1% of the world's population. It is known that in contrast to pharmacotherapy with typical antipsychotics, the atypical antipsychotic agents, e.g., risperidone alleviate not only the positive symptoms of schizophrenia but also the negative ones. Moreover, several clinical reports have suggested that the antidepressant mirtazapine- or escitalopram-induced augmentation of risperidone activity may efficiently improve the treatment of negative and some cognitive symptoms of schizophrenia.

Thus, in the present study, we aimed to evaluate the effect of mirtazapine or escitalopram and risperidone, given separately or jointly, on the MK-801 (a NMDA receptor antagonist)-induced deficits in the social interaction test. The experiments were conducted in a black polyvinyl chloride box (57×67×30 cm). Male Wistar rats (185-200 g) were selected from separate housing cages to make a pair for the study. The social interaction was measured 4 h after the subcutaneous administration of MK-801 (0.1 mg/kg), and 60 or 30 min after administration of antidepressant and risperidone, respectively. Each group consisted of six pairs.

The present results showed that MK-801 (0.1 mg/kg) induced deficits in both parameters studied, the number of episodes and the time of interactions. Risperidone at a higher dose (0.1 mg/kg) reversed that effect. Co-treatment with an ineffective dose of risperidone (0.01 mg/kg) and mirtazapine (2.5 or 5 mg/kg) or escitalopram at a dose of 5 mg/kg only (but not 2.5 and 10 mg/kg) abolished the deficits evoked by MK-801.

The obtained results suggest that especially mirtazapine, and to a smaller degree escitalopram may enhance the antipsychotic-like effect of risperidone in the animal test modeling some negative symptoms of schizophrenia. Further studies are necessary to elucidate its mechanism of action. This study was financially supported by grant NCS 2013/09/N/NZ7/02143.

## P 29.

**INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN A  
NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA**

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Accumulating evidence suggests that a neurodevelopmental dysfunction could be one of the causes of schizophrenia (SCZ). Furthermore, a variety of animal and human studies found a dysregulation of the endocannabinoid system (ECS) in psychosis. In the present study, we aimed to investigate the potential effects of prenatal administration of the mitotoxin methylazoxymethanol acetate (MAM) on neurophenotypic presentations using a battery of behavioral tests at different postnatal ages. We also measured the brain expression of endocannabinoid receptors and metabolic enzymes (such as FAAH and MAGL) and the levels of the endocannabinoids anandamide and 2-AG. Timed-pregnant Sprague Dawley rats were treated with MAM (22 mg/kg) or vehicle (VHC) intraperitoneally on gestational day 17 (GD17). At birth, neonatal reflexes had a delayed onset (i.e. percent of appearance) in prenatally MAM-exposed rats, as compared to the control group ( $P < 0.05$ ;  $P < 0.01$ ;  $P < 0.001$ ). At adolescent age, prenatally MAM exposed rats engaged in less social behavior as suggested by the reduced time of interaction in the social interaction test ( $P < 0.05$ ). In the novel object recognition test prenatally MAM-exposed rats showed an impaired cognitive performance, as described by the decreased discrimination index ( $P < 0.001$ ). By contrast, spatial recognition memory was not affected by prenatally MAM-exposure since no difference between the two groups of rats (MAM vs VHC) was found in the Y-Maze test. Interestingly, the behavioral alterations correlated with both decreased expression of FAAH and MAGL ( $P < 0.05$ ) and with enhancement of anandamide and 2-AG levels ( $P < 0.05$ ) in prenatally MAM-exposed rats. These results suggest that behavioral abnormalities resulting from a MAM environmental challenge, which resemble a SCZ-like phenotype, could be due to abnormalities in the endocannabinoid tone.

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## P 30.

**EVALUATION OF THE CB1 RECEPTOR DENSITY IN AN ANIMAL MODEL OF BINGE EATING DISORDER**

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Binge eating disorder (BED) is characterized by repetitive episodes of uncontrolled and excessive food consumption of highly palatable foods within a short period of time (APA, 2013). The endocannabinoid system has been shown to contribute significantly to the homeostatic and hedonic valuation of food: in fact, cannabinoid type-1 receptors (CB1R) are expressed in several brain areas involved in the regulation of eating behavior and reward processes. Also, we have recently shown that pharmacological modulation of the endocannabinoid system, by CB1R agonists and antagonists, was able to modify the aberrant eating behavior present in a validated rat model of BED (Scherma et al, 2013). On the basis of this findings, this follow-up study was undertaken to evaluate the CB1 receptor alterations in selected feeding-related brain areas (prefrontal cortex, nucleus accumbens, amygdala, hypothalamus and hippocampus) as a consequence of dietary-induced binge eating, through quantitative autoradiographic-binding. Binge eating behavior was induced in female rats (Sprague Dawley) by giving them an optional source of high fat diet (margarine). Animals were divided in three groups: control (C), with no access to margarine; low restriction (LR), with 2 h margarine access 7 days a week; high restriction (HR), with 2 h margarine access 3 days a week, all groups had continuous access to chow and water. In HR animals, margarine intake becomes significantly greater than in animals with limited daily access to margarine (LR) and remains stable over prolonged periods of time. Our results revealed no differences in the CB1R distribution between the control animals and those who were subjected to a continuous access to margarine (LR) as well as those subjected to an intermittent access (HR), except for the cingulate cortex (CG1-CG3), in which we have seen a reduction of CB1R density in both LR and HR groups. This result is in agreement with previous findings (Timofeeva et al, 2009) on the effect of long-term consumption of palatable high-energy diet on reducing CB1R mRNA expression in the cingulate (Cg) prefrontal cortex. These data provide additional information in the comprehension of the role of the endocannabinoid system and its possible dysregulation in BED.

*American Psychiatric Association (2013). Washington, DC: American Psychiatric Press.*

*Scherma et al. (2013) British Journal of Pharmacology 169:820–833.*

*Timofeeva et al. (2009) J Neuroendocrinology 21(12):982-92.*

## P 31.

**THE STANDARDIZED ROOT EXTRACT OF *WITHANIA SOMNIFERA* DUNAL, A PLANT OF THE SARDINIAN AND MEDITERRANEAN FLORA, COUNTERACTS MOTOR IMPAIRMENT IN A *DROSOPHILA* MODEL OF PARKINSON'S DISEASE**

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The treatment of neurodegenerative diseases holds an enormous interest in medicine and the discovery of novel drug targets for long-sought therapeutics is a great challenge for researchers and clinicians. The common fruit fly *Drosophila melanogaster* (*Dm*) is a simple, yet powerful, animal species that contributed significantly to the development of neurobiology and the *Dm* mutants LRRK2 *loss-of-function* in the domain WD40 represent a very interesting tool to look into pathophysiology of Parkinson's disease. Accordingly, LRRK2 *Dm* have also the potential to contribute to reveal innovative therapeutic approaches to this neurodegenerative disease. *Withania somnifera* Dunal, a plant that also grows spontaneously in Mediterranean regions, is known in folk medicine for its anti-inflammatory and neuroregenerative properties. The aim of this study was to evaluate the neuroprotective effects of its standardized root methanolic extract (*Wse*) on the model of Parkinson's disease LRRK2 *Dm*. To this end flies were administered *Wse* through diet at different concentrations steps as adult ( $L^-/A^+$ ) and/or as larvae and adult ( $L^+/A^+$ ). While LRRK2 mutants have a reduced lifespan compared to wild type (WT) flies, the results show that the diet enriched of *Wse* at 1% administered only to adults LRRK2 ( $L^-/A^+$ ) significantly a) increased the lifespan; b) improved the locomotor activity and c) improved the muscle electrophysiological response to stimuli of mutants compared to wild type controls. However, the *Wse* administration, carried on chronically ( $L^+/A^+$ ) had overall toxic effects on mutants and WTs.

Based on our the results we can infer that the LRRK2 *loss-of-function* in the domain WD40 is a cause of Parkinsonism and that *Wse* can be usefully employed to counteract some deficits associated with the disease, but is required a careful assessment of the risks likely related to the impaired endosomal activity.

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## P 32.

**EVALUATION OF REWARDING PROPERTIES OF PRAMIPEXOLE IN A RAT MODEL OF PARKINSON'S DISEASE: ROLE OF GENETIC BACKGROUND**

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Pramipexole (PPX) is a dopamine (DA) D<sub>3</sub>/D<sub>2</sub> receptors agonist widely used as DA replacement therapy (DRT) in the treatment of Parkinson's disease (PD). However, its use has been related to the development of addictive-like behaviors associated to the DA dysregulation syndrome (DDS), particularly in a subpopulation of treated patients, characterized by impulsive-compulsive personality traits as well as previous histories of addiction.

In order to assess such correlation between PPX treatment, impulsive-compulsive personality, PD and the onset of DDS, we studied the effects of PPX on conditioned place preference (CPP) paradigm, after bilateral 6-OHDA lesion of DA striatal terminals, in three different strains of rats: the addiction prone Lewis (LEW) and the addiction resistant Fisher 344 (F344) inbred strains, and the Sprague Dawley (SD) outbred strain. Further, in order to test its rewarding properties PPX was directly infused in the nucleus accumbens shell (NAc), a DA mesolimbic region known to be involved in the rewarding effects of drugs of abuse, in healthy rats belonging to the above mentioned strains.

PPX (1 mg/kg s.c.) was able to induce a significant CPP in SD and LEW lesioned rats but not in F344 and control rats. When injected into the NAc shell, PPX (0,05 µg/0.5 µl) induced CPP in all rat strains, with differences in the persistence of its effect, which was stronger in Lewis compared to SD and F344 rats.

These results suggest that in lesioned rats, the parkinsonian state boosts the rewarding properties of systemic PPX, which do not seem entirely influenced by genetic background. This may be due to the fact that PPX exhibits intrinsic rewarding properties as demonstrated by the induction of CPP in all the strains studied, when the drug has been infused directly in the shell of the NAc.

**P 33.****PARKINSON'S DISEASE AND PATHOLOGICAL GAMBLING:  
ROLE OF NEUROSTEROIDS**

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Pathological gambling (PG) is an impulse control disorder consisting in persistent and maladaptive gambling behavior. In males, PG has been associated with high stress responsiveness and depression. Emerging evidence suggests that the neurotransmitter dopamine is involved in the pathophysiology of PG. In particular, the D2/D3 receptor agonist pramipexole, a therapeutic agent used for the treatment of Parkinson's disease, can cause PG. Our group has recently pursued the hypothesis that neurosteroids may play a key role in the pathogenesis of PG. Indeed, chronic stress exposure affects neurosteroid levels, as well as the expression and function of 5-alpha reductase (5AR), a key enzyme for neurosteroid synthesis. Furthermore, we previously showed that the 5AR inhibitor finasteride (FIN) elicits antidopaminergic responses in animal models. These premises led us to hypothesize that PG is supported by imbalances in the cross-regulation of neurosteroids and dopamine in the brain, and that 5AR inhibitors may serve as therapeutic tools for PG. To verify this possibility, we investigated the changes in PG severity following prolonged FIN treatment in PD patients treated with pramipexole. Consistently with our hypothesis, FIN significantly reduced pathological gambling in patients with Parkinson disease. To study the mechanisms underlying these effects, we trained male rats in the probability discounting task, a paradigm aimed at assessing impulsive choice behavior. After acquisition of the operant task, rats were treated with pramipexole, which elicited a significant increase in the selection of the risky choice. Interestingly, finasteride reversed this effect and restored a level of risky-choice selection equivalent to the baseline. In addition, finasteride did not induce PD-like side effects. These preliminary data strongly support the employment of neurosteroid-based drug as a novel avenue for the treatment of PG. Future studies will help us elucidate the neurochemical mechanisms underpinning the therapeutic effects of finasteride.

## P 34.

**L-DOPA CHRONIC TREATMENT INCREASES REACTIVE MICROGLIA AND TNF-ALPHA PRODUCTION IN THE 6-OHDA LESIONED STRIATUM.**

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L-DOPA is the gold standard medication for Parkinson disease (PD). However, the long-term use of this therapy is associated with the onset of movement alterations, including dyskinesia. Neuroinflammation is a main component of PD neuropathology, where microgliosis and altered microglial functions contribute to neurodegeneration by the release of neurotoxic species such as pro-inflammatory cytokines. Recent evidence suggests that neuroinflammation may also be implicated in the development of L-DOPA-induced dyskinesia. Aim of the present study is to investigate a role of neuroinflammation in the development of dyskinesia in the 6-OHDA rat model of PD. To address this issue emiparkinsonian rats were chronically treated with L-DOPA (6 mg/kg/day s.c. for 15 days) or vehicle. Moreover, as inflammatory insult, one group of rats received a single peripheral dose of Lipopolysaccharide (LPS, 2 mg/kg i.p.) 24 hrs before L-DOPA chronic treatment. Abnormal involuntary movements (AIMs) were evaluated on alternate days during the L-DOPA treatment as an index of dyskinetic responses, quantified as the time spent performing limb, axial, locomotor AIM. Rats preadministered with LPS before L-DOPA treatment showed a faster onset of limb and axial AIMs, and spent more time performing AIMs in each test, as compared to L-DOPA alone. Immunoreactivity (IR) for the microglia marker OX-42 was analyzed to evaluate microglia reactivity in the dopamine-depleted dorsal striatum. Moreover, the proinflammatory cytokine TNF- $\alpha$ -IR was evaluated both in microglia and neurons. L-DOPA-treated rats displayed an increased OX-42-IR as compared to vehicle group, and microglia displayed an activated morphology. Moreover, levels of TNF- $\alpha$  colocalization were increased in both microglia and neurons of L-DOPA-treated rats. These results suggest the L-DOPA-induced AIMs are exacerbated by an inflammatory insult. Moreover, the chronic administration of L-DOPA induces a neuroinflammatory response in the dorsal striatum, which may contribute to the development of AIMs.

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## P 35.

**INHIBITION OF 5ALPHA REDUCTASE COUNTERS LEVODOPA-INDUCED  
DYSKINESIA IN 6-OHDA-LESIONED RATS**

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Evidence shows that neurosteroids regulate dopamine (DA) neurotransmission. Accordingly, we previously reported that inhibition of 5 $\alpha$ -reductase (5AR), the rate-limiting enzyme for neurosteroids synthesis, elicits anti-DAergic actions. In particular, the 5AR inhibitor finasteride (FIN) restored the PPI deficits, the hyperactivity and the stereotyped responses induced by dopaminomimetic agents. Interestingly, all these behavioral effects were mediated by post-synaptic DAergic regulations in the striatum and were not associated to extrapyramidal symptoms. L-DOPA-induced dyskinesia (LID) is the most debilitating motor complication associated with chronic dopamine replacement therapy in Parkinson's disease (PD). As LID is closely related to dysregulation of DA signaling in the striatum, the aim of this study was to investigate the ability of FIN to reduce established L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats. Male and female lesioned rats were subjected to a chronic L-DOPA administration until a stable expressions of dyskinesia was achieved. Based on AIMs score, rats were acutely treated with L-DOPA with or without FIN. In addition, in order to further evaluate the impact of FIN on DA receptor supersensitivity in the striatum, the impact of FIN was also evaluated on dyskinesia induced by the D<sub>1</sub>/D<sub>2</sub> receptors agonist apomorphine. Results indicated that FIN dose-dependently reduced the abnormal involuntary movement scale (as index of LID) in both male and female 6-OHDA-lesioned rats. Despite the key therapeutic effects of FIN in humans rely on the conversion of testosterone in DHT, female rats appear to be more susceptible to FIN actions on LID. Finally, FIN was also able to dampen the apomorphine-mediated dyskinesia in male rats. To our knowledge, this is the first study that highlights a possible role of 5AR and its related neurosteroids in the pathophysiology of LID, and suggests FIN as a promising tool for the treatment of LID.

**P 36.****CHARACTERIZATION OF GAIT DISTURBANCES IN EXPERIMENTAL HUNTINGTON'S DISEASE: A POSSIBLE RELATION WITH CHOREA?**

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by an expanded CAG repeat. The clinical features are progressive motor dysfunction, cognitive deterioration and psychiatric disturbances. Unpredictable choreic movements, among the most characteristic hallmarks, may contribute to gait disturbances and loss of balance in HD patients. Transgenic rat model of HD (tgHD) presents typical neuropathological, neurophysiological, and behavioural features resembling the human condition. This rat model is the only described experimental model of HD that exhibits choreic movements.

In this study, we investigated the gait abnormalities and chorea-like movements in 9 months old tgHD rats. We used the Catwalk, with emphasis on static and dynamic gait parameters to test this hypothesis that choreic movements have an influence on dynamic measures of gait in HD.

As we expected, the dynamic parameters seem to be more affected at this age than static parameters in tgHD rats. Our results showed that the number of steps, step cycles, and swing speed of the paws were increased in tgHD rat in compare to wild-type controls.

Our study demonstrates that tgHD rats present clear abnormalities in the dynamic parameters of gait at early symptomatic phase of the disease. Such deficits appear to be related to the end of the hyperkinetic phase of this model, and the appearance of choreic movements.

## P 37.

**EARLY CHANGE OF THE DOPAMINERGIC SYSTEM IN AN EXPERIMENTAL MODEL OF HUNTINGTON'S DISEASE**

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Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder that has onset usually between 35 and 50 years of age. HD is characterized by motor disability, and psychiatric changes such as impulsivity and depression. The mechanisms underlying these symptoms are not well known. For the motor disorder and in particular for the chorea, however, a link with the dopaminergic system has been suggested by human post-mortem studies and clinical therapy studies. Early post-mortem studies showed that striatal dopamine levels, both in the dorsal and ventral striatum were significantly higher in HD patients compared to controls. In addition, clinical studies have shown that the chorea can be treated with dopamine antagonist or dopamine depleting drugs. Moreover, a recent short-term clinical trial demonstrated that dopamine antagonist tetrabenazine (TBZ) efficiently reduced chorea in HD patients compared with placebo group. Despite multiple experimental and clinical evidence suggesting a potential importance of dopaminergic signaling in HD, the mechanistic basis for these observations is poorly understood.

The substantia nigra pars compacta (SNc), the ventral tegmental area (VTA) and the dorsal raphe nuclei (DRN) are the three main sources of striatal dopamine. In this study, using antibodies raised against tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of dopamine, the number of TH-positive cells in the SNc, VTA and DRN was analysed, in an experimental model of HD with chorea, the transgenic rat model of HD (tgHD) at different time points: 14 days old embryos, 14 days and three months old animals. In addition, electrophysiological single-unit recordings were performed in these structures on three months old animals to establish changes in the dopaminergic system responsible for the onset of HD symptoms.

## P 38.

**PHARMACOLOGICAL EVALUATION OF NOVEL MULTI-TARGET COMPOUNDS DERIVED FROM DONEPEZIL+PROPARGYLAMINE+8-HYDROXYQUINOLINE (DPH) HYBRIDS FOR THE POTENTIAL USE IN ALZHEIMER'S DISEASE**

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Alzheimer's Disease (AD) is a multifactorial age-related neurological disorder characterized by neuropathological hallmarks that irreversibly contribute to a progressive death of neurons such as fibrillar amyloid-beta ( $A\beta$ ) senile plaques and neurofibrillary tangles of hyperphosphorylated tau protein in addition to oxidative stress, inflammation, mitochondrial alteration or cholinergic dysfunction. The current pharmacological therapy of AD is regrettably clung to the use of single-target drugs, mainly focused on the cholinergic enhancement, possessing a lack of efficacy against the multifaceted nature of this neurological disorder. In order to confront this appalling condition, the development of Multi-Target Directed Ligands (MTDL) has lately emerged as a novel rational approach. We have recently designed and reported a new series of MTLs (DPH) derived from the hybridization of the benzylpiperidine, propargylamine and 8-hydroxyquinoline moieties for the use in AD. These components were initially determined as dual cholinesterase (AChE and BuChE) and monoamine oxidase (MAO A and MAO B) inhibitors with equimolar  $IC_{50}$  values. Among all the tested derivatives, both DPH4 and DPH6 exhibited the most promising inhibitory profiles and strong biometal-chelating, anti-amyloid, anti-inflammatory and antioxidant properties were subsequently detected. DPH4 revealed moderate inhibition of both  $A\beta_{1-42}$  self-aggregation and AChE-mediated  $A\beta_{1-42}$  aggregation *in vitro* besides a partial neuroprotection against neurotoxicity induced by  $A\beta_{1-42}$  and  $A\beta_{1-42}$  in presence of  $Cu^{2+}$  in undifferentiated PC12 cells. Antioxidant activity was also observed in some *in vitro* assays as well as in SH-SY5Y cells treated with DPH4 against  $H_2O_2$ -induced toxicity exhibiting a significant depletion on the ROS levels. DPH4 also showed anti-inflammatory properties by reducing nitrite levels when inflammation was induced by LPS+IFN $\gamma$  or  $A\beta_{1-42}$  in microglia BV-2 cells. Additionally, DPH6 produced less toxicity than donepezil at high concentrations in an *in vitro* model of hepatotoxicity in human liver HepG2 cells. In *in vivo* experiments performed in healthy adult mice with experimentally-induced amnesia, DPH6 exhibited a significant decrease in scopolamine-induced learning deficits in a passive avoidance task.

We deeply believe that both the well-balanced inhibition profiles and the other remarkable pharmacological properties of DPH derivatives, as novel multi-target compounds, deserve further investigation for their potential therapeutic use in AD.

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**P 39.****PALMITOYLETHANOLAMIDE CONTROLS ASTROCYTE ACTIVATION AND THE AMYLOIDOGENIC PATHWAY IN AN *IN VIVO* MODEL OF ALZHEIMER'S DISEASE**

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Alzheimer's disease (AD) is one of the most diffuse type of dementia characterized not only by senile plaques and neurofibrillary tangles, but even by a glial activation and a chronic inflammatory status called "reactive gliosis". Nowadays it is well recognized that this phenomenon participates actively to neurodegeneration.

Several markers can be used to characterize reactive gliosis; among them S100B represents one of the most important. This neurotrophin becomes toxic during brain injuries, ischemia and several neurological disorders, including AD. Indeed, it has been established that S100B plays a pivotal role in the amyloidogenic pathway of  $\beta$ -amyloid ( $A\beta$ ). In fact this molecule is able to increase the  $A\beta$  precursor protein and the  $\beta$ -secretase activity, by rising the  $A\beta$  levels which, in turn, further activate astrocytes. This self-sustaining loop drives the progression of pathology.

Pharmacological manipulation of this detrimental loop could be useful to develop new therapies.

In this context, palmitoylethanolamide (PEA), an endogenous lipid compound, exerts many pharmacological activities, especially a marked anti-inflammatory action in peripheral inflammatory models. For this reason we decided to test PEA activity in an *in vivo* model of AD. Obtained results demonstrated that PEA is able to counteract astrocyte activation as well as reduce pro-amyloidogenic pathway through a mechanism partially peroxisome proliferator-activated receptor- $\alpha$ -depending.

## P 40.

THE EFFECT OF ASS234 ON TAU PHOSPHORYLATION *IN VITRO*

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Alzheimer's disease (AD) is the most common cause of dementia in the elderly (affecting over 30 million people worldwide). AD is characterized by the presence in the brain of plaques composed of amyloid beta peptide and of neurofibrillary tangles made of hyperphosphorylated protein tau. In AD, the balance of tau kinase and phosphatase activity is shifted, creating a highly phosphorylated species of tau. This raises the fraction of unbound tau, which is no longer attached to microtubules (MT), allowing for monomeric hyperphosphorylated tau to bind to one another to produce oligomers. Fusion of oligomers leads to the formation of paired helical filaments, the primary constituent of neurofibrillary tangles (NFT). A number of neuroprotective strategies are being suggested to counter tau hyperphosphorylation and to reduce tau aggregates: 1) MT-stabilizing agents, 2) modulation of tau phosphorylation, 3) tau aggregation inhibitors, 4) targeting extracellular tau oligomers, 5) targeting tau clearance, and 6) targeting tau proteolysis. A multipotent, blood-brain barrier-permeable compound ASS234, has already been shown to inhibit A $\beta$  aggregation, possesses antioxidant properties, and protects from A $\beta$ -induced apoptosis *in vitro* (Bolea et al., Curr. Alzheimer Res., 2013), but its properties in regard to tau phosphorylation and aggregation have not been yet studied. The goal of this study was to assess the influence of ASS234 on tau phosphorylation in SH-SY5Y cells. Preliminary results of the study will be presented and discussed during the poster session.

Bolea I., Gella A., Monjas L., Pérez C., Rodríguez-Franco M.I., Marco-Contelles J., et al. (2013). Multipotent, permeable drug ASS234 inhibits A $\beta$  aggregation, possesses antioxidant properties and protects from A $\beta$ -induced apoptosis *in vitro*. *Current Alzheimer Research*. 10(8), 797-808.

## DRUG SEARCH FOR APOPTOSIS INDUCING FACTOR MODULATION IN CANCER AND NEURODEGENERATIVE DISORDERS

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Apoptosis Inducing Factor (AIF) is a phylogenetically preserved mitochondrial FAD flavoenzyme present in all primary kingdoms and involved in mitochondrial energy metabolism and caspase-independent cell death. In mammals, anomalous behaviors of this protein are related to pathological disorders such as cancer and degenerative diseases caused by a defect or an excess of apoptosis. Neurons are the cells suffering larger effects upon AIF deficiency, probably due to their high dependency, from the energetic point of view, on the mitochondrial energy metabolism. AIF deficiency in these cells leads to neurodegeneration and blindness (1,2). So far, four AIF mutations in humans have been related to mitochondrial disorders (3-6), and with the behavior of the protein as a reductase. The recent interest in the design of new therapies to modulate caspase-independent apoptosis pathways makes AIF a potential pharmacological target to treat pathological disorders related with AIF-dependent mitochondriopathies. One of the possibilities for modulating the pro-apoptotic function might be regulating its reductase activity.

In this study, we have screened more than 10000 compounds with high chemical and pharmacological diversity, as well as with known bioavailability and safety in humans. We have performed a High Throughput Screening method based on thermal denaturation. The obtained unfolding curves were analyzed to determine the midpoint unfolding temperature of each compound, using two different approaches designed to complement each other and to avoid false negatives. This method allowed identifying eleven potential binders of human AIF. We have also characterized their interaction with AIF and their toxicity on cells. Further studies are needed with the hit compounds to determine its pharmacological potential on the treatment of diseases caused by a dysregulation of AIF activities.

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## P 42.

**THALAMUS PATHOLOGY IN MULTIPLE SCLEROSIS: FROM BIOLOGY TO CLINICAL APPLICATION**Tim Clarner, Kipp Markus*Institute of Neuroanatomy, RWTH Aachen University, 52074 Aachen, Germany*

There is a broad consensus that Multiple Sclerosis (MS) represents more than an inflammatory disease: it harbors several characteristic aspects of a classical neurodegenerative disorder, i.e. damage to axons, synapses and nerve cell bodies. While the clinician is equipped with appropriate tools to prevent immune-cell driven relapses, effective therapeutic options to ameliorate the simultaneously progressing neurodegeneration are still missing. Furthermore, while several sophisticated paraclinical methods exist to monitor the inflammatory-driven aspects of the disease, techniques to monitor progression of early neurodegeneration are still in their infancy and have not been convincingly validated. Results of several clinical studies suggest that the thalamus displays early changes during the MS disease course and correlates with distinct clinical deficits, among motor impairment and cognitive deficits. It has, thus, been suggested that the thalamus, with its multiple reciprocal connections, is sensitive to pathological processes occurring in different brain regions, thus acting as a "barometer" for diffuse brain parenchymal damage in MS. In this project we elaborate whether or not the thalamus might be an ideal region of interest to monitor during clinical neuroprotective trials. To better understand the effects of widespread (neuro-) inflammation on thalamic pathology, we used two distinct MS animal models, i.e. cuprizone-induced toxic demyelination and experimental autoimmune encephalomyelitis (EAE). In the cuprizone model, specific subregions of the thalamus, namely the ventral posterolateral nucleus, the ventral posteromedial nucleus and the ventral anterior-lateral nucleus are demyelinated, paralleled by microgliosis and acute axonal damage. In contrast, thalamic pathology is less distinct in the EAE-model, despite severe inflammation in the cerebellum and spinal cord. Anterograde transneuronal degeneration occurs when a neuron is damaged and causes the degeneration of a postsynaptic neuron. Since the thalamus is not directly affected in the EAE model, but thalamic afferents/efferents are, we investigated whether neuronal cell death occurs in the thalamus due to axonal transection of efferent or afferent pathways. We are able to show that significant atrophy and neuronal loss occurs in such thalamic regions implicating that transneuronal degeneration is operant in the EAE model.

**P 43.****STRESS GRANULE DYNAMICS IN CULTURED FIBROBLASTS FROM ALS PATIENTS CARRYING A *TARDBP* MUTATION**

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Amyotrophic Lateral Sclerosis (ALS) is a late-onset neurodegenerative disease characterized by the selective loss of upper and lower motor neurons; most ALS cases are sporadic, and only 5-10% are familial. About 4% of familial cases, are due to mutations in *TARDBP*, the gene encoding TDP-43.

TDP-43 is an ubiquitous nuclear protein that regulates mRNA functions and metabolism. It is involved in assembly of stress granules (SGs), transient cytoplasmic aggregates containing non-translating messenger ribonucleoproteins, that rapidly form when cells are exposed to stress conditions.

We investigated SG dynamics in human cultured fibroblasts from ALS patients carrying *TARDBP*<sup>A382T</sup> mutation compare with fibroblasts from healthy subjects.

In order to induce SG formation, cells were treated with sodium arsenite (0,5 mM), for 30 and 60 min. SGs were identified by immunostaining for SG markers (TIA-1 and HuR). After treatments (both 30 and 60 min), we observed a significantly higher number of cells exhibiting SG formation in fibroblasts from healthy controls compare with those from ALS patients. Moreover, fibroblasts from healthy controls showed more SGs per cell compare with those from ALS patients while no differences were observed in SG size between groups. TDP-43 immunostaining was only observed into the nucleus of all the cells. We found that *TARDBP* mutation confers increased vulnerability to acute sodium arsenite exposure, as detected by MTT assay, with a significant higher cytotoxicity in fibroblasts from ALS patients compare with healthy controls. The involvement of TDP-43 in SG assembly was confirmed by silencing *TARDBP* gene in fibroblasts from healthy controls. In fact we observed that sodium arsenite was not able to induce SG formation in *TARDBP* silenced cells.

Our data suggests that the impairment of TDP-43 function may disrupts stress granule dynamics and that may contributes to neuronal vulnerability in ALS.

P 44.

**NEW MGLU RECEPTORS POSITIVE ALLOSTERIC MODULATORS WITH  
POTENTIAL ANXIOLYTIC ACTIVITY?**

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The metabotropic glutamate receptors play important neuromodulatory role throughout the brain. Intervention in glutamatergic neurotransmission through group III of mGlu receptors has been pursued for the treatment of many neurological and psychiatric disorders such as anxiety, schizophrenia, epilepsy, Parkinson disease, addiction. Thus, new mGlu receptors ligands, especially allosteric modulators with improved selectivity and reduced side effects, have become a very attractive target for therapeutic intervention. Since only a few ligands for the mGlu receptors type are known, there is a need for developing more potent and specific compounds that can be used in clinical trials.

Identification and pharmacodynamics characterization of novel chemical scaffolds possessing group III of mGlu receptors positive allosteric modulation activity (PAM).

The screening study and activity of potential PAM was determined using forskolin-induced cAMP production, in T-Rex-293 cell lines stably expressing mGlu receptors (HTRF cAMP detection kit, Cisbio). The potential anxiolytic and antidepressant-like activity of new PAMs was analyzed with modified stress-induced hyperthermia in singly housed mice and tail suspension test, respectively.

We have identified chemical scaffolds possessing mGlu receptors potential PAMs activity. Those compounds in the presence of L-Glutamine decreased the forskolin-induced cAMP production in T-Rex-293 mGlu receptors expressing cells. Moreover, we demonstrated anxiolytic, but not antidepressant-like activity of PAMs in behavioral tests.

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**P 45.****EFFECTS OF ANTIDEPRESSANTS AND KETAMINE ON CATECHOLAMINE TRANSMISSION IN THE RAT BED NUCLEUS OF STRIA TERMINALIS: A MICRODIALYSIS STUDY**

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Antidepressants include a relatively wide spectrum of drugs that, when administered acutely, increase monoamines extracellular concentration in many brain area. Among them, the bed nucleus of stria terminalis (BNST) that is considered a relay station in mediating the activation of stress response but also in the acquisition and expression of emotions, being richly innervated by monoamines may be involved in the mechanism of action of antidepressants. We previously showed that various antidepressants, independently from their mechanism of action, share the property of increasing dose dependently catecholamine transmission in the rat BNST. We further investigated the role of BNST in depression by evaluating the catecholamine transmission in this area by comparing the effect of acute administration of sub-anesthetic doses of ketamine with that of systemic and local administration of several antidepressants.

Ketamine, dose dependently (10-40 mg/Kg i.p.), increased norepinephrine and dopamine output by 186 and 176 respectively, whereas antidepressants (5-20 mg/Kg i.p.), increased norepinephrine and dopamine as follows: desipramine, 239 and 137; citalopram, 95 and 122; bupropion, 255 and 164 (values are maximal increase expressed in percent of basal). These results suggest that catecholamine transmission in the BNST may be part of a common downstream pathway that is involved in the action mechanism of antidepressants and ketamine, and that a dysfunction of neuronal transmission in this brain area may have a role in the aetiology of affective disorders.

## P 46.

**RETINAL SUBSENSITIVITY TO LIGHT ASSOCIATED TO SEASONAL PROFILE AND  
COGNITIVE IMPAIRMENT IN DEPRESSED PATIENTS**

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Seasonal variation sensitivity is a well-established feature of Depression and it can be in its greatest expression a causal factor of a specific clinical entity such as Seasonal Affective Disorder or contribute to the DSM Seasonal Specifier, with a wide interindividual distribution, clinically associated to temporary cognitive impairment and to sleep and circadian rhythms changes. Studies addressed several possible neuroendocrine and neurophysiology mechanisms, without definite conclusions. Following first exploring studies, we investigated the retinal response to light as a possible condition underlying seasonal and cognitive variation in depressed patients.

Abnormal retinal responses to light could be due to altered retinal signalling correlated by differences in the retinohypothalamic pathway, compressed of Melanopsin-containing intrinsically photosensitive retinal ganglion cell (ipRGCs), responding to light intrinsically in absence of rod and cone signalling. It can be investigated by pattern electroretinogram (PERG) to objectively measure the ability to perceive contrast. Previous findings of retinal subsensitivity support a role of melanopsin system in the modulation of light effects on several behaviours such as circadian rhythms, sleep, mood and acute alertness responses. The study aimed to evaluate the correlation between retinal photoreceptorial functionality and severity, seasonal sensitivity and cognitive impairment in Depression.

31 patients with Major Depressive Disorder, aged 30 - 65, males (12) and females (19), were assessed for clinical severity (HAM-D) and seasonal sensitivity (SPAQ) and underwent PERG and CANTAB Battery. PERG-based contrast gain significantly correlated with performance scores on several CANTAB tests assessing visual memory (PAL and DMS), executive functions (SWM) and attention (RVP). Both PERG data and CANTAB tests significantly correlated with HAM-D depression scores and SPAQ seasonal score.

The results support the hypothesis of retinal subsensitivity in depressed patients and underline the role of individual profile in liability to chronobiological changes and treatment indication, both in depression and other psychopathology and distress conditions.

**ENDOCANNABINOID SYSTEM MODULATION OF HIPPOCAMPAL SYNAPTIC PLASTICITY IN NORMAL AND EPILEPTIC CONDITIONS**

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Memory impairments are common among patients with epilepsy, particularly, temporal lobe epilepsy, where memory-related brain structures, such as hippocampus, are directly involved by seizure activity (1). The cognitive decline associated with epilepsy might be reversible when seizures are controlled. However, antiepileptic drugs might increase the risk of memory alterations (2). Cannabinoid compounds are anticonvulsant in several animal models of epilepsy (3,4) and have been reported to affect memory processes (5,6). To test whether the activation of the endocannabinoid system might be antiepileptic without showing detrimental effect on synaptic plasticity, male adult Sprague-Dawley rats were administered with the cannabinoid agonist WIN55,212-2 (WIN) and the fatty acid amide hydrolase inhibitor NF1245 and tested for synaptic plasticity in normal and epileptic conditions. We used two different tetanic high-frequency stimulation protocols to induce either the phenomenon of maximal dentate activation (MDA, 20 Hz), or long-term potentiation (LTP, 200 Hz) at the perforant path-dentate gyrus (PP-DG) synapses. To test the comorbid cognitive impairment we investigated changes in the excitability and synaptic plasticity of the DG before, during and after the MDA protocol. We found alterations of neuronal excitability and synaptic plasticity in rat subjected to MDA. Both WIN (2 mg/kg; i.p.) and NF1245 (10 mg/kg; i.p.) showed antiepileptic effects and prevented the alteration of neuronal excitability and short-term synaptic plasticity induced by repetitive seizures. However, WIN negatively affected LTP. Conversely, antiepileptic dose of NF1245 did not induce any alteration in LTP. These results suggest that endocannabinoids might be a suitable candidate for the treatment of epilepsy without having detrimental effects on memory.

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## P 48.

**CANNABIDIVARINE (CBDV) MODULATES GABA<sub>A</sub>-CURRENT RUN DOWN IN PHARMACORESISTANT EPILEPTIC PATIENTS**

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Mesial Temporal lobe epilepsy (mTLE) is defined as the occurrence of spontaneous seizures involving the limbic system and it is the most prevalent form of adult pharmacoresistant focal onset epilepsy. Recurrent seizures in epilepsy can be caused by a reduced efficacy of the GABAergic inhibitory system, and specifically mTLE has been associated with GABA receptor A (GABA<sub>A</sub>R) dysfunction. We have shown previously that the repetitive activation of GABA<sub>A</sub>R produces a use-dependent decrease (run-down) of the GABA-evoked currents ( $I_{GABA}$ ), which is markedly pronounced in the hippocampus and cortex of patients with drug resistant mTLE. Cannabidivarine (CBDV) was isolated from *Cannabis Sativa* for the first time in 1969 and recently some studies demonstrated a strong anticonvulsivant effect both in vitro and in vivo experiments using animal models of epilepsy. Experiments were thus designed to investigate whether CBDV could affect  $I_{GABA}$  characteristics in mTLE patients. GABA<sub>A</sub> receptors and their surrounding native membrane were microtransplanted into *Xenopus* oocytes from hippocampi of epileptic patients and the GABA-evoked currents were recorded using standard two-microelectrode voltage-clamp technique. We found that CBDV is able to reduce of about 35 % the  $I_{GABA}$  run-down. This effect had a slow kinetic and it was not found when CBDV has been co-applied with GABA. Noteworthy, no effect by CBDV has been found on AMPA evoked current ( $I_{AMPA}$ ) using the same epileptic hippocampal samples. These preliminary evidences suggest that the CBDV effect is specific for GABAergic transmission that it is strongly impaired in mTLE, suggesting new alternative therapeutical approaches for this and, possibly other forms, of pharmacoresistant epilepsies.

**P 49.****COGNITIVE CHANGES IN A RODENT MODEL FOR TEMPORAL LOBE EPILEPSY**

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Temporal lobe epilepsy (TLE) is a central nervous system disorder that is characterized by spontaneous, recurrent seizures and involves the limbic system. Patients often experience neuropsychological comorbidities, such as memory impairment, impairments of executive function, language, depression and anxiety. Hypotheses that may explain the relation between TLE and cognitive comorbidities are: 1. Patient characteristics, such as seizure frequency and severity, age of onset and EEG abnormalities determine the presence and severity of cognitive comorbidities, 2. Cognitive decline is caused by the use of antiepileptic drugs and 3. A shared molecular pathophysiological mechanism may underlie both, cognitive dysfunction and seizures.

In this study we assessed cognitive functioning in a rodent model of TLE. Briefly, in this model a longlasting status epilepticus (SE) was induced by electrical stimulation the ventral hippocampus. After a latent phase of 2 to 4 weeks, the animals developed spontaneous recurrent seizures. Before SE, immediately after, and in the spontaneous recurrent seizure phase, animals were behaviorally assessed for: working and spatial memory in the operant chamber, recognition memory with an object recognition task, anxiety in the open field and elevated zero maze and anhedonic-like behavior using a sucrose preference test.

We showed that animals develop spatial memory impairment after SE, which increase in severity in the chronic phase. This impairment was more pronounced in animals with spontaneous seizures. Furthermore, compared to pre- and post-SE levels, animals were less anxious in the chronic phase. Finally, in none of the 3 assessed stages did the animals show impaired recognition memory or anhedonic-like behavior.

The onset of spatial memory impairment before the occurrence of spontaneous recurrent seizures supports the idea of a shared pathophysiological mechanism. However, the increase in severity in the chronic phase might be related to seizures occurrence. Future molecular analyses are warranted to define the suggested shared pathophysiological mechanisms.

**P 50.****EFFECTS OF THE INTERACTION BETWEEN VULNERABILITY FACTORS AND MAO-A ON PATHOLOGICAL AGGRESSION**

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Gene-environment interactions have been shown to play a critical role in the development of aggression and other neuropsychiatric disorders. Several independent studies have highlighted that pathological aggression in males is often linked to the interaction of early-life abuse and/or neglect with allelic variants associated with low activity of monoamine oxidase (MAO) A, the key enzyme for the degradation of brain serotonin and norepinephrine. MAO-A knockout (KO) mice have been shown to display overt aggressive reactions and perseverative behaviors. To explore the neural underpinnings of the interaction between early stress and low MAO-A, we tested the impact of early maternal separation (MS) in MAO-A(Neo) mice, a newly generated line of MAO-A hypomorphic mutants with highly repetitive behaviors, but no spontaneous aggressive responses. While MS did not significantly affect the aggressive behavior in either MAO-A KO or wild-type (WT) mice, the same manipulation resulted in a robust enhancement of fighting responses in MAO-A(Neo) mice, to a level comparable with that of MAO-A KO counterparts. Furthermore, the behavioral alterations of MAO-A(Neo) mice subjected to MS manipulation were accompanied by significant changes in the prefrontal cortex expression of 5-HT<sub>2A</sub> receptors, but not in the neurochemical levels of 5-HT, NE and DA. These data parallel epidemiological findings on the interaction of low-MAO A allelic variants and early stress in males with respect to the development of antisocial behavior; furthermore, our findings provide a powerful translational platform to investigate the pathophysiology of aggression on a highly isomorphic murine model. Further studies are ongoing to characterize the neurobiological bases of gene-environment interactions on the development of aggression and other emotional disturbances in MAO-A(Neo) mice.

**P 51.****ALTERATIONS OF ZINC TRANSPORTER PROTEINS ZNT-1, ZNT-3 AND ZNT-4 IN THE PREFRONTAL CORTEX AND HIPPOCAMPUS OF SUICIDE VICTIMS**

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Depression is a serious psychiatric illness often accompanied by suicide. Recent clinical and preclinical data indicated that disturbances in zinc homeostasis play a crucial role in the induction of depressive symptoms. Zn homeostasis results from a coordinated action of ZnT and ZIP transporters and metallothioneins. ZnT-1, ZnT-3 and ZnT-4 are the members of ZnT family of transporters responsible for decreasing cytoplasmic zinc level. Taken into account that a considerable percentage of suicide victims had suffered from depression and that zinc is involved in the psychopathology of depression, we hypothesized that alteration in zinc homeostasis regulating proteins such as ZnT-1, ZnT-3 and ZnT-4 might also occur in the brain of suicide.

Brain tissue from 17 suicide victims and six unexpected sudden death control subjects (mean age: 35.8±4.3 years for suicide and 34.3±6.0 for controls) were taken into analysis. Protein level of ZnT-1, ZnT-3 and ZnT-4 transporters was determined using Western blot procedure.

Analysis of the prefrontal cortex (PFC) and hippocampus (Hp) of suicide subjects showed a significant increase in the level of ZnT-1 protein (by 73% in the PFC and by 90% in the Hp,  $p < 0.05$ ); significant increase in the level of ZnT-4 protein (by 214% in the PFC and by 168% in the Hp,  $p < 0.03$ ) and significant decrease in the level of ZnT-3 (by 48% in the PFC  $p < 0.03$ ) but increase in the ZnT-3 protein level in the Hp (by 86%,  $p < 0.04$ ) when compared to controls.

Our results suggest that alterations in Zn transport proteins may contribute to the pathology observed in suicide-related disorder such as depression. A different pattern of changes in the expression of studied ZnTs in the PFC might indicate different and independent role of these proteins in the suicide related processes. More research is needed to confirm this hypotheses.

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## P 52.

**FENOFIBRATE, A CLINICALLY UTILIZED PPAR $\alpha$  AGONIST, ENHANCES ACTIVITY OF DOPAMINE AND SEROTONIN NEURONS IN RATS**

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Preclinical studies demonstrated that activation of peroxisome proliferator-activated receptors-alpha (PPAR $\alpha$ ), ligand-activated nuclear receptor transcription factors widely expressed in the CNS, acutely modulates activity of midbrain dopamine neurons by phosphorylation of  $\beta$ 2 subunit-containing nicotinic acetylcholine receptors. Experiments also suggest that these effects might underlie antidepressant-like properties, as shown by preliminary behavioral experiments in animal models of depression. Little is known, however, how chronic treatments with PPAR $\alpha$  agonists affect neuronal activity, specifically of dopamine and serotonin cells, whose activity is key in affective regulation.

On these bases, we investigated how a chronic administration of fenofibrate, a PPAR $\alpha$  agonist clinically approved as lipid-lowering medication, affects dopamine neurons in the ventral tegmental area (VTA) and serotonin cells in the dorsal raphe nucleus (DRN), two brain regions involved in the neurobiology of psychiatric disorders, including depressive states.

*In vivo* single unit extracellular recordings were carried out from adult anaesthetized rats, which were fed for 14 days with either a standard (control group) or fenofibrate 0.2% (FBR group).

Statistical analysis did not reveal differences in neither the number of spontaneously active VTA dopamine neurons nor their firing rate between control and FBR groups. However, chronic fenofibrate administration enhanced burst activity of dopamine cells (i.e. percent of spikes in burst, mean spikes per burst, burst rate, mean intraburst frequency and mean burst duration), which might correlate with higher dopamine release in terminal regions. Accordingly, DRN serotonin cells recorded from the FBR group showed higher frequency and changes in discharge pattern when compared to controls.

Taken together, our results suggest that chronic exposure to the PPAR $\alpha$  agonist fenofibrate affects activity of VTA dopamine and DRN serotonin neurons. These advances might help understanding the mechanism whereby PPAR $\alpha$  is implicated in the pathophysiology of neuropsychiatric disorders, and represent a novel promising therapeutic target.

## P 53.

**DEEP BRAIN STIMULATION OF THE INFERIOR COLLICULUS REDUCES TINNITUS  
IN A VALIDATED ANIMAL MODEL FOR TINNITUS**

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Tinnitus can cause a serious burden on patients and remains often treatment-resistant. Up to date no effective treatments are available. Deep brain stimulation (DBS) has been widely applied for various neurological disorders, most often in patients with Parkinson's disease. DBS interferes with pathological neuronal activity. The inferior colliculus (IC) shows increased neuronal firing and bursting activity in animal models of tinnitus. We therefore hypothesized that tinnitus can be treated by DBS of the IC. In this study we assessed treatment of experimental tinnitus with DBS in the IC in an animal model with behavioral signs of tinnitus in an intra-individual controlled experimental design. In nine Sprague Dawley rats the startle reflex using the pre-pulse inhibition (PPI) paradigm was measured as a pretest. Tinnitus was defined as an increase in gap:no-gap ratio. After bilateral DBS implantation in the IC the sham situation was assessed including screening of hearing using the auditory brainstem response (ABR). Unilateral tinnitus was induced using a 16 kHz octave-banded noise and the PPI and ABR were again measured. DBS in the IC was then applied at 100 Hz at 100  $\mu$ A. Histological examination showed that the electrodes were positioned within the IC. After noise trauma the rats showed an increase of gap:no gap ratio at 16 and 20 kHz ( $p=0.01$ ). Hearing in the contralateral ear was not impaired by noise trauma as measured by the ABR. During DBS the gap:no gap ratio returned back to baseline ( $p=0.01$ ). This study shows that DBS of the IC is effective in reducing experimental tinnitus in a validated animal model for tinnitus. Further studies are needed to validate this finding and other more accessible brain structures for human application need to be studied.

## **POSTER SESSION II**

# MISCELLANEOUS

**P 01.****THE EFFECTS OF TOPIRAMATE ON THE NEUROPEPTIDE-Y LEVEL AND NEURON NUMBER IN THE ARCUATE NUCLEUS OF THE FEMALE OBESE RATS**

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The increasing prevalence of obesity in the treatment of the disease requires the introduction of alternative solutions. Lately for this purpose, the most commonly used drugs that lead to weight loss as a side effect are anti-epileptics. The aim of the present study is to investigate the effects of topiramate on the hypothalamus of the female obese rats. In this study, twenty-four female rats were divided into four equal groups that non-obese control, obese control, non-obese topiramate and obese topiramate ones. In the none-obese groups commercial rat diet was applied and obese groups were fed a high-fat diet including 40% calories from fat. At the end of 9<sup>th</sup> week, the subjects in the drug treated groups have taken topiramate once a day for 6 weeks. Then, arcuate nucleus containing brain samples were analyzed by using histological and stereological methods. The number of neurons in the non-obese topiramate groups was found to be significantly higher than that of the non-obese control group ( $p<0.001$ ). Neuropeptide-Y levels which were increased in obese rats. Bu, following the topiramate treatment, the levels were decreased. As a result, the use of topiramate and obesity leads to oxidative stress. Also, the mechanisms about the weight loss effect of topiramate on the obesity are thought to be associated with decreased levels of Neuropeptide-Y.

**P 02.****TURMERIC: HOW FAR CAN IT AFFECT AFLTOXICOSIS IN THE RAT BRAIN?**

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Turmeric has been used for 4,000 years to treat a variety of conditions. It may help in fighting infections and some cancers, reducing inflammation, and potentially in treating digestive problems, and it has gotten a lot of press lately. Previous studies showed the protective effect of turmeric on Aflatoxicosis induced by Aflatoxins.

Aflatoxin B1 is one of the most prevalent known mycotoxins (secondary fungal metabolic products), it commonly contaminates maize and other types of crops during production, harvest, storage and processing. It affects human and animals by eating Aflatoxin contaminated foods, ingestion of contaminated dairy products or by inhaling dust particles of Aflatoxin B1.

We wanted to figure out the possible role of turmeric on brain Aflatoxicosis. Therefore, adult rats were intraperitoneally injected by Aflatoxin B1 five days consecutively. To evaluate the effect of turmeric, it was orally administered synergistically in another group of animals. Three weeks after the administration, rats were sacrificed for laboratory analysis.

Histological examination revealed neuronal degeneration and white matter necrosis in the Aflatoxin injected animal group. On the contrary, brain tissue of rats that were subject to the synergetic effect of Aflatoxin and turmeric showed less degeneration and necrosis. It was closely similar to the normal architecture. Moreover, turmeric treated rats showed a decreased level in the NSE (Neuron Specific Enolase) when compared to Aflatoxin treated ones.

In conclusion, our results suggest that turmeric can play an important role against the negative effects of Aflatoxin. We strongly advice turmeric addition to the daily dietary meals.

**P 03.****RECENT DEVELOPMENTS FOR NEUROTRANSMITTER ANALYSIS**

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In the field of neurotransmitter analysis, there is a continuing demand for faster and more sensitive analyses.

- Concentrations of some neurotransmitters from particular brain regions are very low.
- There is a demand for measurements in ever smaller sample volumes (<5 $\mu$ L).
- In each sample the levels of many components are of interest.

The recent development of a new electrochemical detector (DECADE Elite) and a new electrochemical flow cell (Sencell) in combination high efficiency separation columns makes it possible to improve the methods for neurotransmitter analysis. We present an overview these methods for analyzing neurotransmitters in microdialysate samples with the ALEXYS Neurotransmitter Analyzer. The DECADE Elite with an increased data rate and high temperature fully supports fast UHPLC separations. The Sencell from Antec has an Adjustable Spacer Technology (ATS) that enhances the sensitivity and improves detection limits. Optimized applications have been developed for the following neurotransmitters in microdialysate samples:

- Monoamines
- Acidic metabolites
- Acetylcholine
- Amino acids glutamate and GABA

The ALEXYS Neurotransmitter Analyzer is a versatile and flexible UHPLC system dedicated to analyzing a wide range of neurotransmitters in very small samples and at low concentrations.

## P 04.

**EARLY DEPOLARIZING GABA CONTROLS CRITICAL PERIOD PLASTICITY IN THE RAT VISUAL CORTEX**

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GABA, the main inhibitory neurotransmitter in the adult mammalian brain, plays a fundamental role for development and plasticity of the visual system. Conversely, GABA exerts depolarizing action during early development due to high expression of the Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> cotransporter NKCC1. Interestingly, nothing is known about depolarizing-GABA control of plasticity mechanisms in the visual system. Here, we interfered with depolarizing-GABA action by treating rat pups with NKCC1 inhibitor (and FDA-approved diuretic) bumetanide or vehicle from P3 to P8 and examined the time course of plasticity by means of *in vivo* (ocular dominance plasticity) and *in vitro* (long term potentiation, LTP, in acute slices) paradigms. We found that plasticity was high in developing control animals (P20-26) and virtually negligible in young adults (P35). In striking contrast, significant levels of plasticity were still detectable in bumetanide-treated rats at P35, although negligible plasticity was observed in fully adult animals (P75). The effect on plasticity was not due to defects in structural or functional development of the visual system of bumetanide-treated rats. Indeed, migration and morphology of layer II-III pyramidal neurons in the visual cortex and basic physiological parameters developed normally in these animals. Moreover, treatment with the osmotic diuretic mannitol did not mimic bumetanide effects on plasticity, indicating that the latter were specifically due to chloride regulation. To explain the higher level of plasticity in bumetanide-treated rats at P35, we analyzed basal GABAergic transmission and the extracellular matrix, which normally act as brakes for plasticity in the adult. We found a reduction of both GABAergic inhibitory tone and density of perineuronal nets. Finally, as Brain Derived Neurotrophic Factor (BDNF) regulates the time course of critical period plasticity, we analyzed BDNF levels in the visual cortex at P35. We found a significantly lower expression of BDNF in bumetanide-treated animals. In seeking for a possible direct mechanism, we increased BDNF signaling by BDNF-mimetic DHF during bumetanide treatment, as depolarizing GABA has been described to modulate BDNF levels during development. We found that plasticity at P35 was negligible in animals co-treated with bumetanide and DHF, and this was accompanied by a restoration of a normal expression of plasticity brakes. These results demonstrate that depolarizing GABA exerts a long lasting modulation of plasticity of cortical circuits by a strong crosstalk with BDNF, without affecting general development of the visual system.

# **ADDICTION**

## P 05.

**MESOLIMBIC DOPAMINE TRANSMISSION OF ADOLESCENT AND ADULT RATS  
ARE DIFFERENTIALLY AFFECTED BY DRUGS OF ABUSE**

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Increasing evidences suggest a heightened vulnerability to drugs of abuse in adolescence. During this developmental time frame the brain undergoes extensive remodelling affecting particularly reward system. Such changes involve both mesocortical and mesolimbic pathways (1,2). There is evidence for a predominance of ventral striatum (approach system) relative to prefrontal cortex (regulatory system) that produce typical adolescent behaviors (risk-taking, novelty seeking etc.) but, although most of the studies suggest a delayed maturation of the PFC, it is still debated if dopaminergic transmission in the nucleus accumbens (NAc) of adolescents is hyper-(3,4) or hypo-reactive<sup>1</sup>. In rodent models, in spite of overwhelming studies on reward function, tested through conditioned place preference or self-administration paradigms, direct evidences on adolescent dopamine (DA) transmission responsiveness to drugs of abuse are limited (5).

In order to assess differences in mesolimbic DA transmission between adults and adolescents and its responsiveness to different drugs of abuse we used in vivo microdialysis. Male Sprague-Dawley rats of 5, 6, 7 or 10,11,12 weeks of age were implanted with dual probe, aimed at the shell and core of NAc and challenged with nicotine (0.4 mg/kg s.c.),  $\Delta^9$ -tetrahydrocannabinol (THC, 1.0mg/kg i.p.), cocaine (10 mg/kg i.p.) or morphine (1.0 mg/kg s.c.) and extracellular DA levels monitored simultaneously with behaviour.

Although no significant differences were observed between adolescents and adults in basal DA levels, neither in the shell and core of NAc, adolescents showed different effects depending on the drug and on the age of exposure. While no differences were observed in DA transmission responsiveness, both in the shell and in the core of NAc, after morphine or nicotine administration, rats at 6 weeks of age showed greater increase of DA levels in the NAc shell following 1.0 mg/kg i.p. of THC compared to adult rats. Moreover 5 weeks animals appear to be less sensitive to the DA increasing effects of cocaine (10 mg/kg i.p.) compared to adolescents of 6 and 7 weeks and to adults. The differences observed after THC and cocaine challenge might be explained respectively by changes in endocannabinoid system during development (6) and in DA uptake transporter (DAT) levels as reported by previous studies (7,8).

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## P 06.

**DICHOTOMOUS EFFECT OF SEROTONIN<sub>2B</sub> RECEPTOR ANTAGONISTS ON COCAINE-INDUCED HYPERLOCOMOTION AND DOPAMINE RELEASE IN THE RAT STRIATUM AND NUCLEUS ACCUMBENS**

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Serotonin (5-HT) is a well-known modulator of the activity of dopamine (DA) ascending pathways, which participate in reward function and drug addiction. The 5-HT<sub>2B</sub> receptor (5-HT<sub>2B</sub>R) exerts a tonic excitatory control on DA release in the shell subregion of the nucleus accumbens (NAc), a key structure for the effects of drugs of abuse. The present study was aimed at assessing the effect of two selective 5-HT<sub>2B</sub>R antagonists, RS127445 and LY266097, on cocaine-induced hyperlocomotion and DA outflow, measured, using intracerebral microdialysis, in the striatum and the NAc of freely moving rats. Also, to assess a possible postsynaptic interaction, we evaluated the influence of 5-HT<sub>2B</sub>R blockade on the late-onset hyperlocomotion induced by the DA-D<sub>2</sub>R agonist quinpirole, whose effect, at variance with cocaine, occurs independently of changes of DA outflow and is related to direct stimulation of post-synaptic DA-Rs.

RS127445 (0.16 mg/kg, i.p.) or LY266097 (0.63 mg/kg, i.p.) significantly reduced basal DA outflow in the NAc shell, but had no effect on cocaine (10 mg/kg, i.p.)-induced DA outflow. RS127445 was unable to modulate cocaine-induced DA outflow in the NAc core and the striatum. Conversely, RS127445 and LY266097 reduced cocaine-induced hyperlocomotion. Also, RS127445 reduced quinpirole (0.5 mg/kg, s.c.)-induced late-onset hyperlocomotion. The present findings demonstrate that 5-HT<sub>2B</sub>R blockade reduces cocaine-induced hyperlocomotion independently of subcortical DA outflow. Furthermore, they suggest that RS127445 could exert its suppressant effect on cocaine-induced hyperlocomotion by acting downstream to DA neurons, probably by controlling DA transmission in the NAc and/or the striatum.

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## P 07.

**EXTINCTION TRAINING REGULATES COCAINE-INDUCED STRUCTURAL PLASTICITY IN THE ANTERIOR PREFRONTAL CORTEX AFTER WITHDRAWAL FROM COCAINE SELF-ADMINISTRATION**

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Cocaine addiction is a chronic relapsing disorder associated with persistent changes in brain circuits. Recent studies suggested that compulsive drug-seeking after cocaine abuse could be related to inhibitory control impairment in human prefrontal cortex. Persistent changes in behavior seem to be dependent on the reorganization of synaptic connections (structural plasticity). With regard to this, modifications in neuron morphology and dendritic spines after withdrawal from cocaine self-administration have been reported in several brain regions involved in addiction including the prefrontal cortex. Although it is well-known that extinction training modulates cocaine-induced responses after withdrawal, there are no studies evaluating the role of extinction in cocaine-induced alterations on dendritic and spine morphology. In this work, Lewis rats were trained to self-administered intravenous cocaine during daily 2-h sessions for 3 weeks. After cocaine self-administration training, in a group of animals were conducted 15 daily extinction sessions (extinction), while a group-matched remained in their home cages (abstinence). Then, rats were perfused with 4% paraformaldehyde and their brains were removed for processing by means of intracellular injection method. 3D z-Stack images were taken for analysis with a confocal microscope and a 3D morphological analysis of the dendrites and spines in the anterior prefrontal cortex were performed. The results showed increased spine density after abstinence of cocaine self-administration but not immediately after cocaine self-administration or after extinction. By contrast, increased dendritic volume and higher frequency of larger spines in animals that extinguished cocaine self-administration was found.

Our data suggest that extinction training regulates cocaine-induced structural plasticity after withdrawal from cocaine self-administration. The present findings would support extinction-based therapies as a useful approach that could be added to a pharmacological treatment of cocaine addiction.

**P 08.****CHRONIC VOLUNTARY EXERCISE DIFFERENTIALLY AFFECTS THE REWARD-FACILITATING AND THE LOCOMOTOR-STIMULATING EFFECTS OF COCAINE**Vicky Katsidoni, George Panagis*University of Crete, Dept. Psychology, Laboratory of Behavioral Neuroscience, 74100 Rethymno, Crete, Greece*

Several studies demonstrated that physical exercise creates individual behavioral differences leading to a protective-addiction phenotype in rodents. Accordingly, it is possible that exercise affects the sensitivity of brain reward systems and/or interferes with the reinforcing and psychomotor properties of addictive drugs. This study first examined the effects of chronic voluntary wheel-running on intracranial self-stimulation (ICSS) and the reaction to novelty using the open-field test. We then assessed its consequences in the reward-facilitating and locomotor-stimulating effects of cocaine. Male Sprague-Dawley rats were randomly divided into two groups: exercised and non-exercised. At the age of three months, the rats used for the ICSS experiments, were implanted with a monopolar electrode within the medial forebrain bundle (MFB). During the training phase of self-stimulation, we recorded a series of indices in order to compare the sensitivity of MFB self-stimulation in the two groups. After stabilization of the ICSS behavior, the animals of both groups received systemic injections of cocaine (0, 2.5, 5 and 10mg/kg, i.p.) and their ICSS threshold and asymptote were tabulated. The rats' reaction to novelty was measured in an open-field arena. Subsequently, the rats were injected with cocaine (0, 2.5, 5 and 10mg/kg, i.p.) and their motor activity was measured. Exercised rats required significantly increased number of primings, elevated frequency and more time in order to acquire the self-stimulation behavior, while the ICSS threshold frequency was significantly elevated, indicating a decreased sensitivity of the MFB reward pathway. The reward-facilitating effect of cocaine was significantly decreased in the exercised animals compared to the control group. Moreover, the exercised rats showed decreased reaction to novelty. However, psychostimulant-induced hyperlocomotion was significantly increased in the exercised rats at the higher doses of cocaine. Altogether the results reveal that long-term voluntary exercise can reduce the reward-facilitating effects of psychostimulants and modulate their psychomotor activity.

## P 09.

**DIFFERENTIAL EFFECT OF L-DOPA ON COCAINE SELF-ADMINISTRATION AND CUE-INDUCED REINSTATEMENT IN RATS**

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We have recently shown that systemic administration of the dopamine-beta-hydroxylase inhibitors disulfiram and nopicastat suppresses cocaine-induced reinstatement of cocaine-seeking behavior in rats, and contextually increases cocaine-induced dopamine release in the medial prefrontal cortex (mPFC). The behavioral effects are mediated by a supra-maximal stimulation of dopamine D1 receptors that leads to their functional inactivation. Similarly, L-DOPA administration was demonstrated to suppress cocaine-induced reinstatement (Devoto et al., 2014).

In this follow-up study we verified whether the suppressant effect of L-DOPA on cue-induced reinstatement of cocaine-seeking behavior was as effective as on drug-induced reinstatement.

Male rats were trained to self-administer cocaine (0.5 mg/kg/infusion) intravenously for 2 hours under a fixed-ratio 1 schedule of reinforcement, each cocaine infusion being associated with visual and auditory cues. After an extinction training, animals underwent cue-induced reinstatement test sessions. Acute exposure to a cue priming immediately before starting the session promptly reinstated cocaine-seeking behavior to the pre-extinction responding level, an effect efficaciously reversed by pretreatment with L-DOPA (50 mg/kg).

Since extended access to self-administered cocaine may produce symptoms characteristic of addiction that are not seen following more limited drug access (Paterson and Markou, 2003), animals were allowed to resume responding for cocaine during extended (6 hours) sessions. As expected, cocaine self-administration was promptly reacquired. Once a stable drug intake was reached, rats were pretreated with L-DOPA either on the last day of cocaine self-administration training or during cue-induced reinstatement of drug-seeking behavior. Results showed that L-DOPA did not affect cocaine intake but prevented cocaine-seeking reinstatement induced by re-exposure to cue priming.

Our findings showed that L-DOPA does not affect voluntary cocaine intake in rats, yet it may be useful in preventing relapse to cocaine since it is able to prevent both drug- and cue-induced reinstatement of cocaine-seeking.

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**P 10.****EFFECTS OF PRELIMBIC AND CEREBELLAR LESIONS ON THE ACQUISITION OF PREFERENCE MEMORY TOWARDS COCAINE-RELATED CUES**

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Several memory processes underlie motivational trigger of drug-seeking and drug-taking behaviour. One of them is the acquisition of preference memories for drug-related cues. Growing evidence supports that reorganization of the prefronto–striatal–limbic networks underpins storage of these drug-induced memories. However and despite the fact that several data have supported the involvement of the cerebellum in the functional alterations observed after prolonged cocaine use, this brain structure has been traditionally ignored and excluded from the circuitry affected by addictive drugs. Recently, we have shown that after training mice in a cocaine-odour Pavlovian conditioning procedure conditioned preference for the odour associated with cocaine was directly correlated with cFOS expression in cells at the dorsal region of the granule cell layer of the cerebellar vermis. It was a very specific hallmark of conditioned preference for cocaine not seen if mice did not develop preference for the cocaine-related cue. The present study aimed at evaluating the effects of infralimbic and cerebellar deactivations in rats before starting with the conditioning training to acquire preference for an olfactory stimulus paired with cocaine (CS+). One group of rats was subjected ten minutes before training to a temporary prelimbic inactivation by lidocaine. Another group was treated with quinolinic acid for a permanent lesion in the posterior cerebellar vermis (lobules VIII-IX). Remarkably, an against our expectations, the results indicated that either inactivation of prelimbic cortex or cerebellar lesion increased up to 100% the percentage of animals acquiring conditioned preference for cocaine. Therefore, our findings demonstrated that the development of cocaine-induced conditioned preference is promoted when prelimbic or cerebellar activity decreases. Also, these results suggest that the prelimbic cortex and cerebellum work together on inhibiting acquisition of drug-related emotional memories.

## P 11.

**EFFECTS OF INTRA-ACCUMBAL AND INTRA-PREFRONTAL CORTEX  
MICROINJECTIONS OF ADENOSINE 2A RECEPTOR LIGANDS ON RESPONDING  
FOR COCAINE REWARD AND SEEKING IN RATS**

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Cocaine is one of the most addictive substance and its dependence remains a complex social and medical problem. It is well-established that the brain dopamine (DA) mesocorticolimbic pathways play a key role in behavioral responses of cocaine and are related to development of drug addiction (Koob et al., 2001). Many studies indicated that adenosine via its receptors  $A_{2A}$  modulates the behavioral effects of cocaine by controlling DA neurotransmission (Filip et al., 2012).

In the present study, the hypothesis was examined that  $A_{2A}$  receptors in the nucleus accumbens (NAc) or prefrontal cortex (PFC) may control either cocaine self-administration (0.25-0.5 mg/kg/infusion) or the drug-seeking behavior. In male Wistar rats implanted with bilateral cannulae aimed at the PFC or NAc, local microinjections of the selective  $A_{2A}$  agonist (CGS 21680) or antagonists (KW 6002 and SCH 58261) were given to separate group of animals. All doses of ligands used in experiments were inactive in the locomotor activity in rats.

Our findings demonstrated that  $A_{2A}$  receptors located to the NAc or PFC have no role to control rewarding properties of cocaine, as neither KW 6002 (0.25-2.50  $\mu$ g/side) nor SCH 58261 (0.25-2.5  $\mu$ g/side) microinjections altered cocaine self-administration. We also showed that intra-NAc administration of CGS 21680 (1-2.5 ng/side) significantly reduced number of active lever presses and the number of cocaine (0.25 mg/kg/infusion) infusions.

In extinction/reinstatement procedure none of the  $A_{2A}$  receptor antagonists administered into the NAc affected cocaine (10 mg/kg, ip)- or the drug-associated conditioned stimulus-evoked reinstatement of cocaine seeking behavior. On the other hand, local, intra-NAc microinjections of CGS 21680 dose-dependently (1-2.5 ng/side) attenuated the reinstatement of active lever presses induced by cocaine and by the drug-associated conditioned stimuli.

These data indicate that the NAc is a brain site at which the stimulation of  $A_{2A}$  receptors exerts an inhibitory control over the effects of cocaine.

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**P 12.****NUCLEUS ACCUMBENS SHELL AND CORE DOPAMINE RESPONSIVENESS DURING OPERANT RESPONDING FOR SUCROSE: NOSE POKING VERSUS LEVER PRESSING**

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In our study we investigated by microdialysis the responsiveness of dopamine (DA) transmission in the nucleus accumbens shell and core in rats responding for sucrose using two different operant schedules: nose poking versus lever pressing.

In rats trained to respond for sucrose pellets on nose poking fixed ratio 1 (FR1) schedule, dialysate DA increased in the shell but not in the core during active responding as well as under extinction in the presence of sucrose cues. In rats responding for sucrose on lever pressing FR1 schedule we observed a strengthening of DA transmission both in the shell and in the core.

Non-contingent sucrose presentation and feeding in nose poking and lever pressing FR1 trained animals increased dialysate DA to a similar extent in the shell and in the core.

We can conclude that while non contingent sucrose feeding activates dopamine transmission in the shell and core, response-contingent feeding activates without habituation dopamine transmission selectively in the shell in nose poking FR1 trained rats as a result of the action of sucrose conditioned cues. In lever pressing trained rats during responding for sucrose we found an increase of DA in both areas as a result of the higher effort required to obtain the reward.

These findings can explain most of the discrepancies existing in the literature on the responsiveness of shell and core DA during food self administration.

## P 13.

## INFLUENCE OF AGE AND GENETIC BACKGROUND ON ETHANOL INTAKE IN MODEL OF ALCOHOL ABUSE

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Among factors contributing to individual vulnerability to drug addiction genetic background and age of first exposure are critical ones. Increasing evidences show that genetic factors may account for 40-70 % of the variance of substance abuse and addiction (1). Given that alcohol consumption, especially as binge-drinking, among young people is becoming an alarming phenomenon (2,3), it urges to better understand the influence of genetic background and the role of early onset of use in the development of alcohol dependence. To this aim, we used an animal model of genetic vulnerability, the inbred rat strains Lewis (LEW) and Fischer 344 (F344) and an experimental paradigm of intermittent exposure to high concentrations of ethanol (20%) (4,5) to characterize patterns of intake in adult and adolescent rats. High vulnerable LEW and low vulnerable F344 rats were compared with an outbred strain of rat, Sprague-Dawley (SD). Adolescent (6 weeks) or adult (10 weeks) male LEW, F344 and SD rats were individually housed and exposed to a 2-bottle choice regimen (water vs alcohol 20 %) with intermittent alcohol access (three 24 h sessions/week), for 7 weeks (adolescents) or 11 weeks (adults). Results obtained showed significant differences between strains and age groups in ethanol intake and behavioral reactions following consumption and during abstinence days. Both adult SD and LEW, but not F344 rats, escalated their alcohol intake over time reaching stable levels (SD  $3.6 \pm 0.1$ , LEW  $2.3 \pm 0.1$ , F344  $0.8$  g/kg/24h). Adolescent rats consumed significantly higher amount of ethanol at the beginning of exposure, but while SD did increase their intake over time ( $4.57$  g/kg/24h), LEW and F344 kept their intake stable. However, LEW rats, although having a lower ethanol consumption compared to SD rats, showed more compulsive intake from the beginning of ethanol exposure, consuming a higher amount of ethanol during the first hour of exposure, and reducing, significantly more than SD e F344, their water intake over time. Behaviorally LEW rats showed since the first exposure locomotor activation and hedonic reactions absent in the other two strains at the starting of the protocol. Moreover LEW rats, differently from the other two strains, showed high scores of withdrawal and seeking behavior during abstinence. The present results suggest that individual genetic background and early onset of alcohol use are critical factors in progression toward abuse and development of alcohol addiction and related disorders.

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**P 14.****CONCENTRATION-DEPENDENT EFFECTS OF ETHANOL ON *C. elegans*  
BEHAVIOUR EQUIVALENT TO INTOXICATING BLOOD ALCOHOL  
CONCENTRATIONS IN HUMAN**

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Ethanol is one of the most widely used and socially acceptable drugs in the world. Its pharmacology is complex and it acts on many different neurotransmitter circuits including glutamatergic, GABAergic, serotonergic, dopaminergic and opioid peptidergic systems, on voltage-gated ion channels and intracellular signalling cascades. Moreover, these systems can adapt to the chronic presence of ethanol leading to tolerance to ethanol's effects. If ethanol is then removed, these adaptations can lead to withdrawal symptoms.

Here we are using the simple nematode worm *C. elegans* to enable an analysis at all levels of organization from gene, molecule, and neurone through to neuronal circuit and behaviour. In order to achieve this we have first provided evidence that ethanol rapidly equilibrates across the cuticle of *C. elegans* thus enabling experiments in which the concentration-dependent effects of ethanol on behaviour can be defined. Mutations which increase the permeability of the cuticle to drugs do not increase the sensitivity of the animal to alcohol in behavioural assays. We have developed paradigms to delineate the effect of alcohol on *C. elegans* behavioural spanning a concentration-range equivalent to intoxicating through to anaesthetic and lethal blood alcohol concentrations in humans. We report low dose effects on behaviour and provide evidence that these effects are mediated at least in part by neuropeptidergic signalling pathways.

**P 15.****INTERMITTENT ADOLESCENT ALCOHOL EXPOSURE INDUCES COGNITIVE IMPAIRMENT AND SEX DEPENDENT ALTERATIONS IN THE FRONTAL CORTEX AND HIPPOCAMPAL FORMATION OF YOUNG ADULT RATS**

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Alcohol consumption among adolescents is a serious public health concern. In animal models, the most popular pattern of adolescent alcohol consumption nowadays is mimicked by a protocol of intermittent access to alcohol. In the present study we investigated the long-term consequences of adolescent ethanol exposure (20% in drinking water), using a 4 day drinking-in-the-dark procedure, during the adolescence period (4 weeks, from postnatal days, pnd, 28 to 52). Male and female adolescent *Wistar* rats were given access to ethanol (or water), each week, for 2h sessions during three days, and for an additional 4h session on the 4th day. At adulthood, following behavioral assessments – elevated plus-maze (pnd 54) and novel object recognition (pnd 63) – animals were sacrificed (pnd 68), their brains removed, and frontal cortex (FC) and hippocampal formation (HF) dissected out for Western Blotting analysis. Intermittent alcohol exposure during adolescence induced a remarkable cognitive deficit in recognition memory in both sexes as well as significant sex-dependent changes in HF and FC plasticity markers, glial cells (GFAP), and cannabinoid receptor expression (CB1R and CB2R). Alcohol exposure induced a significant increase in FC synaptophysin expression in males and females, but only among females in the HF. Instead, a significant increase in SNAP25 expression was only observed in males' HF. GFAP expression was diminished by alcohol exposure in the HF of males, whereas among females GFAP expression was increased in this region but decreased within the FC. Additionally, a significant decrease in FC CB2R expression was also found in both sexes following alcohol exposure, with no changes being found for this receptor in the HF or in the expression of CB1R in either region. The results indicate that alcohol exposure during adolescence induces a long-term cognitive impairment that might be related, at least partially, with the cortical and hippocampal changes described herein.

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**P 16.****DUAL MOTOR RESPONSES ELICITED BY ETHANOL. EFFECTS OF ETHANOL AFTER MU-OPIOID RECEPTOR BLOCKADE IN THE pVTA**

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Several electrophysiological and behavioral findings have suggested that the ethanol effects on the ventral tegmental area (VTA) dopaminergic (DA) neuron activity could be the result of two concurrent and opposing mechanisms, one promoting and the other reducing GABA release onto VTA DA neurons. Following this idea, the activation of the VTA DA neurons observed after acute ethanol administration could be dependent on disinhibition mediated by salsolinol (an ethanol-derived metabolite) through interactions with  $\mu$ -opioid receptors (MORs), whereas the non-metabolized fraction of ethanol could be responsible for the reduction of the activity of these neurons, in this case through a mechanism involving GABA<sub>A</sub> receptors. Thus, the net effect observed after ethanol intra-VTA administration should be dependent on the balance between the effects provoked by both, the metabolized and non-metabolized ethanol fractions, which, in turn, depending on the ethanol dose. Different pharmacological strategies affecting the local ethanol metabolism have previously been used to disentangle this complex scenario. For example, in our previous experiments, the intra-VTA ethanol administration of 35 nmol did not modify the motor activity of animals since the activating and depressing effects offset each other. In the present study, we have explored the suitability of a new strategy: the blockade of MORs to suppress selectively the activating effects of ethanol without affecting the inhibitory effects mediated by the non-metabolized fraction of ethanol. The consequences of an ineffective dose of ethanol (35 nmol) on the motor activity of rats were analyzed when animals were pretreated with the selective  $\mu$ -opioid antagonist  $\beta$ -Funtaltrexamine ( $\beta$ -FNA). Results showed that the pretreatment with  $\beta$ -FNA selectively blocked the activating effect of ethanol leaving unaltered the concurrent depressing effect. Consequently, the initially ineffective 35 nmol dose became a depressant dose.

**P 17.****EFFECT OF D-PENICILLAMINE ON VOLUNTARY ETHANOL INTAKE: STUDY IN WISTAR RATS WITH NO PREVIOUS EXPERIENCE IN DRUG CONSUMPTION**

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Recent preclinical studies conducted in our laboratory have shown that chemical inactivation of central acetaldehyde (ACD) (the first metabolite of ethanol) by D-penicillamine (DP) could be a valid strategy to treat relapse in alcoholic patients during periods of abstinence (1). The rationale of this therapy is based on the depletion of ACD formed in the brain after alcohol consumption, which is likely to prevent activation of the dopaminergic neurons of the mesocorticolimbic system in the ventral tegmental area (VTA), which play an important role in triggering the relapse process. Furthermore, recently, Karahanian et al. have shown that reducing the amount of ACD available in VTA (using an alternative strategy, but comparable to our, which consisted of an intra-VTA administration of a lentivirus capable of inducing the expression of aldehyde dehydrogenase 2) reduced voluntary ethanol consumption in rats that never had consumed, but not in a chronic alcohol administration model (2). Hence, the main goal of this study was to evaluate whether DP is also able to reduce voluntary ethanol consumption in Wistar rats with no previous experience in alcohol consumption (n=24). DP was administered using the same dose, route and mode (1 mg/h subcutaneously infused through an osmotic mini-pump) that had previously shown efficacy in relapse prevention. The effects were evaluated using a non-operant ethanol self-administration model based on the four-bottle paradigm (water, ethanol 5%, 10% and 20%). According to the obtained results, although voluntary ethanol consumption in these rats was not reduced by DP, yet the treatment significantly reduced the preference for ethanol due to a change in the voluntary consumption pattern.

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Lucía Hipólito, Otilia Peñuela, Alejandro Orrico, Lucía Martí-Prats, M<sup>a</sup> José Cano-Cebrián, Ana Polache, Luis Granero, Teodoro Zornoza

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Recent preclinical studies conducted in our laboratory have shown that chemical inactivation of central acetaldehyde (ACD) (the first metabolite of ethanol) by D-penicillamine (DP) could be a valid strategy to treat relapse in alcoholic patients during periods of abstinence (1). The rationale of this therapy is based on the depletion of ACD formed in the brain after alcohol consumption, which is likely to prevent activation of the dopaminergic neurons of the mesocorticolimbic system in the ventral tegmental area (VTA), which play an important role in triggering the relapse process. Furthermore, recently, Karahanian et al. have shown that reducing the amount of ACD available in VTA (using an alternative strategy, but comparable to our, which consisted of an intra-VTA administration of a lentivirus capable of inducing the expression of aldehyde dehydrogenase 2) reduced voluntary ethanol consumption in rats that never had consumed, but not in a chronic alcohol administration model (2). Hence, the main goal of this study was to evaluate whether DP is also able to reduce voluntary ethanol consumption in Wistar rats with no previous experience in alcohol consumption (n=24). DP was administered using the same dose, route and mode (1 mg/h subcutaneously infused through an osmotic mini-pump) that had previously shown efficacy in relapse prevention. The effects were evaluated using a non-operant ethanol self-administration model based on the four-bottle paradigm (water, ethanol 5%, 10% and 20%). According to the obtained results, although voluntary ethanol consumption in these rats was not reduced by DP, yet the treatment significantly reduced the preference for ethanol due to a change in the voluntary consumption pattern.

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**P 19.****EFFECTS OF THE MEK INHIBITOR, SL327, ON ACQUISITION AND EXPRESSION OF ETHANOL-ELICITED CONDITIONED PLACE PREFERENCE AND CONDITIONED PLACE AVERSION**

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Drug-elicited place conditioning allows to study the acquisition and the expression of associative learning, to characterize the positive or negative motivational properties of drugs and their underpinning neurobiological mechanisms. In particular, drug-elicited conditioned place preference (CPP) is based on the establishment of positive memories and as such allows to model drug-seeking behaviour. On the contrary, based on the establishment of negative memories, drug-elicited conditioned place aversion (CPA) results in the prevention of aversive experiences that have been conditioned to drug-associated stimuli. Previous studies indicated that Mitogen-activating Extracellular Kinase (MEK)/Extracellular signal Regulated Kinase (ERK) cascade is involved in place conditioning. However, while the role of MEK has been shown to be critical for the acquisition of CPP, further investigations are needed to characterize its role in CPP expression as well as in both acquisition and expression of CPA. Accordingly, this study was aimed at investigating, in male CD-1 mice, whether MEK blockade with SL327, a blood brain barrier penetrant MEK inhibitor, would impair acquisition and expression of ethanol (2 g/kg)-elicited CPP and CPA. In the acquisition experiments, SL327 was administered 60 min before the unconditioned stimulus (ethanol) whereas, in the expression experiments, SL327 was administered 60 min before conditioned stimulus (post-conditioning test). The results confirmed that ethanol significantly elicits both CPP and CPA. SL327 significantly prevented the acquisition of ethanol-elicited CPP but not CPA; in addition, SL327 while able to significantly reduce the expression of ethanol-elicited CPP, failed to prevent the expression of ethanol-elicited CPA. These results extend previous data on the differential role of MEK on acquisition and expression of drug-elicited place conditioning and suggest that their activation may be at the basis of different mechanisms as a function of the motivational sign of the unconditioned stimulus (preference vs aversion) and of the experimental phase (acquisition vs expression).

## P 20.

**THE EFFECT OF THE AMPHETAMINE SELF-ADMINISTRATION AND ITS WITHDRAWAL ON THE DENSITY AND EXPRESSION OF THE 5-HT<sub>1B</sub> RECEPTORS PROTEIN IN DESIGNATED STRUCTURES OF THE RAT BRAIN**

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Amphetamine is a powerful psychostimulant drug and its reinforcing and rewarding effects are mediated mainly by the limbic dopamine (DA) system, precisely by enhancing DA efflux from the nerve terminals located in the nucleus accumbens by reversing DA transporters. Interestingly, in parallel to this fact, amphetamine also increases extracellular serotonin (5-HT) level by the same mechanism. Manipulation of the 5-HT system has been demonstrated to modify several effects of amphetamine, including its reinforcing activity. Recent findings gave behavioral evidence that 5-HT<sub>1B</sub> receptors are closely engaged in amphetamine reward in rats and revealed among others, that their tonic activation is not involved in the self-administration (S-A) of amphetamine, whereas pharmacological blockade of this receptor during the amphetamine-induced reinstatement decreases the relapse [Miszkiel et al. 2012, 2013]. However, the molecular background of the above phenomena remain unclear.

In the present work, the effects of extended amphetamine S-A (0.06 mg/kg/infusion) and its early and long withdrawal have been studied on 5-HT<sub>1B</sub> receptor density and expression on brain slices and in brain homogenates obtained from rat's prefrontal cortex, hippocampus, dorsal striatum and nucleus accumbens. To that end immunohistochemical mapping, Western blot and radioligand binding techniques have been used. Control groups for this experiment consisted of rats given passive saline (yoked saline rats) and amphetamine (yoked amphetamine rats) infusions. Latter yoked amphetamine group was introduced in order to distinguish the pharmacological vs. motivational properties induced by active amphetamine S-A.

Results of this study revealed that both density and expression of the 5-HT<sub>1B</sub> receptors protein slightly differ among the experimental group, and those changes depend on the experimental time point. According to obtained data, we hypothesize that 5-HT<sub>1B</sub> receptors may contribute to the etiology of amphetamine addiction.

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**P 21.**  
**RELAPSE OF METHAMPHETAMINE ADDICTION IN ANIMAL MODEL OF  
 DEPRESSION**

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As drug addiction is commonly associated with depression, we combined the olfactory bulbectomy model (OBX) of depression with the operant intravenous drug self-administration procedure in rats to investigate the comorbidity (1). The aim of this study was to assess differences in relapse-like behaviour after a period of forced abstinence. Male Sprague-Dawley rats were divided randomly into two groups; in one group the bilateral ablation of olfactory bulbs was performed while the other group was sham operated. The midline frontal incision was made on the skull and 2 burr holes were drilled 6-7 mm anterior from the bregma. Bulbs were aspirated and dead space was filled with a haemostatic sponge. Sham rats underwent identical procedures but their bulbs were left intact. Subsequently, intracardiac catheter was implanted through the jugular vein. After recovery, intravenous self-administration procedure was conducted in operant boxes using active and inactive nosepokes. Active nosepoke resulted in activation of the infusion pump delivering one infusion of methamphetamine (0.08 mg/kg). After baseline methamphetamine intake was maintained (maintenance phase) and responding was stable, period of forced abstinence was initiated and rats were kept in their home-cages for 14 days. Finally, one reinstatement session was conducted. The relapse rate was assessed as a number of active nosepokes during the reinstatement session expressed as a percentage of active nosepoking during the maintenance phase. OBX group displayed approximately 140 % active nosepokes compared to 48 % in sham group. Bulbectomized rats have been previously reported to self-administer more methamphetamine (1) and WIN 55-212,2 (2) than control rats. In accordance with this evidence depressive-like phenotype in this study has significantly increased reinstatement of methamphetamine seeking behaviour. This indicates higher vulnerability to relapse in this model. This paradigm can be used for preclinical screening for potential medications in the dual diagnose of drug abuse and depression.

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**P 22.****ANTI-CRAVING EFFECTS OF ENVIRONMENTAL ENRICHMENT AFTER METHAMPHETAMINE SELF-ADMINISTRATION**

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Drugs addiction is a chronic, relapsing brain disorder characterized by loss of control over drug-taking and drug-seeking behaviors. Exposure to environmental enrichment (EE), a combination of complex inanimate and social stimulation, during periods of abstinence has been shown to reduce relapse to cocaine in animal models of addiction. In this study, we investigated whether the effects of EE could be generalized to other drugs of abuse such as methamphetamine (METH). In addition, because normalization of stress appears to be a central mechanism of the anti-addiction effects of EE, we measured the levels of glucocorticoids receptors in several brain areas, including cortex, amygdala and hippocampus of these rats. For this, we allowed rats to self-administer METH for 20 long (14h) sessions and then we exposed them to either EE or standard environments (SE) during a 21-day period of forced abstinence. Subsequently, we evaluated relapse induced by the context in a single 3h session. We found that exposure to EE reduced the relapse to METH seeking behavior, which indicates that the curative effect of EE can be generalized to other drugs. Regarding glucocorticoid receptor levels, we found: 1) that exposure to EE reduced the GR levels in the amygdala and hypothalamus and that these effects are blunted by prior self-administration of METH and 2) that METH self-administration increased the levels of GR in the hippocampus and that this effect is reversed by EE in the dorsal part of the hippocampus. Altogether, these results indicate that EE has beneficial effects against relapse to METH and that the regulation of the glucocorticoid system might be involved in these effects.

## P 23.

**NEURONAL ADAPTATIONS IN THE HABENULO-ROSTROMEDIAL TEGMENTAL NUCLEUS PATHWAY DURING CANNABINOID WITHDRAWAL**

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The mesolimbic dopamine (DA) system, which arises from the ventral tegmental area (VTA), shows a reduction in its function during spontaneous and drug-induced withdrawal from chronic cannabinoid exposure<sup>1,2</sup>. DA system activity depends upon the integration of various inputs, among the others the lateral habenula (LHb), which can bidirectionally control DA cells via direct and indirect connections through the rostromedial tegmental nucleus (RMTg)<sup>3</sup>. Both RMTg and LHb cells are activated by aversive/unpleasant events, and inhibited by rewarding/positive stimuli. Therefore, these nuclei may represent an interesting potential convergence point for drug-evoked reward and aversive opponent processes.

On these bases, we tested the possibility that LHB-RMTg pathway is causally involved in the hypodopaminergic state that characterizes cannabinoid withdrawal phase.

To this aim, we used single unit extracellular recordings from either VTA, RMTg and LHb neurons in anesthetized male Sprague-Dawley rats. To induce  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) dependence, rats were chronically treated (15 mg/kg, i.p.) twice daily for 6.5 days<sup>1</sup>. Administration of the cannabinoid antagonist rimonabant (5 mg/kg, i.p.) precipitated an intense behavioral withdrawal syndrome, whereas abrupt  $\Delta^9$ -THC suspension caused milder signs of abstinence. Electrophysiological experiments confirmed that  $\Delta^9$ -THC withdrawal is associated with a profound decrease in the firing rate and burst firing of VTA DA neurons. Remarkably, in  $\Delta^9$ -THC withdrawn rats the duration of RMTg-evoked inhibition lasted longer than controls. By contrast, the spontaneous activity of RMTg GABA neurons was reduced in cannabinoid-withdrawn rats. Consistent with results, we also found that firing rate of RMTg-projecting LHb neurons was markedly suppressed after cannabinoid withdrawal.

These findings support the hypothesis that enhanced GABA inputs from RMTg might contribute to the hypodopaminergia induced by cannabinoid withdrawal, and confirm that LHB-RMTg pathway takes part in the neuronal circuits underlying drug dependence and addiction.

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P 24.

**STRAIN DIFFERENCES IN THE DOSE-RESPONSE RELATIONSHIP DURING HEROIN SELF-ADMINISTRATION INDUCED BY ADOLESCENT  $\Delta^9$ -THC EXPOSURE IN A GENETIC ANIMAL MODEL OF OPIATE VULNERABILITY**

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Starting from Gateway hypothesis (GH), which states that adolescent exposure to cannabis has been widely hypothesized as a predisposing factor to opiate abuse, the aim of this study has been to investigate the role of genetic background on heroin self-administration (SA) behavior of Lewis (LEW) and Fischer (F344) rats, pre-exposed to  $\Delta^9$ -THC in adolescence.

Lewis (LEW) and Fischer (F344) rat strains represent an important animal model which allow to study the genetic components involved in different stages of addiction characterized by neurochemical and behavioral differences in their response to the reinforcing properties of drugs of abuse. Rats were administered with increasing doses of  $\Delta^9$ -THC (2,4,8 mg/kg, i.p.), during the 6<sup>th</sup> post-natal (PN) week, twice daily, for three consecutive days. In adulthood (10<sup>th</sup> PN), LEW and F344 rats were trained to acquire heroin SA behavior (0.025 mg/kg/48  $\mu$ l, 1-h daily session), under Fixed Ratio-1 (FR-1) (1NP = 1 infusion) schedule of responding. When criterion of acquisition was met, all groups were subjected to daily 4-h SA (long access, LA), with increasing doses of heroin (0.025, 0.050 and 0.100 mg/kg), under FR-1 schedule of responding.

Adolescent pre-exposure to  $\Delta^9$ -THC induced higher operant responding activity and greater adaptation to the changes in experimental conditions (LA) of opiate-reinforced SA behavior in adult LEW rats as well as progressive escalation of heroin intake when exposed to higher doses of heroin, compared to LEW vehicle as well as to F344 strain. No such differences were observed in the F344 rats. The results strongly confirm that LEW rats are more sensitive to the reinforcing properties of heroin compared to the F344 strain, suggesting the importance of the genetic background in the vulnerability of drug addiction and that adolescent  $\Delta^9$ -THC pre-exposure influences opiate reinforcing properties in adulthood only a strain-related way.

## P 25.

**EFFECTS OF INDIAN GINSENG, *WITHANIA SOMNIFERA* DUNAL, ON THE MOTIVATIONAL PROPERTIES OF MORPHINE AND ON MORPHINE-ELICITED ERK PHOSPHORYLATION: A BEHAVIOURAL AND IMMUNOHISTOCHEMICAL STUDY**

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Previous studies reported critical interactions between morphine and the standardized root extract of *Withania somnifera* Dunal (WSE), Indian ginseng, a plant safely used in traditional medicine for its anti-inflammatory properties. In particular, WSE was reported to prevent the development of tolerance and dependence in mice chronically treated with morphine (Kulkarni and Ninan, 1997), to prevent the reduction of dendritic spines density in the rat nucleus accumbens shell (AcbSh) upon morphine withdrawal (Kasture et al., 2009) and to protract the analgesic effects of morphine and prevent morphine-induced hyperalgesia in the low intensity tail-flick test (Orru et al., 2014). The aim of the present experiments was to further investigate, in CD-1 mice, the interaction between morphine and WSE by studying the ability of WSE 1) to interfere with the motivational properties of morphine, as determined by conditioned place preference (CPP), and 2) to affect morphine-elicited Extracellular signal Regulated Kinase (ERK) phosphorylation (pERK) in the AcbSh and core (AcbC). Notably, besides representing a kinase involved in neuronal plasticity and associative learning, pERK has been shown to play a critical role in the acute effects of drugs of abuse as well as in their ability to elicit associative learning-based CPP (Beninger and Gerdjikov, 2004). The results of the present study show that WSE administration (25-100 mg/kg) either during conditioning and before post-conditioning test prevented, respectively, the acquisition and the expression of morphine (5 mg/kg)-elicited CPP. In addition, we found that WSE administration (50 mg/kg) significantly reduced the increased expression of pERK-positive neurons in AcbSh and AcbC. Overall, these results extend to the motivational properties of morphine the ability of WSE to interfere with its central effects and, by decreasing morphine-elicited pERK expression in AcbSh and AcbC, suggest a possible mechanism by which WSE may impair morphine-elicited CPP.

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## P 26.

**NEUROCHEMICAL ASPECTS OF 5-METHOXY-DIISOPROPYLTRYPTAMINE (5-MEO-DIPT, 'FOXY') IN RAT BRAIN REGIONS**

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5-methoxydiisopropyltryptamine (5-MeO-DIPT) is one of the most popular 'club drug'. A comparison of the chemical structure of 5-MeO-DIPT shows structural similarities with serotonin (5-HT). That suggests possible influence on dopamine (DA) and 5-HT system while, hallucinations noted after 'foxy' intoxication indicate on involvement of glutamate (GLU). However mechanism of 5-MeO-DIPT action in the brain is not fully understood.

The aim of our study was to examine the extracellular level of DA, 5-HT,  $\gamma$ -aminobutyric acid (GABA) and GLU after 5-MeO-DIPT administration in freely moving rats.

Determination of extracellular levels of DA, 5-HT, GABA and GLU was carried out using microdialysis technique and HPLC with coulochemical and electrochemical detection.

Intraperitoneal administration of 5-MeO-DIPT at doses of 20, 2 x 10 or 10 mg/kg produced increase in DA and 5-HT release in the rat striatum and the frontal cortex. Moreover, GLU release was increased in both structures after 5-MeO-DIPT given at a dose of 20 and 2 x 10 mg/kg, while extracellular GABA was enhanced in the striatum after a dose of 20 mg/kg and in the frontal cortex after a dose of 2 x 10 mg/kg.

Hallucinogenic properties of 5-MeO-DIPT may be related with disturbed balance between glutamatergic and GABAergic neurotransmission as result of D1/D2 or 5-HT<sub>2A/2C</sub> receptors stimulation by released DA or 5-HT in rat frontal cortex or striatum.

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## P 27.

**EFFECTS OF 25I-NBOME, A NEW POTENT 5HT2A AGONIST, ON THE CENTRAL NERVOUS SYSTEM OF RODENTS**

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25I-NBOMe (4-iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine), commonly called “N-Bomb”, is a new psychoactive synthetic compound broadly available as “legal” alternative to LSD. 25I-NBOMe acts as a full agonist of 5HT2A receptor, a receptor highly expressed in the prefrontal cortex that mediates the primary effect of hallucinogenic drugs. It is usually ingested sublingually or insufflated. Users are often unaware of ingesting fake LSD, and several episodes of acute intoxication have been reported with severe effects such as confusion, agitation, hypertension, tachycardia, hyperthermia, heart failure, generalized seizure, loss of consciousness, acute kidney. In order to investigate the pharmaco-toxicological central effect and the abuse potential of 25I-NBOMe, we performed behavioral tests (motor activity by open field, drag and accelerod test and reaction time task) and Tyrosine hydroxylase (TH)- and DAT-immunoreactive fibers analysis in dopaminergic (DA) terminals in male C57BL/6 mice, and *in vivo* brain microdialysis in male Sprague-Dawley rats. Behavioral tests results showed no significant differences between controls and treated animals in accelerod, drag and open field tests for all the doses tested (0.1-1 mg/kg i.p.). However, animals exhibited a significant increase of reaction time within 30 minutes after the administration of 25I-NBOMe. TH- and DAT-ir fibers analysis were performed in dorsal striatum (Cpu) and ventral striatum (NAc shell), after a sub-acute administration of 25I-NBOMe (2 mg/kg/i.p.; once a day, 3 consecutive days). No significant modifications in TH and DAT-ir fibers density were observed. By brain microdialysis, we evaluated the effect of 25I-NBOMe (0.3-1.0 mg/kg i.p.) on DA transmission in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) shell/core. Preliminary results show a tendency for a preferential increase of DA in the NAc shell vs NAc core and mPFC. Taking together the present results suggest that 25I-NBOMe does not show relevant pharmaco-toxicological central effect. Further experiments following different doses and repeated administrations are in progress.

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**P 28.****CREATION OF TRANSGENIC RATS FOR THE  $\alpha 5$  SUBUNIT OF NICOTINIC RECEPTORS USING ZINC FINGER NUCLEASE TECHNOLOGY**

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Tobacco smoking is a major public health issue. Nicotine is the main psychoactive component of tobacco smoke that leads to addiction and exerts its initial effect on neuronal nicotinic acetylcholine ion channel receptors (nAChRs).

Recently, human genome-wide association studies have identified a single nucleotide polymorphism in the gene of the  $\alpha 5$  subunit of nAChRs that increases the risk for nicotine dependence ( $\alpha 5$ SNP). The rat is a better model than the mouse to study complex behaviours related to drug addiction. Thus, we decided to create Long-Evans rat with the  $\alpha 5$ SNP or without the  $\alpha 5$  subunit ( $\alpha 5$ KO) by using Zinc Finger Nuclease (ZFN) technology to study their behaviours in a model of nicotine addiction.

To create  $\alpha 5$ KO and  $\alpha 5$ SNP rats, we have injected the pronucleus of rat's single cell embryos with a ZFN pair targeting the exon 5 of the  $\alpha 5$  subunit with the addition of a plasmid donor containing the  $\alpha 5$ SNP, respectively.

We have obtained  $\alpha 5$ KO and  $\alpha 5$ SNP rats without detectable off-target activity and with heritable transmission to the next generation. We have confirmed by immunoprecipitation the absence of  $\alpha 5$  protein in the brain of  $\alpha 5$ KO rats and its presence in the brain of  $\alpha 5$ SNP rats. We have then investigated the behavior of those transgenic rats in an intravenous nicotine self-administration paradigm to evaluate the specific behaviours potentially modified by the presence of the  $\alpha 5$ SNP or the absence of the  $\alpha 5$  subunit.

In conclusion, the use of ZFN technology has led to the successful creation of homozygous  $\alpha 5$ KO and  $\alpha 5$ SNP rats with good rate and specificity in a short time frame. These models are valuable to study the impact of the  $\alpha 5$  subunit and of the  $\alpha 5$ SNP on complex behaviours related to drug addiction that are not attainable with mice.

**P 29.****MESOLIMBIC REGULATION OF PREFRONTAL GLUTAMATE AND DOPAMINE  
RELEASE: ROLE OF LOCAL NICOTINE RECEPTORS**

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Reciprocal interactions, within a distributed neural system containing the nucleus accumbens (NAC), basal forebrain (BF), medial dorsal thalamus, and prefrontal cortex (PFC) are dysregulated in several neuropsychiatric disorders (e.g. schizophrenia, ADD, drug addiction). Interactions between PFC and NAC shell are critical under conditions when increasing demands of a cognitive task must recruit motivational processes in an attempt to maintain appropriate performance. Consistent with this linkage, NMDA-mediated stimulation of the NAC shell evokes ACh release in the PFC, thereby facilitating cue detection and improving performance on a sustained attention task. Moreover, it has been recently demonstrated, using a biosensor with sec-to-sec resolution, that such NMDA activation elevates prefrontal glutamate levels hypothesizing the role of alpha7 nicotinic receptors in this regulation.

The aim of the present experiment was to extend this analysis by determining the effects of NMDA stimulation of the NAC shell on prefrontal glutamate and dopamine release using microdialysis methods and investigate if the nicotinic (and alpha7) receptor activation, from the enhanced cholinergic transmission, was necessary for elevations in glutamate and DA levels.

Adult male Wistar rats were implanted with an infusion cannula into the shell region of the NAC and a microdialysis probe in the ipsilateral mPFC. NMDA (0.30 µg/0.5 µL) was infused and extracellular levels of glutamate and DA were measured. In a separate group of animals, the role of nicotinic (alpha7) receptors in this stimulated release was determined following local perfusions of mecamylamine (10.0 or 100.0 µM); DHβE (10 µM) or α-bungarotoxin (1.0 µM).

We found that activation of NAC stimulates Glutamate and DA release in PFC. The evoked release of glutamate and DA appear to be secondary to the release of ACh and a subsequent activation of local nicotinic (alpha7) receptors on glutamate and DA terminals.

# **STRESS, EMOTION & BEHAVIOR**

**P 30.****THE INVOLVEMENT OF THE MEDIAL PREFRONTAL CORTEX AND  
BASOLATERAL AMYGDALA IN THE REGULATION OF COPING WITH STRESSORS**

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Recent reports showed that endocannabinoid signaling plays an important role in the regulation of behavioral responses to stressful challenges: enhanced signaling of the endocannabinoid anandamide (AEA) by the blockade of its degrading enzyme fatty acid amide hydrolase (FAAH) decreases behavioral reactivity to, and promotes active coping with stressors. Brain areas involved in this important function of endocannabinoids has not yet been studied.

In the present work, we aimed to assess the involvement of two relevant brain regions, the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) in AEA-mediated regulation of reactivity to stressors. FAAH blocker URB597 was chronically implanted in the mPFC or BLA of male Wistar rats, then behavioral reactivity was measured to a mild stressor (high illumination) or to a combination of a severe and mild stressor (contextual reminder to a footshock presented earlier; high illumination).

According to our results, enhanced AEA signaling in the mPFC decreased reactivity to a mild stressor in a similar manner described in our previous work employing systemic URB597 administration. No effects on severe stressors were observed although implantation dampened the effects of a mild stressor in the combination of two stressors. No such behavioral changes occurred after URB597 implantation into the BLA.

These findings suggest that previously shown endocannabinoid-mediated mechanisms in the regulation of coping with mild stressors are mPFC-dependent, while the BLA is not similarly involved in the endocannabinoid control of stress coping.

**P 31.****PRENATAL STRESS PROCEDURE ELEVATED THE PRO-INFLAMMATORY MONOCYTE CHEMOATTRACTANT PROTEIN (MCP-1/CCL2) AND CCR2 RECEPTOR EXPRESSION IN BRAIN OF ADULT RATS: A LINK TO DEPRESSION**

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Recently it has been demonstrated an important role of chemokines in regulation of cell migration, activity of neuropeptides and neurotransmitters action in brain. Among chemokines, especially interesting seems to be monocyte chemoattractant protein (MCP-1/CCL2) involved in regulation of brain inflammatory processes and communication between damaged neurons and surrounding glial cells. Therefore it has been suggested that CCL2/CCR2 pathway may be important in the development of brain inflammatory diseases like depression.

The main purpose of study was to determine whether prenatal stress procedure (an animal model of depression) affects the chemokine CCL2-CCR2 system as well as the pro-inflammatory cytokines expression in two main areas involved in the pathogenesis of depression.

Pregnant Sprague- Dawley rats were subjected daily to 3 stress sessions from 14th day of pregnancy until delivery. At 3 months of age male offspring from both control and prenatally stressed groups were tested behaviorally (Porsolt test, sucrose preference test). Two days later animals were decapitated and the hippocampi and frontal cortices were dissected out. Next, the mRNA expression and protein levels of CCL2 and CCR2 were determined. Furthermore the inflammatory status was indicated by the measurement of the expression of cytokines: IL-1b, TNF-a and IL-6.

Behavioral tests revealed depressive-like behaviors in prenatally stressed adult animals. Interestingly, in animal model of depression we demonstrated an enhanced expression of CCL2 and CCR2 receptor in both examined brain structures. Moreover, the elevation of IL-1b, TNF-a, IL-6 expression has been shown in offspring after prenatal stress.

Summarizing, it may be suggested that disturbances, caused by prenatal stress, lead to dysfunction in CCL2-CCR2 system. The prolonged inflammatory activation might be one of the key factor resulting in development of depressive disorders in adulthood.

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## P 32.

**DECREASED ALLOPREGNANOLONE INDUCED BY NEONATAL ESTRADIOL INCREASES  $\alpha 4\beta\delta$  GABA<sub>A</sub> RECEPTORS, STRESS SENSITIVITY AND SPATIAL LEARNING IN ADULT FEMALE RATS**

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We have previously shown that exposure of developing female rats to estradiol during the perinatal period induced a long-lasting dysregulation of the gonadal axis and decreased brain allopregnanolone concentrations. We now examined whether these changes were associated with altered sensitivity to stress, learning and memory and expression of hippocampal extrasynaptic GABA<sub>A</sub> receptors ( $\alpha 4\beta\delta$  and  $\alpha 5\beta\gamma 2$ ) that are involved in anxiety and memory consolidation.

A single administration of  $\beta$ -estradiol 3-benzoate (EB) on the day of birth decreased allopregnanolone concentration in the hippocampus of adult female rats. Neonatal EB administration also increased the expression of  $\alpha 4$  and  $\delta$  subunits of the GABA<sub>A</sub> receptor in the extrasynaptic membrane fraction of hippocampus from adult female rats;  $\alpha 5$  and  $\gamma 2$  subunit expression was not altered in the same membrane fraction. Neonatal EB treatment decreased the latency and the cumulative search error to reach the platform in the Morris water maze suggesting improved learning in adult female rats. Neonatal EB treatment also enhanced memory performance during the probe trial.

Finally, neonatal EB treatment induced a greater enhancement (4 fold) in the extracellular concentrations of dopamine in the prefrontal cortex of adult EB-treated rats exposed to foot-shock stress, an effect that was normalized by restoring allopregnanolone concentrations with progesterone administration.

These results suggest that neonatal exposure to estradiol plays a major role in the regulation of hippocampal allopregnanolone concentrations, expression of extrasynaptic GABA<sub>A</sub> receptors, stress sensitivity and cognition during adulthood. The increased expression of  $\alpha 4\beta\delta$  GABA<sub>A</sub> receptors in the hippocampus may represent a homeostatic response to counteract the persistent decrease in allopregnanolone levels induced by neonatal treatment. Given that allopregnanolone has been reported to compensate response to stress and impair learning and memory, the persistent decrease in its concentrations may account for the improved cognition and higher sensitivity to stress observed in neonatal EB-treated rats.

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**P 33.****ENVIRONMENTAL ENRICHMENT IN MICE: CAN HOUSING IN ENRICHED ENVIRONMENTS COUNTERACT THE EFFECTS OF SOCIAL STRESS ON ANXIETY-LIKE BEHAVIOR AND CORTICOSTERONE LEVELS?**

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Our main aim was to compare short and long-term effects of exposure to environmental enrichment in mice submitted to chronic social stress. 128 NMRI male mice arrived to our laboratory at postnatal day (PND) 21. During Phase I of the study, which began at PND 28, mice were assigned to four experimental groups: 1) EE+STRESS: mice housed in EE and submitted to stress; 2) EE+NO STRESS: mice housed in EE and not submitted to stress; 3) SE+STRESS: mice housed in standard environment (SE) and submitted to stress, and 4) SE+NO STRESS: mice housed in SE and not submitted to stress. At the end of this phase, mice were evaluated in the elevated plus-maze (EPM) (n=32) and corticosterone levels were measured (n=32). Phase II began on PND 83 and mice were assigned to two different experimental housing conditions: EE (n=48) or SE (n=48). At the end of this phase, both behavioral and corticosterone measures were obtained again. Results of Phase I indicated that “Housing” factor reached statistical significance: mice housed in EE displayed lower anxiety-like behavior than SE. The group EE+STRESS showed higher corticosterone levels than EE+NO STRESS whereas EE+NO STRESS displayed lower levels than SE+NO STRESS. At the end of the study, animals housed in EE displayed some emotional changes in the EPM but not significant differences between groups were obtained in corticosterone levels. These results suggest that behavioral and physiological effects of social stress may be influenced by the type of housing although further studies are needed in order to evaluate more in depth long-term consequences of social stress.

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## P 34.

**CHANGES IN THE DYNAMIC REGULATION OF NEGATIVE FEEDBACK OF HPA AXIS INDUCED BY POSTWEANING SOCIAL ISOLATION IN RATS**

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Rats deprived of social contact from weaning experience a form of prolonged stress that leads to long-lasting behavioural alterations. It has been demonstrated in our laboratory that social isolation induced a marked decrease in brain and plasma concentration of 3a,5a-THPROG (which is paralleled with a decrease in GABA<sub>A</sub> transmission), corticosterone and adrenocorticotropin plasma levels, enhanced the steroidogenic effect of acute stress, acute ethanol and corticotrophin-releasing-factor. These data suggest that social isolation induces changes in stress responsiveness and impaired negative feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis.

To better characterized dynamic changes of HPA axis activity of socially isolated rats following stress exposure, we evaluated the time course response in plasma corticosterone and in hippocampal and hypothalamic glucocorticoid receptors (GR) expression.

Corticosterone levels increased in both groups after 5 min, to return to baseline 60 min in group-housed rats, while it was still high 7 hours later in isolated animals.

Re-exposure of animals to foot-shock stress 120 min after the first one increased corticosterone levels to the same extent induced by the first stress exposure in group-housed rats, while it was blunted in isolated rats.

Hippocampal and hypothalamic GR were increased in isolated rats. Acute stress exposure progressively enhanced GR expression in group-housed rats; this increase was not present in isolated animals.

We evaluated the effect of social isolation and acute stress on hypothalamic CB1 receptors levels: social isolation increased basal levels while foot-shock stress failed to modify CB1 expression. At variance, in group-housed animals hypothalamic CB1 expression was increase 60 min after acute stress exposure.

These results suggest that socially isolation stress causes modifications in the dynamic changes of glucocorticoid feedback resulting in a prolonged response to stress. Experiments are in progress to clarify if alterations of endocannabinoid signaling in HPA axis may be involved in the deficient glucocorticoid feedback.

## P 35.

**SOCIAL ISOLATION CHANGES THE RESPONSE OF MESOCORTICOLIMBIC DOPAMINERGIC NEURONS TO FOOD RESTRICTION IN RATS**

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The mesolimbic dopaminergic pathway plays an important role in the genesis of emotional arousal and behavioral activation in response to stimuli that provide a reward. This neural circuitry is also active in the early stages of learning and stabilization of addictive behavior due to substances abuse. Isolated animals have a different sensitivity to natural or artificial reinforcers. Accordingly, experimental evidences suggest that exposure to stress can deeply modify eating behavior. In light of these evidences the aim of this study was to investigate the influence of a chronic stress, like social isolation at weaning, on the sensitivity of mesocorticolimbic dopaminergic neurons to anticipation and consumption of food. Rats have been food restricted using a protocol that consists in training the animals to consume their meal for only two hours for day. Using vertical microdialysis, extracellular concentrations of dopamine in response to anticipation and consumption of food were measured both in the mPFC and the NAC.

In PFC of GH rats extracellular DA increased (+180%) 80 minutes before food presentation showing the maximal increase (+350%) during food intake. On the contrary, in the NAc of GH rats no significant changes were observed. In SI animals trained to food restriction the increase in mPFC DA output observed in GH animals was completely blunted, while, in the NAc, 40 min before the presentation of the food, a significant increase in extracellular concentrations of DA was observed. Our results show that exposure to chronic stress modified the response of mesocortico-limbic dopaminergic neurons to an enjoyable stimulus and suggest that these changes might be important to explain the greater sensitivity to abuse that is observed in individuals subjected to stressful stimuli. This underlying alteration in brain function might be a crucial mechanism that predisposes individuals to impulsive behavior and increases the risk of developing addiction.

## P 36.

**LONG-TERM, SEX-DEPENDENT NEUROBEHAVIORAL EFFECTS OF MATERNAL DEPRIVATION AND NEONATAL LEPTIN TREATMENT IN MALE AND FEMALE RATS**

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Maternal deprivation (MD) during neonatal life has diverse long-term behavioral effects and alters the development of hippocampus (HC) and frontal cortex (FC), with several of these effects being sexually dimorphic. MD animals show a marked reduction in their circulating leptin (LEPT) levels, not only during the MD period, but also several days later (PND 13). A neonatal LEPT surge occurs in rodents (beginning around PND 5 and peaking between PND 9 and 10) that has an important neurotrophic role. We hypothesized that the deficient neonatal LEPT signaling of MD rats could alter the development of their HC and FC. Accordingly, replacement of neonatal LEPT in MD rats would at least in part counteract their neurobehavioural alterations. MD was carried out in Wistar rats for 24 h on PND 9. Male and female MD and control rats were treated from PND 9 to 13 with rat leptin (3 mg/kg/day sc) or vehicle. In adulthood, the animals were submitted to the novel object memory test and the elevated plus maze test of anxiety. Neuronal and glial population markers, components of the glutamatergic and cannabinoid systems and diverse synaptic plasticity markers were evaluated by PCR and/or western blot. Main results: 1) In some but not all the parameters analyzed the neonatal LEPT treatment reversed the effects of MD (for example, mRNA expression of hippocampal BDNF GLAST and IGF1 in males) confirming partially our hypothesis; 2) The neonatal LEPT treatment, per se, exerted a number of behavioral (increased anxiety) and neural effects (for example, sex and region-dependent effects on mRNA expression of LEPT and CB1 cannabinoid receptors, GFAP, CRH, GLAST and on the expression of the following proteins: NG2, NeuN, PSD95, NCAM, synaptophysin. An adequate neonatal LEPT level (avoiding excess and deficiency) appears to be necessary for its correct neuro-programing effect.

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## P 37.

**GABAB-GIRK COMPLEX IN THE LATERAL HABENULA: A PROMINENT TARGET FOR AVERSIVE STATES**

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The lateral Habenula (LHb) has been described to play a crucial role in motivated behavior, likely through its control of midbrain neuronal populations. LHb neurons encode aversive stimuli. Work in primates and rodents indicate that aversive stimuli phasically increase LHb neurons firing rate (1) and optogenetic activation of LHb terminals onto midbrain neurons induces avoidance behavior in mice (2,3). However, little is known about the cellular changes in the LHb occurring as a consequence of an aversive event and whether they can causally contribute to specific behaviors.

In order to test the hypothesis that aversive events can trigger cellular modifications in the LHb underlying changes in neuronal excitability, we took advantage of the inescapable footshock paradigm and ex-vivo patch-clamp recordings in acute slices from mice.

To obtain information on the spontaneous output firing of neurons we recorded neurons of the LHb in the cell-attached configurations, one hour after the aversive procedure. We found that in slices obtained from footshock exposed mice (FsE), neurons presented a significantly higher firing frequency than control mice. We sought to understand the molecular mechanisms responsible for the increased firing rate. Performing recordings in whole-cell mode, we find that glutamate and GABA<sub>A</sub> transmission remained unchanged. Instead, the pharmacological activation of GABA<sub>B</sub>-Rs by baclofen, elicited a GIRK-mediated outward current, which was reduced in FsE. This effect was mimicked by other protocols inducing aversive behaviors in mice (odor predator and restraint stress). Moreover, in FsE mice the modulatory effect of GABA<sub>B</sub>-Rs on the firing activity resulted strongly weakened.

These data indicate that aversive events drive a functional reduction of the GABA<sub>B</sub>-GIRK signal along with a loss of inhibitory control on firing activity of LHb neurons, providing a sensitive cellular mechanism underlying LHb neurons hyperexcitability recorded after an aversive event.

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**P 38.****EVALUATION OF THE ROLE OF DIFFERENT NMDA RECEPTOR SUBUNITS IN THE DEVELOPMENT OF BEHAVIORAL DYSFUNCTIONS INDUCED BY TRAUMATIC EXPERIENCE**

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Stressful and traumatic life events often lead to the development of post-traumatic stress disorder (PTSD) a highly debilitating psychiatric condition with long-lasting symptomatology. Recent reports suggest that glutamatergic receptor function in limbic regions, particularly N-methyl-D-aspartate receptors (NMDAR) containing different subunits play an important role in the development of trauma evoked behavioral dysfunctions.

In the present study we aimed to evaluate the effects of general NMDAR blockade and blockade of NMDARs containing NR2C/D, NR2B and NR2A subunits on the expression of contextual conditioned fear, a widely applied model of PTSD and on trauma-induced social withdrawal.

Rats were submitted to a single series of footshocks and were underwent a contextual reminder and the social avoidance test 28 days later following a previous treatment of NMDAR antagonist MK-801, NR2A antagonist PEAQX, NR2C/D antagonist PPDA or NR2B antagonist Ro25-6981, respectively. Neither PEAQX, nor PPDA altered conditioned fear, while both MK-801 and Ro25-6981 was able to reduce this response. In contrast to MK-801, Ro25-6981 did not induce locomotor side effects. Surprisingly, besides its effects on conditioned fear, Ro25-6981-induced blockade of NR2B subunit containing NMDARs enhanced trauma-evoked social withdrawal. Real-time PCR measurements showed that NR2A and NR2B subunit mRNA expression was increased in the prefrontal cortex, a relevant subregion in PTSD, on the first day after the traumatic event, while only NR2B mRNA levels remained elevated 28 days after shock exposure.

Our pharmacological study suggests that glutamatergic neurotransmission is involved in trauma-induced behavioral dysfunctions in a subunit-selective manner. Trauma exposure evoked increases in NR2B subunit containing NMDA receptor expression, which possibly contributed to behavioral changes. Intriguingly, signaling mechanisms mediated by these receptors alter different behavioral effects of trauma in different directions. Our findings may contribute to a better understanding of the pathomechanisms of PTSD.

## P 39.

**DISSOCIATION OF FEAR LEARNING AND EXPRESSION BY A PSYCHEDELIC  
SEROTONIN 5-HT<sub>2A</sub> RECEPTOR AGONIST**

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Serotonin 5-HT<sub>2a</sub> receptors are the principal targets of psychedelic drugs, which are presently reconsidered for therapeutic use in the treatment of anxiety disorders. 5-HT<sub>2a</sub> receptor polymorphisms are associated with anxiety disorders, but it remains unclear how activation of 5-HT<sub>2a</sub> receptors may suppress core symptoms such as excessive fear. In the present study we investigated the effects of the synthetic psychedelic 5-HT<sub>2a</sub> receptor agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) and the non-psychedelic 5-HT<sub>2a</sub> agonist ergotamine (ERG) on fear learning and expression in mice using an auditory fear conditioning task. Systemic administration of DOI (2 mg/kg) before conditioning did not affect fear learning but administration before testing abolished fear expression. In contrast, systemic administration of ERG (0.5 mg/kg) had no effect on either fear learning or fear expression. Specific 5-HT<sub>2a</sub> receptor involvement in the observed effects of DOI was demonstrated by the absence of effects in 5-HT<sub>2a</sub> receptor knockout mice. We found that systemic administration of DOI (2 mg/kg) induced c-Fos expression in the medial prefrontal cortex and in the amygdala. Both brain regions express 5-HT<sub>2a</sub> receptors. However, fear expression was unaffected by infusion of DOI into the medial prefrontal cortex but was suppressed by infusion of DOI (1 µg/µl) into the amygdala. Our data demonstrate that the psychedelic DOI suppresses fear expression specifically through activation of amygdala 5-HT<sub>2a</sub> receptors.

**P 40.**  
**RESPONSE TO SOCIAL CHALLENGES: THE ROLE OF 2-ARACHIDONOYLGLYCEROL SIGNALING**

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In the previous years the impact of endocannabinoid signaling on emotional behavior was widely studied as this signaling pathway presents a promising target in the pharmacological treatment of emotional disorders. Recent studies suggest that endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) affect behavior differentially. Anandamide has been investigated extensively but studies on the specific behavioral roles of 2-AG only recently became possible and its involvement in social behaviors has not yet been investigated. In the present work we conducted a comprehensive investigation on the social impact of 2-AG. With the employment of JZL184 a monoacylglycerol lipase (MAGL) inhibitor 2-AG signaling was enhanced in mice who were later submitted to the resident-intruder, sociability and social interaction paradigms, respectively. In the resident-intruder paradigm JZL184 near completely abolished aggressiveness and increased victimization in the residents while the level of defensiveness remained unaltered despite the large increase in bites received. In the case of intruders, JZL184 exerted similar negative effects on bites and offensive behavior, while interestingly agitation and defensiveness during, and the corticosterone response to aggressive encounters were also increased. Our results regarding sociability and social interactions suggest that JZL184 treatment has broader effects on social behavior and deeply affects the way in which the animal responds social challenges. Taken together our findings show that 2-AG has an unusually strong negative influence on aggressive behavior and plays an important role in the modulation of social behavior.

**P 41.**  
**FOXP IN *Octopus vulgaris***

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We report evidence of the presence of FoxP in the transcriptome of the cephalopod mollusc *Octopus vulgaris*. We studied the expression of this gene in the central and peripheral nervous system of the animal at different life stages, and describe the pattern of distribution in several areas of the nervous system. We also explored the presence in these genes - we fished in the octopus transcriptome - of functional protein domains typical of FoxP2 and other FoxP proteins of higher vertebrates by *in silico* approaches. Finally, based on our preliminary results, we tested the hypothesis of involvement of *O. vulgaris* FoxP in the control of body patterning, i.e. the capability animals possess to change their appearance for mimicry and communication due to neural control of chromatophores and muscles in their skin.

**P 42.****OPTIMISTIC EXPECTANCIES INCREASE CELL MEDIATED IMMUNITY IN RATS**

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It has been shown that psychological factors can influence the onset and progression of immunologically mediated diseases. Most papers has focused on the determination of impact of negative emotion on disease susceptibility and course of illness. In contrast, the effects of dispositional optimism, as defined by generalized positive expectation for the future, on the immune system is poorly recognized and few human studies yield mixed results. Recent development of the ambiguous-cue interpretation (ACI) test, a task that can be used to measure the natural propensity of rats to interpret environmental stimuli in a positive or negative manner established possibility to study the impact of optimism and pessimism on immunological system in animals. The behavioral tasks were performed in computer-controlled Skinner boxes. During positive tone training phase, the rats were trained to press the lever located on the left side to feeder to receive the sucrose solution when a tone signaled reward availability. During negative tone training stage, the rats were trained to press the lever located on the right said to the feeder to avoid an electric shock when another tone signaled a forthcoming punishment. The ambiguous-cue interpretation testing session consisted of 20 positive, 20 negative, and 10 intermediate tone presentations. On the basis of the average cognitive bias index obtained from series of 10 consecutive ACI tests performed in 1-week intervals, the rats were divided into two subgroups of “optimistic” and “pessimistic” animals. In “optimistic” animals it was observed increase in relative spleen and thymus weight, increase in proliferative activity of T and B lymphocytes in response to mitogens and increase in metabolic activity of splenocytes in comparison to “pessimistic” animals. These initial finding suggest that individuals in trait positive emotional state show increase in some parameters of cell-mediated immunity.

**P 43.****ROLE OF THE ENDOCANNABINOID SYSTEM IN THE BEHAVIORAL ALTERATIONS OBSERVED IN THE RAT VALPROIC ACID MODEL OF AUTISM**

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Autism spectrum disorders (ASD) are characterized by impaired social behaviors and communication associated with stereotyped behaviors, for which no effective treatments are yet available. Prenatal exposure to valproic acid (VPA) has been proposed as a rodent model of ASD. The first purpose of this study was to investigate whether VPA prenatal exposure alters ultrasonic vocalization (USV) emission and homing behavior in the rat offspring. Furthermore, since several studies showed that endocannabinoids modulates socio-emotional behaviors, the second purpose of this study was to test the ability of cannabinoid drugs to correct the behavioral deficits found in VPA-exposed rats. Wistar pregnant rats were treated intraperitoneally with VPA (500mg/Kg/2ml) or saline (SAL) at post natal day 12 (PND12). After delivery, at PND 5, 9 and 13, pup USVs were registered during 3 minutes of isolation from the nest. At PND13, homing behavior was assessed, defined as the ability of the pup to recognize nest bedding compared with fresh bedding. VPA-prenatally exposed male rats vocalized less compared to SAL-exposed males at PND5 ( $p<0,05$ ) and 9 ( $p<0,05$ ). In the homing test, VPA-prenatally exposed male animals showed a decrease in the latency to reach the familiar bedding ( $p<0,05$ ) and in the time spent in the familiar litter ( $p<0,05$ ) compared to SAL-treated animals. No differences in USV emission and homing behavior were found in the female offspring. Interestingly, the anandamide hydrolysis inhibitor URB597 corrected the altered USV emission displayed by VPA-exposed rats. Ongoing experiments are evaluating the effects of URB597 on the altered homing behavior displayed by VPA-treated offspring. Altogether, these findings reveal that early embryonic exposure to VPA in rats provides a good model for specific aspects of ASD and is a valuable tool to explore potential pharmacological targets for this disease.

## P 44.

**PHARMACOLOGICAL INSIGHTS INTO SOCIAL PLAY BEHAVIOR: THE ROLE OF NUCLEUS ACCUMBENS DOPAMINE**

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Social play behavior is highly rewarding and is mediated by nucleus accumbens (NAc) opioid and endocannabinoid neurotransmission. Dopaminergic neurotransmission in the NAc modulates the rewarding properties of both natural and drug rewards. Therefore, the present study had a twofold aim: 1. investigate the role of NAc dopamine in social play behavior; 2. investigate the crosstalk between dopamine, opioid and endocannabinoid neurotransmission in the regulation of social play behavior.

We equipped adolescent Wistar rats with bilateral guide cannulae aimed at the NAc, and briefly isolated them before testing. Intra-NAc infusion of low doses of the psychostimulant amphetamine increased social play behavior. The effects of amphetamine were antagonized by intra-NAc co-infusion of a non-effective dose of the non selective dopamine receptor antagonist alpha-flupentixol and by intra-NAc co-infusion of a non-effective dose of the D2 receptor antagonist eticlopride. Intra-NAc infusion of either alpha-flupentixol or eticlopride in rats isolated for 2 h before test did not affect social play behavior; conversely, intra-NAc infusion of alpha-flupentixol and eticlopride after a 24 h or 7days social isolation decreased social play behavior. Intra-NAc infusion of alpha-flupentixol also antagonized the play-enhancing effects induced by systemic treatment with the anandamide hydrolysis inhibitor URB597 and the opioid receptor agonist morphine. Altogether, these findings show that NAc dopaminergic neurotransmission plays an important role in social play and that it interacts with endocannabinoid and opioid systems in the modulation of this behavior.

## P 45.

**EFFECTS OF MEMANTINE ON ACTIVITY AND SLEEP PATTERNS IN DROSOPHILA SHAKER MUTANTS**

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In the last times, the depth knowledge of simple organisms like the fruit fly *Drosophila melanogaster* has expanded. The *Drosophila* model is used not only for genetic analysis but, also, as a tool for studying the mechanisms of neurodegenerative diseases, sleep disorders and, recently, mood disorders. In Sardinia the prevalence of such severe psychiatric illness is doubled compared to other Italian regions.

Ion dysregulation is thought to be involved in the pathophysiology of bipolar illness and, in acute manic patients, increased glutamate levels have been shown. Clinical studies demonstrated the efficacy of memantine (MEM, a non competitive NMDA receptor antagonist), as antidepressant-like and mood-stabilizing drug in patients resistant to classical therapy, although its specific mechanism is not clear.

The present study aims to characterize this model for exploring the potential neuroprotective effects MEM on waking activity and sleep patterns in *Drosophila Shaker* (*Sh*) mutants. Such *Sh* mutants, carrying an alteration in the potassium Shaker channel, show reduced sleep duration, motor hyperactivity and shorter lifespan, all of these could be considered as an endophenotype of bipolar disorder.

In *Sh* mutants and their controls, both sexes, memantine (5 and 10 mg/100 g diet) was tested for three weeks. Twenty-four hour motor activity and sleep at the beginning and every week of treatment were measured by the DAMS infrared system and then analyzed by pySolo dedicated software. After treatment, lifespan was measured calculating the percentage of survivorship daily. In *Sh* mutants both doses of MEM significantly affect their sleep patterns improving sleep episodes, quality and rhythm. Reduced motor activity and increased the life span have been observed, with opposite effects in wild type flies.

Western blot analysis showed increased expression of NMDA receptor NR1 subunit in untreated *Sh* mutant compared with wild type brains, suggesting that NMDA neurotransmission may play a role in their behavior.

## P 46.

**24 H TOTAL SLEEP DEPRIVATION EFFECTS ON SLEEP RECOVERY PERIOD AND GENES INVOLVED IN GLUCOSE METABOLISM EXPRESSION IN MICE**

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Total sleep deprivation (TSD) effects on sleep are well described, however no consistent data are available about their long terms effects. Ventrolateral preoptic nucleus (VLPO) is identified as the structure containing neurons responsible of slow wave sleep (SWS) which is involved in restauration mechanisms of energy reserves. Metabolism response to prolonged wakefulness is suspected to influence transition from wakefulness to SWS. Our first aim is to determine TSD effects on homeostatic and circadian sleep during the recovery period. Our second aim is to determine TSD effects on genes involved in glucose metabolism expression by studying 4 genes of interest (*hk4*, *glut1*, *glut3*, *irs4*) in different cerebral structures (VLPO, hypothalamus, hippocampus, cortex, MPO).

24 h TSD was produced by slowly rotating wheel in 13 C57BL/6 mice. 8 mice were implanted with telemetry transmitter recording EEG, locomotor activity and temperature before (baseline 24h), during (24h), and after TSD (48h). A second group of 5 mice were sacrificed to collect cerebral structures for RT-PCR genes expression determination. A third group of 5 mice were not sleep deprived (control) and sacrificed to collect cerebral structures for RT-PCR.

TSD increased non-REM sleep and delta power with no effect on REM sleep during the 48h recovery period as compared to baseline. Parameters of the circadian rhythms of temperature and locomotor activity were not different between baseline, TSD and recovery. TSD decreased (mRNA) *glut3* expression in the VLPO, and (mRNA) *hk4* expression in the MPO. No effect of TSD on (mRNA) *irs4* and *glut1* expression was observed.

In conclusion, 24h TSD has induced significant effects on homeostatic sleep with no effect on circadian sleep during recovery period and modulated *glut3* and *hk4* expressions in the cerebral structures involved in homeostatic sleep regulation process, indicating that glucose metabolism neuronal pathway is impacted by TSD.

## P 47.

**BEHAVIORAL EFFECTS OF THE OLIVE COMPOUND OLEUROPEINE**

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Olives and olive products, as part of the Mediterranean diet have been suggested to be beneficial against cognitive decline, dementia and Alzheimer's disease. Oleuropein (oleu) is a natural phenolic antioxidant, which is present in elevated concentration in olives, olive oil and olive tree leaves (*Olea europaea* L.). In the current study we investigated the effects of the compound Oleuropein, on the behavioral phenotype of an Alzheimer disease mouse model (3xTg), which expresses mutated forms of human proteins Tau, APP and Presenilin1. This model has high validity because it exhibits the main pathological aspects of Alzheimer's disease. Male and female wild type (wt) and transgenic (3xTg) mice were used at the age of 9-11 months old. The behavioral screening included activity, anxiety, depression and memory/learning tests, such as the open field, light/dark, Y maze, novel object recognition and tail suspension tests. The 3xTg mice exhibit reduced locomotor activity, which was not reversed by oleuropein treatment. However, oleuropein treatment resulted in the improvement of the reduced exploratory activity of 3xTg mice towards a novel environment. Sex differences were only observed in stereotypic behavior of 3xTg mice. Anxiety levels and "depressive-like" behavior were not affected by genotype, sex or treatment. In novel object recognition task 3xTg mice exhibited mild cognitive deficits, which were reversed by oleuropein treatment. Conclusively, it seems that oleuropein exerts a mild beneficial effect in cognition. However, more studies are required, in order to elucidate its role of in Alzheimer's disease.

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## P 48.

**TARGETING SUBSTANTIA NIGRA THROUGH OXYTOCIN RECEPTORS**

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The paraventricular nucleus of the hypothalamus (PVN) contains parvocellular oxytocinergic neurons projecting to extrahypothalamic brain areas such as the hippocampus, amygdala, ventral tegmental area (VTA) and substantia nigra (SN). Some oxytocin functions in any of these areas have already been identified whereas the role of oxytocinergic projections to the SN and the nature of the interaction between oxytocin and the nigral dopaminergic neurons involved in the voluntary control of movement are still unclear. The aim of this study was to investigate the effect, on the dopaminergic nigrostriatal function, of the injection into the SN of a novel cytotoxin that selectively targets and destroys cells expressing oxytocin receptors.

Male Sprague Dawley rats (n=24) were monilaterally or bilaterally injected into the SN with the toxin or vehicle (PBS). Locomotor activity was assessed before and two and four weeks after injection using a Digiscan Activity Analyser. Immunohistochemistry was used to verify the presence and extent of the lesion in the dopaminergic neurons and to investigate any modifications in the GABAergic and the glutamatergic systems.

After four weeks, animals bilaterally injected with the toxin showed a significant increase in locomotor activity (60%), not observed in monilaterally injected animals, whereas PBS injection had no effect. In toxin injected animals, Tyrosine Hydroxylase immunoreactivity was reduced in SN compacta somas and in their dendrites coursing into the pars reticulata while no evident variation in Glutamate Decarboxylase immunoreactivity was observed. Interestingly, preliminary results showed a tendency towards reduction in SN Vesicular Glutamate Transporters (VGluT1, VGluT2 and VGluT3) immunoreactivity, possibly correlated with the dopaminergic lesion extent, suggesting the potential induction of alterations also in the glutamatergic system.

In conclusion, the increase in locomotor activity observed despite the dopaminergic degeneration suggests that oxytocin function in SN might involve other neuronal systems in addition to the dopaminergic one.

P 49.

**AUTOMATED GAIT ANALYSIS FAILS TO DETECT CONTROLLED CORTICAL  
IMPACT-INDUCED MOTOR DEFICIT IN RATS**

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Animal models with constant, long-lasting motor abnormalities are needed together with testing methods, which are capable of assessing this deficit. In the current study controlled cortical impact (CCI) was applied in rats to induce damage of the forelimb area of the motor cortex to induce functional impairment of the contralateral limb.

Motor performance was assessed pre- and post-operatively for a period of 6 weeks using the automated gait analysis system CatWalk XT, the cylinder test, the Montoya staircase test and the adhesive removal test.

Lesion volumes were measured stereologically and CCI created robust histopathological lesions in the motor cortex. However, no behavioral abnormalities were detected at any time point after surgery using the CatWalk, while the cylinder test, staircase and adhesive removal test did show a clear behavioral deficit in the contralateral forelimb of CCI rats compared to controls.

CCI on the motor cortex has proven to be a reliable model for induction of chronic motor deficits restricted to the contralateral forelimb. The CatWalk gait analysis system appeared not to be a suitable test to detect motor deficits and other tests, tailored to measure fine motor behavior, must be used to reliably measure functional impairment.

**P 50.*****IN VITRO* ELECTROTAXIS: LOW CURRENT DENSITIES ATTRACT  
NEURAL PRECURSOR CELLS**

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Neural precursor cells (NPCs) have the capacity to sense and react to electrical fields (EFs) by migrating towards the cathode *in vitro*, a process called electrotaxis. The present study elucidated the effect of a number of varying current amplitudes on migration of NPCs.

SH-SY5Y cells, positive for stem cell markers Sox-2 and DCX, were chronically exposed to an EF for a period of 24 h in a live cell imaging setup to visualize and evaluate cell migration. Electrical stimulation took place in a 12-well plate where SH-SY5Y cells adhered to the bottom of the wells. Monophasic cathodal current pulses were delivered with varying current densities of 5, 10, 20 and 40  $\mu\text{A}$  at a fixed frequency of 50 Hz and a pulse width of 0.1 ms. Non-stimulated cells were used as a negative control to account for random patterns of cell migration. Pictures taken during the 24 h stimulation period were stacked and the migration paths of individual cells were tracked manually using ImageJ. Displacement of SH-SY5Y cells towards the cathode was evaluated by assessing direction, velocity and distance of migration.

Depending on the delivered current, SH-SY5Y cells showed increased displacement towards the cathode. When compared to non-stimulated cells, cells stimulated with either 5 or 10  $\mu\text{A}$  ended up significantly closer to the cathode after 24 h of EF exposure. Furthermore, exposure to the highest current density of 40  $\mu\text{A}$  led to a visible decrease in cell viability.

In the current study usage of the lowest stimulation currents (e.g. 5 or 10  $\mu\text{A}$ ) was found most effective in inducing electrotactic migration of SH-SY5Y cells. Studies assessing the effect of those stimulation parameters on cell phenotype and viability are still ongoing. Furthermore, the exact mechanism how extracellular EFs are translated into cytoskeletal changes needs to be identified.



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