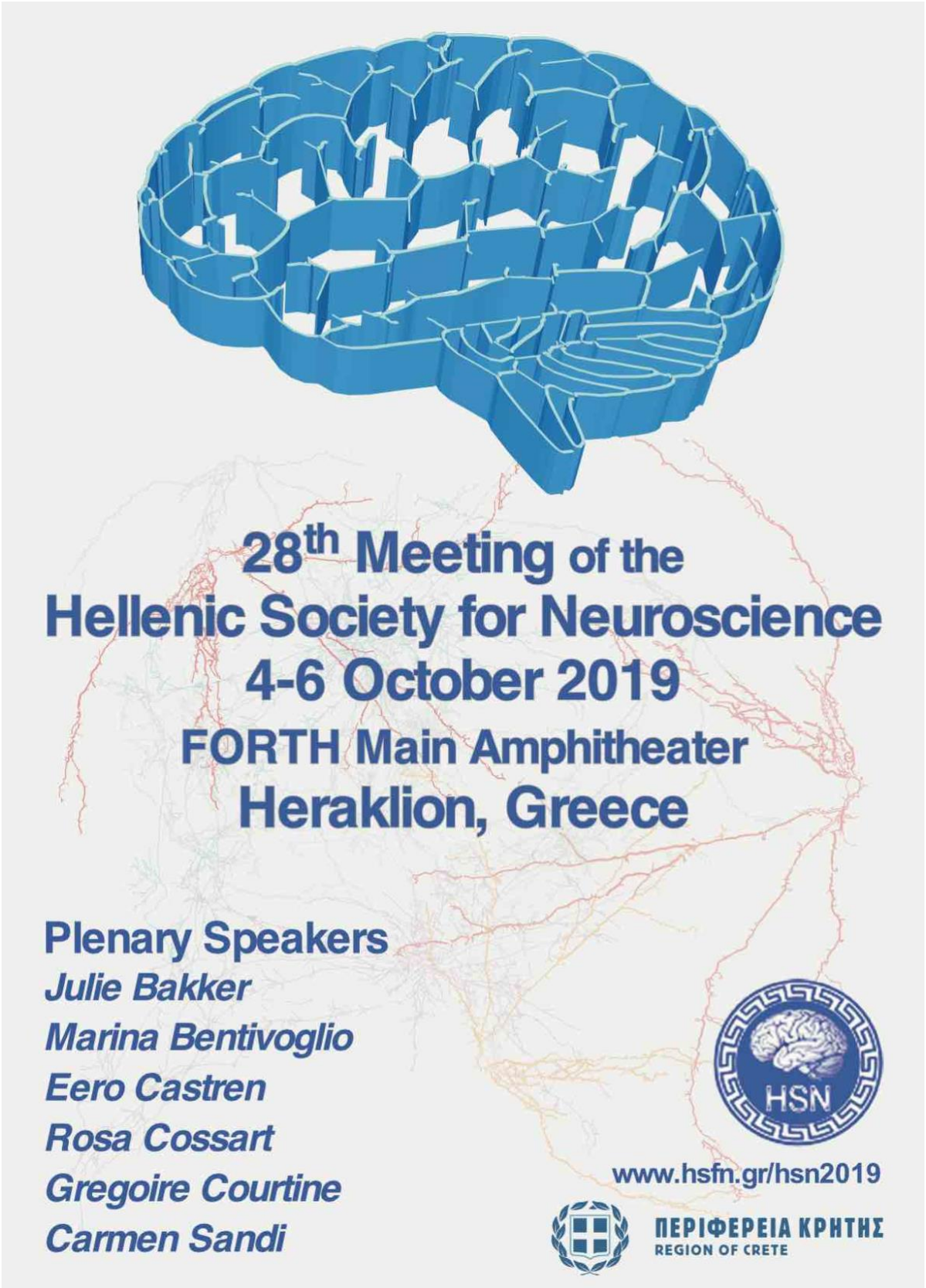

Book of abstracts

PLENARY LECTURES

Plenary lecture 1

Friday, 4 October 2019
10:00-11:00am

Chair: Prof. Domna Karagogeos
Faculty of Medicine, University of Crete and Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas

Eero Castren
Neuroscience Center, HiLIFE
University of Helsinki
Finland.

Title: Neurotrophic factors, neuronal plasticity and the antidepressant effect

Abstract
Experience-dependent plasticity tunes developing neuronal networks to optimally represent external and internal milieu. Brain-derived neurotrophic factor (BDNF) through its receptor TRKB is a critical regulator of activity-dependent plasticity. We have shown that antidepressant drugs activate TRKB signaling and thereby reactivate juvenile-like plasticity in the adult mammalian brain, including mood-related networks. We recently found that both typical and fast-acting antidepressants directly bind to a cholesterol interaction motif in TRKB. Mutation of this TRKB cholesterol interaction site or cholesterol depletion by pravastatin impair BDNF-mediated plasticity and cellular and behavioral responses to antidepressants *in vitro* and *in vivo*. Using light-activatable TRKB receptors have demonstrated a critical role for TRKB signaling in parvalbumin-containing interneurons as orchestrator of cortical network plasticity. We suggest that binding to and activation of TRKB is the common mechanism of antidepressant action, and propose that for optimal results, plasticity-inducing antidepressant treatment should be combined with physical or psychological rehabilitation that guides the plastic networks towards recovery.

PLENARY LECTURES

Plenary lecture 2

Friday, 4 October 2019
19:00-20:00am

Chair: Assoc. Prof. Christina Dalla, Faculty of Medicine, National Kapodistrian University of Athens

Julie Bakker
Neuroendocrinology, GIGA Neurosciences
Liège University, Belgium

Title: Gender identity, sex hormones, and the developing brain

Abstract

Gender incongruence is defined as a marked and persistent incongruence between an individual's experienced gender and the at birth assigned sex. This talk will provide an overview on the possible neurobiological mechanisms underlying gender incongruence. A prominent hypothesis on the etiology of gender incongruence has proposed that the condition is related to an altered or less pronounced sexual differentiation of the brain. Indeed, evidence was obtained for sex-atypical responses in adolescents diagnosed with gender incongruence, i.e., hypothalamic responses to the male-typical chemosignal androstadienone were in line with their experienced gender in both transgender boys and girls, as well as masculinized neural activity patterns while performing a mental rotation task in trans boys. By contrast, at the structural level, gray matter volumes were largely concordant with their sex assigned at birth. This suggests that there are most likely additional neural mechanisms underlying gender incongruence. An alternative hypothesis on gender incongruence was recently proposed suggesting that the body dysphoria experienced by transgender individuals might be related to different own-body perception and altered underlying neural networks involved in self-referential thinking. This hypothesis is based primarily on transgender-specific patterns in functional connectivity (FC), and in particularly those involving the pregenual anterior cingulate cortex, which is important for self-perception. Accordingly, a trans-specific FC pattern was observed in the visual network, for which no sex differences were observed, in adolescent trans girls. Taken together, gender incongruence could be related to an altered sexual differentiation of the brain or to an altered cerebral network involved in own body perception, or to both. More research is clearly needed in particularly in light of the dramatic but unexplained rise in number of referrals to specialized gender identity clinics worldwide and thus increasing demand for hormonal interventions.

PLENARY LECTURES

Plenary lecture 3

Saturday, 5 October 2019
9:00-10:00am

Chair: Ass. Prof. Kyriaki Sidiropoulou, Dept of Biology, University of Crete and Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas

Rosa Cossart
Neuroendocrinology, GIGA Neurosciences
Liège University, Belgium

Title: How development scaffolds internal hippocampal dynamics

The hippocampus spontaneously generates sequences of assembly activation that mirror its function in organizing experience in time and space. These sequences arise from the interaction between external sensory inputs and internally-generated *self-organized* activity.

During development, spontaneous hippocampal dynamics are tightly correlated to sensorimotor inputs and highly coordinated among most neurons through the action of early born GABA hub cells (Bonifazi et al. 2009, Picardo et al. 2012). In contrast, internal dynamics are a strong organizing factor in the adult hippocampus and spontaneous activity is dominated by a sparse, but stable subset of active neurons organized in functionally orthogonal "assemblies" (Malvache et al. 2016).

In sum, there is both an **internalization** and a **sparsification** of hippocampal dynamics as development proceeds with the emergence of basic functional modules in the form of **hippocampal assemblies**. Both processes are likely to rely on the development of functional GABAergic circuits.

Our lab studies the circuit basis of hippocampal assemblies from development into adulthood and across different states of the normal and pathological brain. To describe the circuit basis of cortical dynamics, we have developed a unique multidisciplinary approach that combines *in vitro* and *in vivo* calcium imaging, electrophysiology, holographic photo-stimulation, neuroanatomy, data mining, mouse genetics and behavior.

In this talk I will present both published and unpublished data from my lab indicating that internal hippocampal dynamics are remarkably structured and organized and that this functional organization is set very early during development.

PLENARY LECTURES

Plenary lecture 4

Saturday, 5 October 2019
14:30-15:30am

Chair: Ass. Prof. Foteini Stylianopoulou, Director, Hellenic Pasteur Institute

Carmen Sandi
Brain Mind Institute, Ecole Polytechnique Federal de Lausanne (EPFL), Switzerland

Title: *Neural circuits for anxious actions*

Abstract
There is important inter-individual variation in coping responses and motivated behavior under stress, and trait anxiety is revealing as a key moderator of this variation. Our work in animals and humans implicates a key role of the mesolimbic system and identifies the involvement of metabolism and mitochondrial function in nucleus accumbens circuits in the link between anxiety and motivated actions. Our findings have implications for the understanding of the mechanisms involved in individual differences in motivated behavior and vulnerability to stress.

PLENARY LECTURES

Plenary lecture 5

Sunday, 6 October 2019
9:00-10:00am

Chair: Assoc. Prof. Georgia Gregoriou, Faculty of Medicine, University of Crete and Institute of Applied and Computational Mathematics - Foundation for Research and Technology Hellas

Gregoire Courtine
Center for Neuroprosthetics and Brain Mind Institute, School of Life Sciences, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland
Department of Neurosurgery, Lausanne University Hospital (CHUV) and University of Lausanne (Unil), Lausanne, Switzerland

Title: *Neurotechnologies restoring motor control after paralysis*

Over the past 15 years, my research team have developed a multipronged intervention that reestablished voluntary control of paralyzed legs in animal models of spinal cord injury, and recently in humans. This intervention acts over two time-windows. Immediately, electrical and chemical stimulations applied to the lumbar spinal cord reactivate lumbar execute centers located below the injury that coordinate leg movements, enabling voluntary control of paralyzed muscles during locomotion. In the long term, will-powered training regimens enabled by these electrochemical stimulations and cutting-edge robotic assistance promote the reorganization of residual connections that restores voluntary movements without stimulation. During my talk, I will discuss the technological and conceptual development of these interventions in preclinical models, how we translated these developments in humans with SCI, and how we envision the next steps to establish a clinically viable treatment.

SYMPOSIA

S1: Brain-Gut and Mood

Friday, 4 October 2019
11:00-12:30

Chair: Arjan Brokland, Maastricht University, The Netherlands

Speaker 1
Dietmar Fuchs
Innsburk University
Austria

Title: Cognitive Aging: insights from functional and molecular neuroimaging

The lecture will give a neuroscientist perspective on cognitive aging, emphasizing the contribution of human brain imaging to reveal the biological underpinnings of the cognitive changes observed with age. Functional magnetic resonance imaging has allowed investigating how brain activity and connectivity between regions change as we age. Positron emission tomography imaging has demonstrated the high prevalence of neurodegenerative lesions, such as amyloid plaques and tau tangles, in older adults with normal cognition for their age. The development of Alzheimer's biomarkers has redefined the concept of the most frequent age-associated cognitive disorder as a biological entity. In the same time, it questions our understanding of cognitive aging, as asymptomatic Alzheimer's pathology is present in most seniors. Tracking the progressive accumulation of Alzheimer's pathology in clinically normal older adults now allows investigating the impact of this pathology on cognition, and opens up new research avenues to test therapeutic targets in preventive clinical trials.

Speaker 2
Gerard Clarke
[1]Department of Psychiatry and Neurobehavioural Science, University College Cork, [2]APC Microbiome Ireland, University College, [3]INFANT Research Centre, University College Cork
Cork, Ireland

Title The Gut Microbiome, Brain Function and Behaviour: Moving the Goalposts in Psychiatry?

Experimental evidence continues to accumulate from both preclinical and clinical studies to support the concept that the gut microbiota can regulate brain function, structure and behaviour. Within this conceptual framework, the gut microbiome can signal along the gut-brain axis to influence many fundamental aspects of the central nervous system. This includes a major influence on host stress physiology as well as a broad range of behaviours relevant to stress-related psychiatric disorders including depression and anxiety, and functional gastrointestinal disorders such as irritable bowel syndrome. Stress exposures across the life span can also alter microbiome-gut-brain axis signalling, including gut microbiome composition and function, illustrating the bidirectional nature of this host-microbe dialogue.
Research efforts continue to identify the precise mechanisms underpinning these effects and possible routes of communication include the vagus nerve, the neuroendocrine system and immune factors. There is now an increasing focus on microbial regulation of tryptophan metabolism and the serotonergic system arising from a number of key observations taken from a variety of strategies used to parse the role of microbiota in brain function. These include germ free animals, antibiotic treatments, dietary manipulations, probiotics and prebiotics. Microbiota-deficient animals in particular consistently show alterations in the availability of tryptophan as well as serotonergic alterations in the gastrointestinal tract and the CNS. Many of the behaviours influenced by the gut microbiota rely on intact serotonergic neurotransmission and serotonin is a key stress-responsive neurotransmitter at both terminals of this bidirectional communication network.
Taken together, these studies support the possibility of microbial-directed management strategies to improve brain function and behaviour. An important option in this regard is the use of psychobiotics to modulate signalling along the microbiome-gut-brain axis. To date, this approach has met with some success mixed with failures to translate in healthy study participants. Further translational insights and well-controlled human studies in stress-related disorders will be critical as we transition from promising preclinical research towards mechanisms and therapeutic targeting of the gut microbiome in the clinical setting.
Research Support: GC is a faculty member of APC Microbiome Ireland and an associate investigator in the INFANT research centre which are funded by Science Foundation Ireland (SFI; grant numbers: SFI/12/RC/2273 and SFI/12/RC/2272 respectively). Other funding sources include the Health Research Board (HRB) through Health Research Awards (grant numbers HRA_POR/2011/23 and HRA-POR-2-14-647). GC has also been

supported by a NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation (Grant Number 20771) and by the by European Office of Aerospace Research and Development/Air Force Office of Scientific Research (grant number FA9550-17-1-0016).

Speaker 3
Nora Schneider
Neurocognition Group, Nestlé Research
Lausanne, Switzerland

Title: Food & Brain Development

Early childhood is an important and dynamic period of brain growth and development where the brain forms neural networks that serve as a basis of how we think, feel and act. Neural networks connect structurally and functionally in a way that it facilitates fast and efficient brain communication, with myelination, the process that wraps nerve fibers with a lipid-rich sheath, being one of the key neurodevelopmental processes involved in the refinement and shaping of those brain connections.
Early feeding practices and nutrition play a fundamental role in supporting these physiological processes and thus optimal brain and cognitive development, however, more research is needed to identify key nutritional factors and the nature of specific nutrients that support neurodevelopmental processes like brain connectivity and myelination.
Today's lecture will describes the role of infant nutrition in brain development, myelination and related cognitive development, with a special focus on how lipids may play a crucial role in myelination.

SYMPOSIA

S2: The shape of treatments to come: disease-modifying therapies in neurodegenerative diseases

Sponsored By Biogen

Friday, 4 October 2019
13:00-14:30

Chairs: Leonidas Stefanis, University of Athens Medical School and Alexia Polissidis, Collaborating Researcher, Laboratory of Neurodegenerative Diseases, Biomedical Research Foundation Academy of Athens

Speaker 1
George Nomikos, Biogen
USA

Title: Alpha-synuclein antibodies "SPARK" interest in mitigating Parkinson's disease progression

Aggregated alpha-synuclein (α-syn) is a major constituent of Lewy bodies, found in Parkinson's disease (PD), and is thought to play a central role in the pathology and progression of the disease. BIIB054 is a recombinant human monoclonal antibody (mAb) under development by Biogen that binds with subnanomolar affinity to the N-terminal region of aggregated α-syn with much lower affinity for the monomer. Favorable toxicology has been observed in chronic exposure studies in rats and monkeys. A first-in-human, single ascending dose study of BIIB054 in healthy controls and PD patients demonstrated a favorable safety and pharmacokinetic profile. The SPARK trial is an adaptively-designed, randomized, double-blind, placebo-controlled, parallel-group study testing the safety and biological effects of three dosages of BIIB054 compared to placebo studied in two staggered cohorts (24 in cohort A, 287 in cohort B). Participants with early, untreated PD recruited at approximately 90 international sites will receive randomized treatment (monthly IV infusions) for two years. The primary objective is to evaluate dose-related safety of BIIB054. Secondary objectives are to assess the pharmacokinetic and pharmacodynamic effects of BIIB054 through dopaminergic imaging and clinical symptom burden. The first subject in the SPARK trial was enrolled in January 2018, the primary endpoint is estimated to be completed by May 2021 and the trial's completion is anticipated by June 2021. The design and status of the study will be presented. It is hoped that results of the study may support further evaluation of BIIB054 as a potential treatment for PD.

Speaker 2
Jos Prickaerts
Maastricht University, The Netherlands

Title: Phosphodiesterase inhibitors: re-erecting their therapeutic potential for the treatment of dementia

Cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) have been suggested to play specific roles in processes of memory. These cyclic nucleotides are hydrolyzed by specific enzymes, i.e. phosphodiesterases (PDEs). Thus, selective PDE inhibitors preventing the breakdown of cAMP and/or cGMP could improve memory. Rodent studies with different timing of treatment with specific PDE inhibitors indicated that distinct underlying signaling pathways for early and late consolidation processes exist corresponding to specific time-windows for memory consolidation into long-term memory. Most likely the underlying mechanisms are a cGMP/PKG pathway for early consolidation processes and a cAMP/PKA pathway for late consolidation processes. In addition, the early-phase cGMP/PKG signaling actually requires late-phase cAMP/PKA-signaling in long-term memory formation. Recently, it has been shown that elevation of central cGMP levels or cAMP levels after treatment with a specific PDE inhibitor both improve acquisition processes/short-term memory as well. In vitro studying the effects of PDE inhibitors on long-term potentiation, the physiological substrate of memory, support the in vivo data and further show that AMPA receptor trafficking very likely mediates the memory enhancing effects.
In a translational approach we initially also investigated the effects of cGMP elevation via PDE5 inhibition with vardenafil or sildenafil on cognition in humans. However, in contrast to studies with rodents and monkeys, PDE5 inhibition had no effect in humans on cognition including memory processes. It is clear that the transition of a drug from preclinical to clinical creates translational hurdles. Based on the expression patterns of its isoforms in the brain, PDE4, which is cAMP specific, could be more interesting for CNS targeting than PDE5. Indeed we found that a low dose of the PDE inhibitor roflumilast clearly improved cognition in humans. Interestingly, this pro-cognitive effect was not associated with emetic side effects (nausea, vomiting), which are commonly associated with PDE4 inhibition. Based on our data we suggest that the future for disease-specific PDE enzyme inhibition lies in the development of PDE isoform-specific inhibitors without adverse effects. Within the context as described above, the latest results of specific PDE inhibitors on cognitive processes will be presented.

Speaker 3
Georgia Dermentzaki
Columbia University, USA

Title: The newcomer: m6A's role in motor neuron function
Role of m6A RNA methylation in motor neuron function

m6A (N6-methyladenosine) is a highly pervasive modification in RNA that regulates splicing, translation and stability of transcripts. Recent advances in studying RNA modifications have revealed that m6A, exerts a critical role in stem cell self-renewal and differentiation in the mouse nervous system. However, the role of m6A in the adult mammalian nervous system under physiological and pathological conditions remains to a great extent unexplored. A recent study revealed that components of the m6A machinery play an important role in axon regeneration in the peripheral nervous system *in vivo* following nerve injury. In the present study we aim to determine the importance of m6A RNA modification in motor neuron function under normal conditions as well as in neurodegeneration. By downregulating different determinants of the m6A pathway in embryonic stem cell-derived motor neurons (ES-MNs) we identified the components of the m6A machinery that are important for motor neuron survival and neurite outgrowth. In particular, CRISPR-mediated knockout of *Mettl3*, the core enzyme catalyzing the m6A modification, in two different ES lines, severely affected survival and neurite outgrowth of motor neurons, while had a little effect on the rest of the neuronal subtypes. In addition, mice with conditional, MN-specific KO of *Mettl3* exhibit muscle weakness as assessed by grip strength behavioral tests. We are currently using our conditional *Mettl3* KO model to examine whether motor neuron survival and neuromuscular junctions are impaired. In parallel, we are using MNs derived from *Mettl3* KO ES lines to identify m6A downstream targets mediating the selective motor neuron degeneration. We anticipate that the identified targets will have far-reaching implications for our understanding not only of motor neuron function but also of the molecular mechanisms underlying neurodegeneration in motor neuron disorders.

SYMPOSIA

Speaker 4
Panagiota Mavroeidi
Biomedical Research Foundation of the Academy of Athens, Greece

Title: It takes two to tangle: α-synuclein and p25α orchestrate pathology in Multiple System Atrophy

Multiple system atrophy (MSA) is characterized by the presence of distinctive glial cytoplasmic inclusions (GCIs) within oligodendrocytes that contain the neuronal protein alpha-synuclein (aSyn) and the oligodendroglia-specifc phosphoprotein TPPP/p25α. However, the role of oligodendroglial aSyn and p25α in the formation of aSyn-rich GCIs remains unclear. To address this conundrum, we have applied human aSyn (haSyn) pre-formed fbrils (PFFs) to rat wild-type (WT)-, haSyn-, or p25α-overexpressing oligodendroglial cells and to primary differentiated oligodendrocytes derived from WT, knockout (KO)-aSyn, and PLP-haSyn-transgenic mice. HaSyn PFFs are readily taken up by oligodendroglial cells and can recruit minute amounts of endogenous aSyn into the formation of insoluble, highly aggregated, pathological assemblies. The overexpression of haSyn or p25α accelerates the recruitment of endogenous protein and the generation of such aberrant species. In haSyn PFF-treated primary oligodendrocytes, the microtubule and myelin networks are disrupted, thus recapitulating a pathological hallmark of MSA, in a manner totally dependent upon the seeding of endogenous aSyn. Finally, delivery of haSyn PFFs into the mouse brain led to the formation of aberrant aSyn forms, including the endogenous protein, within oligodendroglia and evoked myelin decompaction in WT mice, but not in KO-aSyn mice. This line of research highlights the role of endogenous aSyn and p25α in the formation of pathological aSyn assemblies in oligodendrocytes and provides in vivo evidence of the contribution of oligodendroglial aSyn in the establishment of aSyn pathology in MSA.

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY) and by the MSA Coalition.

SYMPOSIA

S3: Systems neuroscience: from cells to circuits and behavior"

Friday, 4 October 2019
13:00-14:30

Chairs: Dinos Meletis and Andreas Kardamakis, Karolinska Institute, Sweden

Speaker 1
Sten Grillner
Karolinska Institute, Sweden

Title: The computational logics of networks in motion – evolutionary conserved forebrain strategies to control behavior

Vertebrates have an astounding ability to coordinate the complex movements required for adaptive behavior utilizing a set of motor programs, like those coordinating eye, head and locomotor movements. These motor programs have been conserved throughout vertebrate phylogeny and we now understand how many of these motor programs are designed at the microcircuit level. Moreover, the neural mechanisms that determine when a motor program is turned on depend on evolutionary conserved structures that regulate both selection and value-based decisions. The detailed circuitry of both the basal ganglia and lateral habenulae and the dopamine innervation had been developed already at the dawn of vertebrate evolution some 560 million years ago, when the lamprey diverged from the line of evolution leading up to mammals and man. Moreover, lamprey "cortex" targets the same motor areas as in mammals, and contains a visual retinotopic and somatosensory area.

Speaker 2
Gilad Silberberg
Karolinska Institute, Stockholm

Title: Dopaminergic Modulation of Sensory Circuits in the Striatum

Striatal activity is dynamically modulated by acetylcholine and dopamine, both of which are essential for proper basal ganglia function. Synchronized pauses in the activity of striatal cholinergic interneurons (ChINs) are correlated with elevated activity of midbrain dopaminergic neurons, whereas synchronous firing of ChINs induces local release of dopamine. The mechanisms underlying ChIN synchronization and its interplay with dopamine release are not fully understood. Here we show using multineuron patch-clamp recordings, voltammetry, optogenetics, chemogenetics, and in vivo recordings, that dopamine shapes striatal responses to tactile stimuli in striatal projection neurons. We also demonstrate that robust disynaptic inhibition between ChINs acts as an efficient synchronization mechanism. Inhibitory disynaptic responses were elicited by single action potentials in ChINs and showed a high degree of recurrence within the ChIN network. Disynaptic inhibition was attenuated by dopaminergic midbrain afferents acting on D2 receptors. Our results present a mechanism supporting synchronization of activity and pauses across the ChIN population and a novel form of interaction between striatal acetylcholine and dopamine.

Speaker 3
Jens Hjerling-Leffler
Karolinska Institute, Stockholm

Title: Diversity of Interneurons in the Dorsal Striatum Revealed by Single-Cell RNA Sequencing and PatchSeq

Striatal locally projecting neurons, or interneurons, act on nearby circuits and shape functional output to the rest of the basal ganglia. We performed single cell RNA sequencing of striatal cells enriching for interneurons. We find seven discrete interneurontypes, six of which are GABAergic. In addition to providing specific markers for the populations previouslydescribed, including those expressing Sst/Npy, Th, Npy without Sst, and Chat, we identify two small populations of cells expressing Cck with or without Vip. Surprisingly, the Pvalb-expressing cells do not constitute a discrete cluster but rather are part of a larger group of cells expressingPthlh with a spatial gradient of Pvalb expression. Using PatchSeq, we show that Pthlh cells exhibit a continuum of electrophysiological properties correlated with expression of Pvalb. We also show that additional parameters such as morphology, anatomical location and long-range input differ across this molecular and electrophysiological gradient, suggesting that the extremes of this gradient exhibit distinct circuit function. Furthermore, we find significant molecular differences that correlate with differences in electrophysiological properties between Pvalb-expressing cells of the striatum and those of the cortex.

Speaker 4
Andreas Kardamakis
Karolinska Institute, Stockholm

Title: Exploring visuomotor circuit dynamics in the mouse superior colliculus

The superior colliculus has been well known for its role in the control of overt orienting behaviour, and is increasingly implicated in cognitive process such as visual spatial attention. Its densely connected to the neocortex and tightly looped with several subcortical areas, making it challenging to causally attribute global function to specific inputs or cell types. I will talk about our progress in developing approaches for dissecting local circuit function and long-range interactions, i.e. bottom-up & top-down inputs to the mouse superior colliculus, by using cell-type-specific optogenetic manipulation, whole-cell electrophysiology, viral-based mapping and behaviour. Ultimately, our long-term goal is to create and probe mechanistic models for understanding and interfering with processes in the control of visual attention at the level of the individual neuron.

Speaker 5
Konstantinos Ampatzis
Karolinska Institute, Stockholm

Title: Adult spinal motoneurons change their neurotransmitter phenotype to control locomotion

A particularly essential determinant of a neuron's functionality is its neurotransmitter phenotype. While the prevailing view is that neurotransmitter phenotypes are fixed and determined early during development, a growing body of evidence suggests that neurons retain the ability to switch between different neurotransmitters. However, such changes are considered unlikely in motoneurons due to their crucial functional role in animals' behavior. Here we describe the expression and dynamics of glutamatergic neurotransmission in the adult zebrafish spinal motoneuron circuit assembly. We demonstrate that part of the fast motoneurons retain the ability to switch their neurotransmitter phenotype under physiological (exercise/training) and pathophysiological (spinal cord injury) conditions to corelease glutamate in the neuromuscular junctions to enhance animals' motor output. Our findings suggest that motoneuron neurotransmitter switching is an important plasticity-bestowing mechanism in the reconfiguration of spinal circuits that control movements.

SYMPOSIA

S4: Programming and reprogramming in the embryonic and adult brain

Saturday, 5 October 2019
10:30-12:00

Chairs: Dimitra Thomaidou, Hellenic Pasteur Institute, and Stavros Taraviras, Univeristy of Patras

Speaker 1
Christina Kyrousi, Postdoctoral Research Fellow,
Max Planck Institute of Psychiatry, Germany

Title: Modelling human brain development and disease using cerebral organoids

Basal radial glial cells (bRGs) are a neural progenitor type enriched in primates and humans and contribute to the expansion of neurons generated during cortical development in gyrencephalic species. Shortly after their generation, bRGs delaminate towards the outer subventricular zone, where they divide multiple times before terminal differentiation. The regulation of bRGs generation is essential for the establishment of correct gyrification within the human cortex. Here, we study the role of LGALS3BP, a secreted protein whose RNA expression is enriched in bRGs. By using cerebral organoids, human fetal tissues and mice, we show that manipulation of *LGALS3BP* regulated bRG generation. Additionally, individuals with unique *de novo* variants in *LGALS3BP* demonstrate abnormal gyrification and thickness at multiple sites over the cerebral cortex. Single-cell-RNA-sequencing reveals the extracellular matrix involvement in the LGALS3BP mediated mechanisms. We find that LGALS3BP is required for bRGs delamination and influences cortical development and gyrification in humans

Speaker 2
Maria Vidaki
Massachusetts Institute of Technology, USA

Title: Axonal Local Translation: Novel mechanisms of regulation during development and beyond

Developing axons receive continuous guidance signals from their environment, as they travel long distances to reach their targets and form synapses, and mature axons depend on external cues for maintenance and repair. In either case, axons must quickly process environmental information, often without enough time to communicate with the soma. Local mRNA translation, namely the ability of axons to synthesize their proteins of need independently of the soma, is a key mechanism in this autonomous signalling. Disruption of the process during development is implicated in aberrant axon guidance, defective synaptic function, and numerous neurodevelopmental disorders, while increasing evidence supports that local translation is one of the first processes activated in adult axons after injury. However, the regulatory mechanisms that drive local translation during development and in the adult nervous system remain elusive. Here, we discuss our recent findings on the regulation of axonal local translation during neuronal development. We identified a ribonucleoprotein (RNP) complex, involving the actin-regulatory protein Mena, RNA-binding proteins that are known translational regulators (HnrnpK and PCBP1) and cytosolic mRNAs (Mena-RNP). Upon stimulation with growth factors, like BDNF, the complex disassembles, releasing the associated mRNAs and allowing their local translation. We demonstrated that the presence of Mena in the complex is essential for this process, thus uncovering one of the few known mechanisms that regulate translation in developing axons. Moving forward, we aim to study the conservation and function of the specific mechanism in adult axons, and its potential implication in axon regeneration after injury.

Speaker 3
Stavros Taraviras
University of Patras, Greece

Title: Geminin superfamily members in programming and reprogramming of adult NSCs

A small number of heterogeneous populations of neural stem/progenitor cells (NSCs) is specified into distinct cell fates giving rise to central nervous system. Intrinsic and extrinsic signals govern mammalian NSCs fate specification and subsequent differentiation. It has been suggested that modification in chromatin architecture, epigenetic marks and transcription are crucial to determine fate commitment programs into a specific lineage.

Our experimental evidence suggests that a novel family of proteins homologous to Geminin plays a key role on understanding how cell fates of adult NSCs and ependymal cells are established in the developing cortex and links these findings to the pathogenetic mechanisms of hydrocephalus. In addition, our findings provide indications for the ability of Geminin family members to reprogram astrocytes into multiciliated ependymal cells.

Speaker 4
Dimitra Thomaidou
Hellenic Pasteur Institute, Greece

Title: Direct reprogramming of astrocytes to induced-neurons by miRNAs and small neurogenic molecules: molecular mechanism and in vivo therapeutic potential

Direct neuronal reprogramming of glial cells has emerged as a promising approach for neuronal replacement using resident brain cells, to alleviate neuronal loss due to neurodegeneration or trauma. Accordingly, astrocytic reprogramming to induced-neurons (iNs) has been achieved *in vitro* and to some extent *in vivo* using combinations of transcription factors (TFs), miRNAs and chemical cocktails, however little is known about the mechanisms governing the reprogramming process. Recently we have investigated the role of the brain enriched miRNA, miR-124 and the small neurogenic molecule isoexasole-9 (ISX9) in inducing *in vitro* direct neuronal reprogramming of astrocytes, focusing on the elucidation of the core transcriptional mechanisms instructing the process. Our data indicate that miR-124 is sufficient to induce the neurogenic switch of mouse cortical astrocytes to immature iNs baring forebrain identity, while addition of ISX9 promotes iNs' maturation and *in vitro* expands iNs' potential regional identity by highly up-regulating genes participating in the specification of neuronal subtypes present in midbrain and hindbrain areas, an effect that is not maintained *in vivo* following cortical injury, where iNs obtain a cortical identity. Collectively our data indicate that ISX9 has the ability to potentiate multiple regional neuronal identities *in vitro*, but its action is not maintained *in vivo* following cortical injury, implying that the injured cortex microenvironment has a profound impact on the obtained regional neuronal identity of iNs.

SYMPOSIA

S5: Behavioural aspects in animal models: Genetic and environmental impact.

Saturday, 5/10/19
12:00-13:30

Chairs: Antonis Stamatakis, National Kapodistrian University of Athens and Myrto Denaxa, BSRC 'Alexander Fleming'

Speaker 1
Nicoletta Kessaris
University of London, UK

Title: Hyperinhibition in the early postnatal cortex and autism-like behaviour in mice

Decreased inhibition in the cortex is associated with epilepsy and neurodevelopmental disorders in humans and animal models. By contrast, reports of excess interneurons are scarce, and the consequences of increased cortical inhibition remain unknown. Here we show that excess Parvalbumin cortical interneurons, generated as a result of abnormal proliferation in the medial ganglionic eminence during embryogenesis, integrate into the early postnatal network and persist for several weeks after birth. However, their presence is only transient and interneuron numbers and their synapses are normalized at later stages. Yet, despite this correction, mutant mice exhibit lasting behavioural alterations. The transiency of interneuron overabundance may explain why increased interneuron numbers are not routinely described and suggests that such abnormalities in cortical interneuron development may constitute a cryptic developmental alteration that negatively impacts brain maturation.

Speaker 2
Antonis Stamatakis
National Kapodistrian University of Athens, Greece

Title: Effects of an adverse early postnatal experience on rat prefrontal cortex structure and function

Aversive early life experiences in humans have been shown to result in deficits in the function of the prefrontal cortex (PFC). In an effort to elucidate possible neurobiological mechanisms involved, we investigated in rats the effects of a mildly aversive early experience on PFC structure and function. The early experience involved exposure of rat pups during postnatal days (PND) 10–13 to a T-maze in which they search for their mother, but upon finding her are prohibited contact with her, thus being denied the expected reward (DER).

We found that the DER experience resulted in adulthood in impaired PFC function, as assessed in PFC-dependent behavioral tests measuring behavioral flexibility. Furthermore, the DER experience resulted in decreased PFC dopamine levels as well as in reduced glutamatergic neuronal density in the medial orbital (MO) and infralimbic cortex (IL) in adulthood. The decreased neuronal density was detected as early as PND12 and was accompanied by increased micro- and astroglia density in the MO/IL, while dopamine levels were reduced on PND10 and 11.The DER-induced behavioral and cellular deficits were reversed by intracerebral dopamine administration during the DER experience. Notably, a DER-like phenotype has been induced by intracerebral D1 antagonist administration to control pups on PND10-13.

These results point to a morphogenetic role for dopamine in the rat PFC during early postnatal life. If a similar situation holds for humans this could provide additional evidence regarding the aitiopathogenesis of PFC-related psychopathology.

Speaker 3
Katerina Segklia, Hellenic Pasteur Institute, Greece

Title: Cend1 in GABAergic neuron generation: effects on brain structure and function

Speaker 4
Maria Plataki
University of Crete and IMBB-FORTH, Greece

Title: Critical periods during the postnatal development regulate adult prefrontal cortical and hippocampal function

During postnatal development, there are specific temporal windows during which environmental changes affect the maturation of brain circuits and adult neuronal function, which are called critical periods. The critical period and its underlying mechanisms for the development of the visual system has been extensively studied. However, clear evidence for the existence of critical periods in brain areas related to memory systems has not been identified. There have been some data showing that MK-801 treatment during P7 to P14 affect both hippocampal function and prefrontal cortical function, indicative of a critical period. We know that the PFC has a more delayed maturation and does not mature until adulthood. However, we do not know whether there are specific temporal periods during early postnatal life that can be critical specifically for PFC function. To this end, we injected mice with MK-801, an NMDA receptor antagonist, for two different temporal periods: a) from postnatal day (P)7-P14 and b) from P11-P15. Our data show that MK-801(p7-p14)-treated mice perform poorly in the contextual fear conditioning, the novel object recognition, the object-to-place recognition and the temporal order object recognition tasks, indicative of both hippocampal and prefrontal cortical dysfunction. On the other hand, MK-801(p11-p15)-treated mice perform poorly only in the temporal order object recognition task, which is a PFC-dependent task. MK-801(p11-p15)-treated mice perform equally well with control mice in all the hippocampal dependent tasks, such as the novel object recognition, the object to place recognition and the contextual fear conditioning. Our results indicate that the critical period for PFC and hippocampal function are different.

SYMPOSIA

S6: Cognitive functions in the prefrontal cortex: from local circuits to long-range interactions.

Saturday, 5/10/19
15:30-17:00

Chair: Georgia Gregoriou, University of Crete and IACM-FORTH, Greece

Speaker 1
Marie Carlen
Karolinska Institute, Sweden

Title: Structure and function of the mouse prefrontal cortex

The structure and function of the prefrontal cortex across species remain unresolved. Further, it is unclear what characteristics have been conserved versus diversified throughout evolution. Taking advantage of the technological toolbox available to studies in mice (and rats), we are investigating the structure and function of the rodent prefrontal cortex, with a focus on the role of specific neuronal subtypes in executive functions such as attention and working memory. I will present our findings on the connectivity of the mouse prefrontal cortex based on cell-type specific viral tracing, and our work on prefrontal information processing in transgenic mice and rats using large-scale electrophysiological recordings and optogenetics.

Speaker 2
Georgia G. Gregoriou
University of Crete and IACM-FORTH, Greece

Title: The role of the non-human primate prefrontal cortex in the control of visual attention

Converging evidence from electrophysiological recordings and lesion studies in non-human primates (NHPs) has revealed that in NHPs, as in humans, the prefrontal cortex (PFC) is critical for the control of executive functions including selective attention. In humans, impairments in cognitive function have often been associated with dysfunction of prefrontal circuits. However, two questions have remained largely unanswered. First, how is functional connectivity between PFC and other brain areas modulated by behavioral demands during cognitive functions? Second, what is the role of distinct PFC areas in different aspects of cognitive control?

In this talk, I will present results from work in our lab that helps answer these questions. I will show data, which demonstrate that PFC modulates processing in visual cortex with attention through frequency-specific oscillatory coupling. Furthermore, I will show work that aims to identify critical PFC sources of attentional feedback to mid-level visual cortical areas. Finally, I will compare results from prefrontal and parietal cortical areas, which are also thought to control attentional processes and discuss their contribution in large scale networks in the brain.

Speaker 3
Albert Compte
Institut D' Investigacions Biomediques August Pi I Sunayer, Spain,

Title: The synaptic basis of history biases in spatial working memory

Working memory includes memory processes operating at time scales beyond one trial, as revealed by serial attractive biases in delayed response tasks. By investigating monkey and human electrophysiology data, we show that underlying this behavior is the interaction between PFC attractor dynamics in active mnemonic periods, and selective latent subthreshold mechanisms between trials. Stimulus information disappeared from electrophysiological signals between trials, but it was reactivated in anticipation of the new trial. We found evidence that this reactivation was related to the enhancement of serial biases, by using both trial-by-trial correlational analyses and causal perturbation experiments with transcranial magnetic stimulation in PFC. Finally, we report drastically reduced serial biases in anti-NMDAR encephalitis and schizophrenia. By integrating all these findings in a biophysical computational model, we conclude that PFC NMDAR-dependent mechanisms (such as short-term potentiation) support serial biases in working memory, and their deficit underlies working memory alterations in anti-NMDAR encephalitis and schizophrenia.

SYMPOSIA

S7: Time representation in Brain and Brain representation in Time

Saturday, 5/10/19
17:30-19:00

Chairs: Konstantina Kilteni, Karolinska Institute and Argiro Vatakis, Panteion University of Social and Political Sciences

Speaker 1
Konstantina Kilteni
Karolinska Institute, Sweden

Title: Rapid learning and unlearning of sensory delays in self-generated movements

Self-generated touch feels less intense and less ticklish than identical externally generated touch. This somatosensory attenuation occurs because the brain predicts the tactile consequences of our self-generated movements. To produce attenuation, the tactile predictions need to be time-locked to the movement, but how the brain maintains this temporal tuning remains unknown. Using a bimanual self-touch paradigm, we demonstrate that people can rapidly unlearn to attenuate touch immediately after their movement and learn to attenuate delayed touch instead, after repeated exposure to a systematic delay between the movement and the resulting touch. The magnitudes of the unlearning and learning effects are correlated and dependent on the number of trials that participants have been exposed to. We further show that delayed touches feel less ticklish and non-delayed touches more ticklish after exposure to the systematic delay. These findings demonstrate that the brain rapidly recalibrates its internal models to incorporate the new temporal relationship between the motor command and the sensory feedback.

Speaker 2
Argiro Vatakis
University of Social and Political Sciences, Greece

Title: Multisensory binding: The contribution of synchrony and semantic congruency in Multisensory Binding

We perceive the world as a unified whole with multisensory events being 'aligned' in every possible sense. This 'aligned' sense is a complex orchestration of multiple factors and underlying mechanisms, in this talk we focus on two: synchrony and semantic/informational congruency. These factors, the former structural and the latter cognitive, appear to favor the binding of multisensory stimuli, leading in a coherent unified percept. A longstanding debate in the field concerns the contribution of low- and high-level factors in the merging operation (i.e., unity assumption/effect). Recent neuroimaging studies propose the existence of a brain network responsible for multisensory integration, consisting of frontal, temporal, and primary sensory areas, each responding to different stimulus properties. Converging evidence suggests the dissociation of integration and synchrony perception, which is consistent with the view that these processes entail distinct mechanisms, both anatomically and functionally.

Speaker 3
Vassilis Thanopoulos
National and Kapodistrian University of Athens, Greece,

Title: Causal and temporally predictable associations between actions and their effects can induce Intentional Binding

The subjective temporal "attraction" between an action its effect, when both the action is intentional and the effect is temporally predictable, is known as the intentional binding (i.e., IB) phenomenon. To date, the existing literature in IB has mostly focused on the use of abstract stimuli of low informational content (e.g., tones or flashes), which lack an inherent causal relationship between the action and its effects. Therefore, in our research we investigated the role of causality in the induction of IB, by adopting multisensory and naturalistic events (i.e., a hand hitting a wooden surface) through a series of five experiments. In these experiments, we manipulated the levels of causality (different action-effect causal relations) and found that the IB was induced only when the naturalistic sequence of action cue effect was casually linked and predictable in terms of timing. This causal and predictable association of an event from the cue to act to the consequence of that action, can be seen in everyday multisensory events.

Speaker 4
Thomas Karantinos
University Mental Health Research Institute, Greece

Title: Intra-subject reaction time variability in schizophrenia

Increased reaction time intra-subject variability (RT-ISV) in fast decision tasks has been confirmed in patients with schizophrenia and has been hypothesized to result from a deficit in the control of attention. RT-ISV may be a possible behavioural biomarker for schizophrenia as it has been shown to dissociate schizophrenia patients from patients with borderline personality disorder, major depression, obsessive compulsive disorder and bipolar disorder. The distribution of RT in such simple sensorimotor decision tasks carries more information than can be captured by mean RT and therefore distributional analysis techniques and RT modelling must be employed in order to extract it. We will focus on the results of four fast decision tasks that rely on varying degrees of cognitive load. Differences between patients and controls are going to be discussed in terms of information, decision and sensorimotor processing and how these could be related with a dysfunction in distributed neural networks.

S8: New insights/ challenges in CNS and PNS myelination

Sunday, 6 October 2018
10:30-12:00

Chair: Maria Savvaki, IMBB-FORTH

Speaker 1
Catherine Faivre-Sarrailh
CNRS, Institut de Neurobiologie de la Méditerranée, France

Title: The Caspr/Contactin family as autoimmune target at the node of Ranvier

The field of chronic inflammatory demyelinating polyneuropathy (CIDP) has undergone a major advance with the identification of autoantibodies against cell adhesion molecules (CAMs) of the node of Ranvier. CIDP is a very heterogenous disorder with sensori-motor alterations, predominantly proximal weakness and relapsing or progressive disease. Autoantibodies of the IgG4 subtype directed against Contactin, Caspr or Neurofascin-155 were identified in patients with severe CIDP and poor response to intravenous immunoglobulin[1]. The anti-Contactin IgG4 from these patients are pathogenic in a rat model of autoimmune neuropathy, and induce loss of paranodal markers without demyelination, strong nerve activity loss and clinical deterioration[2]. The mechanisms by which anti-Contactin IgG4 cause paranode dismantling have been analyzed *in vitro* in myelinating culture of sensory neurons[3]. Cell aggregation assays indicate that anti-Contactin autoantibodies prevent the trans-interaction of glial Neurofascin-155 together with axonal Caspr/Contactin. By disrupting the paranodal complex, autoantibodies may provoke the lateral dispersion of CAMs along the axon. Epitope mapping of autoantibodies combined with mutagenesis of N-glycosylation sites of Contactin pointed to the critical role of N-linked carbohydrates in CAM interaction. The IgG of one CIDP patient were selectively directed against Contactin bearing mannose-rich N-glycans. Strikingly, the oligomannose type sugars of Contactin are required for association with its glial partner Neurofascin-155[4]. Next, a cluster of 3 N-glycosylation site (N_{467}, N_{473}, N_{494}) has been characterized on the Ig5 domain of Contactin, which is implicated in Neurofascin-155 binding[3]. Interestingly, the reactivity of IgG from another CIDP patient was precisely dependent on this cluster of N-glycans. Worth noting, the anti-Contactin IgG4 may be pathogenic by interfering with the assemblage of the axo-glial junctional complex.

Speaker 2
Kleopas A. Kleopa
The Cyprus Institute of Neurology & Genetics, Cyprus

Title: Gene therapy approaches for PNS and CNS demyelinating disorders

Mutations in the *GJB1* gene, encoding the gap junction (GJ) protein connexin32 (Cx32), expressed by myelinating Schwann cells, cause X-linked Charcot-Marie-Tooth (CMT1X) disease, one of the most common forms of inherited demyelinating peripheral neuropathy. Likewise, recessive mutation in the *GJC2* gene, encoding Cx47, the major GJ protein in oligodendrocytes, cause hypomyelinating leukodystrophy type 2 (HLD2). Study of disease-causing mutations and authentic animal models of these disorders have shown that a loss of function mechanism leads to the disease. In order to develop a gene therapy for these myelin disorders, we developed a cell-targeted approach using viral vectors driven by myelin-specific promoters to replace the connexin genes. We generated the oligodendrocyte-specific short myelin basic protein (*Mbp*) promoter driven *GJC2* construct and the Schwann cell-specific myelin protein zero (*Mpz*) promoter driven *GJB1* expression cassettes into lentiviral or AAV vectors. Expression of reporter gene *Egfp* as well as of therapeutic genes was demonstrated specifically in myelinating cells of the CNS or PNS, respectively. Intracerebral treatment of the Cx32/Cx47 double knockout (KO) model of HLD2 with the *Mbp-GJC2* vector resulted in re-establishment of GJ formation in oligodendrocytes and significant therapeutic rescue of its early severe phenotype. Lumbar intrathecal injection of LV or AAV vectors expressing the *Mpz.GJB1* construct resulted in restoration of Cx32 expression in Schwann cells of lumbar roots, sciatic and femoral nerves. A therapeutic trial in the Cx32 KO model of the disease provided phenotypic improvement both in motor performance, nerve conduction velocities, and peripheral nerve morphology, when administered both pre- as well as post-onset of the peripheral neuropathy. Our studies provide proof of principle for the therapeutic potential of cell-targeted gene delivery to myelinating cells of the CNS and PNS to treat inherited hypo- and demyelinating disorders.

Funding: Muscular Dystrophy Association, Charcot-Marie-Tooth Association, European Leukodystrophy Association, AFM-Telethon

Speaker 3
Maria Savvaki
IMBB – FORTH, Greece

Title: The role of autophagy in the development of CNS myelin

Autophagy comprises a major lysosome-dependent degradation mechanism which engulfs, removes, and recycles unwanted cellular material including damaged organelles and toxic protein aggregates. Its defective regulation has been linked to several disorders, including neurodegenerative diseases and peripheral demyelinating disorders. More specifically, autophagy deficiency results in the toxic accumulation of aggregate-prone proteins in neurons that are associated with these diseases. Although a few studies implicate autophagy in CNS demyelinating pathologies, its role, particularly in oligodendrocytes, the cell type that produces myelin, remains poorly characterized.

We are interested in investigating the contribution of autophagy in the myelin component of the CNS. In our study we employ both *in vivo* and *in vitro* approaches. More specifically, we have generated two transgenic mouse lines where a key autophagy gene (*Atg5*) is specifically ablated in the CNS and oligodendrocytes respectively. Our analysis so far has shown significant myelin defects early in the development of oligodendrocytes. These data are further supported by in vitro study of primary oligodendrocyte cultures under normal and autophagy-regulated conditions.

Deciphering the role of autophagy will be helpful in understanding more about survival pathways in oligodendrocytes and will hopefully reveal novel therapeutic perspectives towards the treatment of myelin damage that occurs in de-, dys-myelinating disorders.

Acknowledgement
This project has received funding from the Hellenic Foundation for Research and Innovation (HFRI), the General Secretariat for Research and Technology (GSRT), under grant agreement No 1676 and from the National Multiple Sclerosis Society (NMSS, pilot Research Grant).

SYMPOSIA

S9: EURONEUROTROPHIN

Sunday, 6 October 2018
12:00-13:30

Chair: Theodora Calogeropoulou, Natioanl Hellenic Research Foundation

Speaker 1
Antonio Cattaneo
Scuola Normale Superiore,, EBRI-Rita Levi Montalcini, Italy

Title: "Taking pain out of NGF

Speaker 2
Eran Perlson
Tel-Aviv University, Israel
Title: Sema3A Facilitates a Retrograde Death Signal in ALS-Diseased Motor Neurons via CRMP4-Dynein Complex Formation

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease with selective dysfunction; it causes the death of motor neurons (MNs). In spite of some progress, currently no effective treatment is available for ALS. Before such treatment can be developed, a more thorough understanding of ALS pathogenesis is required. Recently, we demonstrated that ALS-mutated muscles contribute to ALS pathology via secretion of destabilizing factors such as Sema3A; these factors trigger axon degeneration and Neuromuscular Junction (NMJ) disruption. Here, we focus on the molecular mechanism by which muscle contribute to MNs loss in ALS. We identified CRMP4 as part of a retrograde death signal generated in response to muscle-secreted Sema3A, in ALS-diseased MNs. Exposing distal axons to Sema3A induces CRMP4-dynein complex formation and MN loss in both mouse ($SOD1^{G93A}$) and human-derived (C9orf72) ALS models. Introducing peptides that interfere with CRMP4-dynein interaction in MN axons profoundly reduces Sema3A-dependent MN loss. Thus, we discovered a novel retrograde death signal mechanism underlying MN loss in ALS.

Speaker 3
Kanelina Karali, IMBB-FORTH, Greece

Title: Synthetic microneurotrophins: neurogenic and neuroprotective effects in Alzheimer's disease

Alzheimer's disease (AD) is characterized by progressive neuronal loss and cognitive decline. Its major neuropathological hallmarks are the accumulation of ß-amyloid (Aß) peptide and neurofibrillary tangles within the brain as well as neuronal and synaptic loss. AD affects 36 million people worldwide and 7 million in Europe and has no effective treatment so far. Neurotrophins are a family of closely related secreted proteins that have been shown to control a number of aspects of survival, development and function of neurons and changes in their expression and/or of their receptors have been linked with AD- related degeneration. Neurotrophins have been proposed as therapeutic agents for AD, however their poor pharmacokinetic properties renders them unsuitable candidates. Thus the development of small molecules that harvest aspects of neurotrophins' biological actions and have suitable pharmacokinetic properties is a potential approach.

BNN27 is a newly developed 17-spiro-steroid analog (microneurotrophin) of dehydroepiandrosterone (DHEA) that mimics the neuroprotective effects of NGF, acting as selective activator of its receptors, TrkA and p75NTR, regulating neuronal survival and differentiation as well as promoting neuroprotection [1,2]. We examined the ability of BNN27 to ameliorate AD-related cognitive deficits and neuropathology in the 5xFAD mouse model of AD. BNN27 treatment showed to significantly improve working memory tested in the T-maze and decreased the formation of Aß plaques and the loss of synapses within the hippocampus. Additionally, BNN27 effectively promoted adult hippocampal neurogenesis within the dentate gyrus of the hippocampus and reduced the cholinergic atrophy in basal forebrain of 5xFAD mice by significantly increasing the mean soma size of cholinergic neurons.

Our findings suggest that microneurotrophin BNN27 blocks amyloid deposition and promotes hippocampal neuroregeneration in the 5xFAD mice, most probably affecting the pathways downstream to NGF receptors.

References

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Speaker 4
Theodora Calogeropoulou
National Hellenic Research Foundation, Greece

Titel: "EuroNeurotrophin": A European training network for the discovery of neurotrophins small molecule mimetics as potential therapeutic agents for neurodegeneration and neuroinflammation

Neurodegenerative diseases (ND) like AD, PD, MS and ALS/MND are on the rise in developed societies worldwide. Currently, there exists no cure for any ND, and most of the available drugs fail to tackle ND pathogenesis. Preclinical studies point to the therapeutic potential of neurotrophins, which have been shown to control a number of aspects of survival, development and function of neurons. However, the poor pharmacokinetic properties of neurotrophins render their use as drugs prohibitive. The key idea behind "EuroNeurotrophin" is to address this limitation by developing novel small molecule, neurotrophin mimetics with favourable profiles of stability, tissue penetration and targeted biological actions. Motivated by seminal (patented) results of beneficiaries NHRF and FORTH,[1-4] "EuroNeurotrophin" is the first European consortium to study small molecule neurotrophin mimetics in depth, use them as molecular probes to interrogate neurotrophins, and emphasise their clinical translation. The network has been designed to address the need for the development of new ND treatments through the collaborative training of 14 early-stage researchers. It builds on previous successful collaborations enriched with key specialists, and consists of 10 leading European research groups and 7 private companies of complementary expertise covering all steps of drug discovery and development.

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Acknowledgement

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Symposium 10: From cellular to physiological stress: common mechanisms to neuropsychiatric and neurological pathologies

Sunday, 6 October 2018
14:30-16:00

Chairs: Christina Dalla, Department of Pharmacology, National and Kapodistrian University of Athens, Greece and Ioannis Sotiropoulos, ICVS Institute, University of Minho, Portugal

Speaker 1
Ehud Cohen
The Hebrew University of Jerusalem, Israel

Title: Deciphering the proteostasis network response to the accumulation of toxic protein aggregates in the aging brain

The ability to maintain proper protein homeostasis (proteostasis) is critical for organismal functionality and viability. Cells have evolved sophisticated mechanisms that act in concert to maintain the integrity of the proteome. These mechanisms, which are known as "the proteostasis network", assist protein folding, supervise the integrity of mature proteins and direct damaged polypeptides for degradation. Nevertheless, as the organism ages, subsets of aggregation-prone proteins challenge the proteostasis network by escaping degradation and forming insoluble aggregates. In some cases, these aggregates underlie the development of late-onset neurodegenerative disorders such as Alzheimer's disease (AD) and Huntington's disease (HD). Accordingly, maintaining the proteostasis network active in late stages of life could prevent the manifestation of these devastating diseases. To to assess the therapeutic potential of this approach it is critical to explore how the proteostasis network responds to dissimilar challenges and whether the nature of the aggregating protein or the cell type where aggregates accumulate, shape this response. Employing nematodes that express different neurodegeneration-linked, aggregative proteins, we found that Torsin chaperones (Torsin 1 and 2) protect model worms from the aggregation of the HD-causing, abnormally long poly-glutamine (polyQ) stretches and of a mutated super oxide dismutase 1 (*sod-1*) which leads to the development of Amyotrophic lateral sclerosis (ALS). In contrast, the same chaperones expose nematodes to the toxicity of the AD-causing, Aß peptide. These opposing effects were observed in both, neurons and muscles cells, indicating that the nature of the toxic polypeptide rather than the tissue of expression shapes the proteostasis network's response to dissimilar aggregative proteins. These effects are accompanied by differential modulations of gene expression, including of a subset of neuropeptides. Our results indicate that the proteostasis network differentially responds to dissimilar proteotoxic

challenges and highlight the importance of understanding the accurate response to specific aggregative proteins, to design specifically tailored therapies for neurodegenerative maladies.

Speaker 2
Christina Dalla
National and Kapodistrian University of Athens, Greece

Title: Sex differences in animal models of depression: from behavior to neural networks

Women are more vulnerable than men in most stress-related neuropsychiatric disorders. Moreover, recent preclinical findings link estrogens' signaling with mood and cognition. Our group has studied preclinical sex differences in stress models of depression [1, 2]. Furthermore, we have investigated the behavioral and neurochemical effects of estrogen depletion by aromatase inhibition in male and female rats exposed to forced swim test. We have demonstrated that treatment with letrozole, which is an aromatase inhibitor, decreases noradrenaline levels and dopaminergic ratio in the hippocampus and prefrontal cortex of male and female rats. Also, it enhances the serotonergic ratio in the hippocampus of males and females [3]. Finally, we have recently shown that the integrity of the circuit hippocampus – prefrontal cortex is necessary for stress-induced deficits in neuroplasticity indices and for the expression of "depressive" behaviors [4]. Overall, our studies highlight the importance of inclusion of both male and female animals in preclinical neuroscience research and implicate the critical role of the hippocampal-cortical circuit in stress response.

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Speaker 3
Sheela Vyas
CNRS UMR 8246/ INSERM U1130/ UPMC & Université Pierre et Marie Curie, France

Title: Inflammation, stress and glucocorticoid receptors in neurodegeneration

Following environmental challenges such as stress or trauma, glucocorticoids (GCs) through glucocorticoid receptors (GRs) exert wide-ranging adaptive responses in many tissues, altering physiological and behavioural processes. GC-GR dysfunction is associated with long-term chronic diseases, however their role in neurodegenerative diseases is not well understood.
Both GC (cortisol) and GR levels are altered in Parkinson's disease (PD) patients. Thus circulating levels of cortisol were reported to be chronically high whereas we have found an overall reduction of GR in substantia nigra. Further analyses showed significant reduction in expression of GR in microglia and in astrocytes of substantia nigra brain samples from PD patients.
Due to pleiotropic nature of GR signalling, we are using mouse models in which GR can be conditionally inactivated in one cell type. Our work on its actions in microglia, astrocytes and dopamine neurons revealed its regulatory role in survival of dopaminergic neurons in experimental models of PD. Interestingly, it exerts specific actions in these cell types, thus in microglia it impacts innate immune functions and microglial reactivity whereas in astrocytes it acts not only to regulate inflammation through connexin hemichannels but is also involved in behavioural anomalies as observed in PD. Work on GR in dopamine neurons in experimental Parkinsonism revealed its pro-survival actions only after treatment with exogenous corticosterone. In chronic mild stress conditions, dopamine vulnerability increases together with establishment of pro-inflammatory state. Overall, our approach has allowed us to dissect mechanisms by which GCs and GR can exert cell and context-specific actions in experimental parkinsonism.

Speaker 4
Dr Ioannis Sotiropoulos
ICVS Institute, University of Minho , Portugal

Title: Endolysosomal sorting and RNA-protein biology in stress-driven brain pathology: exploring the link between depression to Alzheimer´s disease.

Despite emerging studies implicating Tau in neuronal atrophy and cognitive impairment associated with Alzheimer's disease (AD), the physiopathology of the disorder is complex and poorly understood. Chronic environmental stress and the major stress hormones, glucocorticoids (GC), are suggested precipitating factors for AD, and have been shown to trigger Tau hyperphosphorylation, accumulation and downstream Tau-dependent neuronal malfunction. However, the mechanisms that regulate Tau clearance and degradation remain unclear. In the current studies, we use *in vitro* and *in vivo* studies to uncover the role of two essential degradation mechanisms, the endolysosomal pathway and autophagy, in Tau proteostasis under control and pathological conditions. We demonstrate for the first time that Tau undergoes degradation via endolysosomal sorting in a pathway requiring the small GTPase Rab35 and the endosomal sorting complex required for transport (ESCRT) machinery. Interestingly, we detect a phospho-dependent selectivity of Tau sorting into the Rab35/ESCRT pathway. Furthermore, we find that chronic stress and high GC levels impair Tau degradation by suppressing Rab35 expression, while Rab35 gain-of-function rescues GC-induced Tau accumulation and related neurostructural deficits (*Vaz-Silva et al. EMBO J 2018*). In addition, stress and high GC trigger an mTOR-dependent inhibition of autophagy, leading to accumulation of Tau aggregates and cell death in AD Tg mouse and cell models. In parallel, we found that environmental stress and GC disturb cellular homeostasis and trigger insoluble accumulation of different RNA-Binding Proteins forming Stress granules(SGs). Interestingly, an mTOR-driven pharmacological stimulation of autophagy attenuates the GC-driven accumulation of Tau and SG-related proteins as well as the related cell death, suggesting a critical interface between autophagy and the SG-related proteins response in the neurodegenerative role of chronic stress (*Silva et al., Cell Death & Diff 2018*). Conclusively, these studies indicate that the Rab35/ESCRT pathway and autophagy are essential for Tau clearance and part of the mechanism through which chronic stress precipitates AD.

SYMPOSIA

S11: LRRK2…15 years from its discovery to becoming a viable drug target for Parkinson's disease.

Sunday, 6 October 2018
16:00-17:30

Chair: Hardy J Rideout, PhD; Collaborating Scientist, Biomedical Research Foundation of the Academy of Athens.

Speaker 1
Elisa Greggio, PhD; University of Padova, Italy

Title: LRRK2 function and dysfunction beyond the neuron

Leucine-rich repeat kinase 2 (LRRK2), a kinase/GTPase mutated in familiar Parkinson's disease, is expressed in neuronal and non-neuronal cells. Increasing evidence suggests that LRRK2 controls molecular pathways linked to inflammatory response, phagocytosis and autophagic processes in microglia and macrophages. Here I will discuss our recent data from *in vitro* and *in vivo* studies supporting a role for LRRK2 in microglia and macrohages physiology and the potential contribution of LRRK2 mutations in increasing the neuroinflammatory process in Parkinson's disease.

Speaker 2
R. Jeremy Nichols
Stanford University, USA

Title: The path towards identifying novel LRRK2 substrates

Inherited mutations in the gene Leucine-rich repeat kinase 2 (LRRK2) are a common cause of familial Parkinson's disease (PD). Importantly, these mutations result in a disease course that is largely indistinguishable from idiopathic PD. In order to gain insight into the physiologic activities performed by this kinase, we turned to coexpression analysis using the rich transcriptional data found in the Genotype-Tissue Expression (GTEx) database. This analysis highlights a select set of genes which were either positively (GO Terms-endomembrane system organization, cellular localization and intracellular transport) or negatively correlated (Heat shock proteins) with LRRK2 expression across several tissues from human donors. Based on these findings, we reasoned that heat shock could provide insight into LRRK2 regulation. To test this hypothesis, we employed a cellular model of Type II pneumocytes which endogenously express LRRK2 and exhibit pathology in the absence of LRRK2 protein or activity in animal models ranging from mouse to primate. Exposure of these cells to mild heat shock results in decreases in LRRK2 kinase activity, dephosphorylation at Ser935 and a prolonged decrease in its protein abundance, consistent with LRRK2 transcriptional regulation in human tissue. Using a line of CRISPR engineered A549 cells bearing a GFP tag knocked-in to the LRRK2 locus, we tracked endogenously expressed protein by live imaging confocal microscopy at the subcellular level to identify a subset of vesicles highly enriched for LRRK2. LRRK2 maintains its membrane association with these vesicles during heat shock. However, their cellular localization and dynamics are significantly altered in a way that mimics treatment with selective LRRK2 kinase inhibitors. This provides critical insight into the vesicle residence, activity and phosphorylation status of LRRK2. In summary, these data have corroborated findings that place LRRK2 as a key regulator of membrane trafficking and uncovered an unexpected impact of the heat shock response on LRRK2.

Speaker 3
Arjan Kortholt
University of Groningen, The Netherlands

Title: Understanding the GTP/GDP cycle of LRRK2 and regulation of its conformation and activity

Mutations in Leucine-rich repeat kinase 2 (LRRK2) are thus far the most frequent cause of late-onset and idiopathic Parkinson's disease (PD). LRRK2 belongs to the group of Roco proteins, which are characterized by the presence of a Ras-like G-domain (Roc), a C-terminal of Roc domain (COR), a kinase and several protein-protein interaction domains. LRRK2 has a complex activation mechanism, involving intra-molecular signalling, dimerization and protein-protein interactions.
PD mutations in LRRK2 have been linked to decreased GTPase activity and increased kinase activity. A number of LRRK2-specific brain penetrant kinase inhibitors have been developed, but most of them have major side effects, and none of the inhibitors can yet be used for the treatment of PD. In addition, different PD mutations in LRRK2 most likely trigger different defects in LRRK2 function. Therefore, alternative approaches that target other domains of LRRK2, leading to dimerization or allosteric modulation of the kinase domain, may have significantly improved therapeutic benefits.

To fully explore these potential targets, it is crucial to get a detailed understanding of the LRRK2 activation mechanism. However, it has been a major challenge to obtain sufficient amounts of LRRK2 protein, either full length or domain constructs, for detailed biochemical and biophysical studies. Recently we were able to purify high quality full-length LRRK2 protein and with a novel directed evolution approach we were able to express various properly folded wild-type and PD-related LRRK2 fragments. Additionally, assays have been developed to analyze LRRK2 activation and function in cells. We use these unique tools to investigate the mechanism and function of LRRK2 dimerization in cells and, for the first time, explore allosteric targeting of LRRK2 dimerization and a proof of concept of allosteric modulators as therapeutics for LRRK2-mediated PD.

Speaker 4
Lilian Petropoulou-Vathi
Biomedical Research Foundation of the Academy of Athens, Greece

Title: LRRK2-focussed biomarkers of disease progression in PD patients

Mutations in the gene encoding LRRK2 are the most common genetic cause of Parkinson's disease (PD), and are also found in a significant number of idiopathic PD cases as well. We have previously observed increased in vitro kinase activity in LRRK2 purified from PBMCs obtained from healthy and affected carriers of the G2019S-LRRK2 mutation. Additionally, apart from the genetic linkage between LRRK2 and Parkinson's disease, changes in the activation state of LRRK2 have been found in post-mortem brain tissue, as well as exosomes purified from both CSF and urine. Increased levels of auto-phosphorylation, a direct index of its kinase activity, have been found in carriers of the G2019S-LRRK2 mutation; and, perhaps more interestingly, in idiopathic PD cases as well. This led us to speculate that LRRK2 kinase activation may be a wise spread quantifiable marker of PD progression. Thus we applied our ELISA-based methods of assessing LRRK2 function to an expanded range of biosamples from idiopathic PD patients, PD patients harbouring a mutation in the gene encoding alpha-synuclein (A53T-ASYN), or healthy control subjects. We will assess LRRK2 function in: PBMCs and monocytes, urinary exosomes, as well as neuronal-derived exosomes present in plasma. The outcome measures will include total LRRK2 levels, phosphorylated LRRK2, in vitro kinase activity, and phosphorylation of two key substrates, Rab10 & Rab29. We hope that this study will help our understanding of the nature and source of changes in LRRK2 activation associated with PD more broadly.

Junior Scientists Symposium 1

Friday, 4/10/19
15:30-17:00

Chair: Antonis Stamatakis, National Kapodistrian University of Athens

Speaker 1
Theodora Velona
Aix-Marseille Université, France

Title: PlexinD1 and Sema3E determine laminar positioning of heterotopically projecting callosal neurons

The corpus callosum is the largest bundle of commissural fibres that transfer information between the two cerebral hemispheres. Callosal projection neurons (CPNs) are a diverse population of pyramidal neurons within the neocortex that mainly interconnect homotopic regions of the opposite cortices. Nevertheless, some CPNs are involved in heterotopic projections between distinct cortical areas or to subcortical regions such as the striatum. In this study, we showed that the axon guidance receptor PlexinD1 is expressed by a large proportion of heterotopically projecting CPNs in layer 5A of the primary somatosensory (S1) and motor (M1) areas. Retrograde tracing of M1 CPNs projecting to the contralateral striatum revealed the presence of ectopic neurons aberrantly located in layers 2/3 of Plxnd1 and Sema3e mutant cortices. These results showed that Sema3E/PlexinD1 signalling controls the laminar distribution of heterotopically projecting CPNs.

Speaker 2
Elpinickie Ninou
BFRAA

Title: Long non-coding RNA – Transcription factor regulatory networks in mammalian brain development

AIMS: With the advent of new generation technologies, a growing list of formerly unknown regulatory RNA species have come into spotlight. Among them, long non-coding RNAs (lncRNAs) have been found to control stem cell pluripotency, carcinogenesis, development and function of several tissues and organs. Although thousands of lncRNAs are expressed in adult mammalian brain in a highly patterned and specific manner, they remain poorly characterized and their roles in brain development have not yet been studied.
METHODS: To tackle this question, we initially performed RNA-Seq analysis in the developing nervous system of mouse embryo. Based on this analysis, we identified many lncRNAs highly expressed in neural cells. We focused on lncRNAs, which are transcribed from genomic loci in close proximity with protein coding genes, encoding for critical transcription factors (TFs) in brain development. We hypothesized that these lncRNAs may be implicated in the regulation of neighboring TF genes.
RESULTS: We characterized the changes in the expression profile of the most interesting from the identified lncRNAs-TF pairs during development of mouse brain. In this study, we further investigated the functional role of lncRNA TCONS_00034309 in the differentiation of neural stem cells by in vitro and in vivo overexpression and knock-down studies, using CRISPR- dCas9-KRAB Effector System.
CONCLUSIONS: Our data suggest critical roles for this lncRNA in neuronal differentiation and astrogliogenesis during brain development. Our study provides insights into the involvement of lncRNAs in organogenesis and shows how lncRNAs and protein-coding genes form regulatory networks with important functions in neural cells.

Iakovos Lazaridis
Karolinska Institute, Sweden

Title: Disappointment and decision making: A functional segregation of pallidal and hypothalamic pathways to the lateral habenula

Encoding and predicting aversive events is a critical function of basal ganglia and limbic circuits that support survival and emotional well-being. Maladaptive circuit changes in aversive signal processing can underlie the pathophysiology of affective disorders. The lateral habenula (LHb) has been linked to aversion and mood regulation through activity modulation of the dopamine and serotonin systems. We have defined the identity and function of glutamatergic (Vglut2+) control of LHb, focusing on the role of inputs originating from the globus pallidus internal segment (GPi) and lateral hypothalamus (LHA). We found through optogenetic targeting of the two pathways that aversive signals in LHb originate from glutamatergic LHA neurons and not the GABA/glutamate co-releasing GPi neurons. In addition, presynaptic connectivity organization supported a primarily limbic input to LHA neurons, in contrast to the sensorimotor input to GPi. Imaging of the LHb-projecting Vglut2+ LHA neurons revealed a signal of the aversive event that rapidly develop a prediction signal. These findings establish the glutamatergic LHA-LHb circuit as a critical node in signaling and learning about aversive events. Lastly, our resent anatomical and functional work on GPi Vglut2+/Vgat+ neurons is revealing a new feedback pathway in the basal ganglia and highlights the role of the GPi-LHb pathway as a part of the sensorimotor system.

Lazaridis I, Tzortzi O, Weglage M, Märtin A, Xuan Y, Parent M, Johansson Y, Fuzik J, Fürth D, Fenno L, Ramakrishnan C, Silberberg G, Deisseroth K, Carlén M, Meletis K. A hypothalamus-habenula circuit controls aversion. *Molecular Psychiatry*; 2019; doi: 10.1038/s41380-019-0369-5.

Speaker 4
Matina Bitzidou
University of Sussex and Francis Crick Institute, UK

Title: Participation of cortical areas in whisker-mediated recognition of elementary tactile sequences

Sequential temporal patterning is a key feature of natural signals, used by the brain to decode stimuli and perceive them as sensory objects. To dissect how neuronal activity distinguishes between behaviorally relevant sequential patterns and to begin exploring whether the cortex uses generic mechanisms for sequence recognition across modalities, we developed a GO/NOGO discrimination task in which mice must distinguish between tactile "words" constructed from distinct vibrations assembled in different orders. In the task, tactile sequences can be recognized through several cues, including the arrival of a specific vibration or the presence of transitions between adjacent vibrations.
Adult male mice learned this elementary sequence recognition task after a few weeks of training. Animals often responded to the earliest possible cues allowing discrimination, i.e. they effectively solved the task as a "detection of change" problem. However, mice enhanced their performance when collecting sensory evidence or deliberating for longer (30 mice, 122 sessions, Spearman rho = 0.24, p = 0.0083).
Optogenetic inactivation of cortical population activity was performed to determine the cortical sites where tactile information is processed. Inactivating the somatosensory "barrel" cortex (S1BC) and secondary somatosensory cortex (S2) abolished sequence discrimination on interspersed light-on trials. Suppression of posterior parietal cortex (PPC) had no detectable effect, while suppression of primary motor cortex (wM1) disinhibited lick responses. These results suggest that the sensory input used for sequence recognition flows through S1BC and S2, but that sequence selectivity is learned in higher areas.
Two-photon imaging in S1BC layer 2/3 of well-trained animals revealed heterogeneous neurons with mixed selectivity to task variables including sensory input, the animal's decision to lick, and trial outcome (rewards and their departure from prediction). These experiments begin to uncover features of cortical activity that sequence recognition shares with other goal- directed sensory discrimination tasks.

Speaker 5
Jagoda Jęczmień-Łazur
Jagiellonian University in Krakow, Poland

Title: Ultraviolet and blue light sensitivity of the visual thalamus

Short wavelength light detection in rodents originates with two classes of photoreceptors: melanopsin cells and UVS-cones, which are activated by blue and ultraviolet light, respectively. Despite the fact that these two classes constitute no more than 1% of all retinal photoreceptors, they act as the principal conduits for circadian and visual functions in rodents by sending signal to many subcortical areas. One of them is the dorsal lateral geniculate nucleus of the thalamus (dLGN), which responsiveness to short wavelengths of light has not received much attention. Thus, the aim of the present work was to characterize the dLGN sensitivity to short wavelength light. We performed series of light stimulations, while recording neuronal activity in the dLGN by using custom-designed illuminator and *in vivo* multi-channel recordings. Light-induced activity was recorded under the dark or yellow background lightening, while high-irradiance stimuli (3 s pulses; 340-390 nm range; 10 nm step) were presented to the rats' eye. Overall, 205 neurons were recorded in the dLGN. In terms of responses recorded against dark background, 51% of neurons responded to the ultraviolet light (340-390 nm), whereas the remaining were more sensitive to the blue light spectrum (400-490 nm). Statistical analysis showed that the 380 nm and 480 nm light pulses produce the maximum response of UV- and blue-light spectrum, respectively. Moreover, colour opponent cells constitute 55% of all recorded neurons. These neurons were activated by blue–yellow and UV-yellow light pulses in an antagonistic manner. Presented study is an electrophysiological characterization of thalamic responses elicited by ultraviolet and blue light, which presumably derived from retinal melanopsin cells and UVS-cones inputs. This particular connection is especially important for rodents to see and use UV/blue light spectrum to communicate, identify food or, as recently proved, to synchronize to the circadian rhythms. Supported by: 2013/08/W/N23/00700.

Speaker 6
Athanasia Papoutsi
IMBB-FORTH, Greece

Title: Modeling of orientation preference in the apical and basal tress of L2/3 V1 pyramidal neurons

Cortical pyramidal neurons receive inputs in two anatomically and functionally distinct domains, the apical and the basal tree. Inputs to the latter, due to their proximity to the soma, greatly influence neuronal output. The more remote apical tree on the other hand has a smaller potential to influence somatic activity. How these inputs co-operate to form the functional output of neurons, however, is currently unknown. In this work we focused on how inputs to these trees shape orientation tuning in L2/3 V1 pyramidal neurons. In particular, we investigated under which conditions orientation tuned inputs to the apical versus the basal trees allow for the emergence of stable neuronal orientation preference. Towards this goal a biophysical model of a L2/3 V1 pyramidal neuron was implemented in the NEURON simulation environment. The passive and active properties of the model neuron were extensively validated against experimental data. Synaptic properties, number and distribution were also constrained according to available data. Using this model neuron we investigated a) the differences in the mean orientation preferences of the two trees and b) the distribution of orientation preferences to individual synapses that allow for the emergence of orientation tuning. Through an extensive exploration of the parameter space we found that the orientation tuning of the model neuron is dominated primarily by that of the basal rather than the apical tree. The model also replicates experimental findings of orientation tuning properties of neurons whose apical or one basal dendrite were removed with in vivo two photon microscopy. Overall, model simulations provide new insights regarding the influence of apical versus basal inputs on orientation preference and predict that a distributed, variably tuned input structure along the basal dendrites is optimal for a flexible orientation tuning.

SYMPOSIA

Junior Scientists Symposium 2

Saturday, 5/10/19
19:00-20:00

Chair: Anastasia Tsingotjidou, Aristotle University of Thessaloniki

Katerina Kalemaki
University of Crete and IMBB-FORTH, Greece

Title: Developmental changes of GABA inhibitory function in the mPFC

The medial prefrontal cortex (mPFC) controls higher cognitive abilities, many of which are affected in neurodevelopmental disorders. The mPFC is characterised by delayed development and matures during adolescence, allowing for significant perturbations by environmental factors. The early developmental mechanisms controlling mPFC circuits, before adolescence, are still largely unsolved. Our study aim to delineate the developmental changes that are on-going in the mPFC from neonatal to pre-juvenile period, i.e. between the second and third postnatal weeks, in comparison to the better-studied barrel cortex (BC). Specifically, we show that the basal synaptic transmission is decreased from the second to the third postnatal weeks, which can be explained by a concurrent decrease in the sEPSCs and increase in the sIPSCs. Although cell density in the mPFC is reduced from P10 to P20, the number of parvalbumin (PVA) and serotonin receptor (5HT3R) positive interneurons are increased. An additional component to the enhaced excitation during the second postnatal week in the mPFC could stem from $GABA_AR$ activity which is found to be excitatory only in the mPFC. Increasing $GABA_AR$ activity leads to increased basal synaptic transmission of mPFC, but not BC. Additionally, the expression of KCC2, an integral transporter involved in the switch between excitatory and inhibitory action of GABA, is decreased mPFC during the second postnatal week, compared to the third one and compared to BC at both time points. Furthermore, the intrinsic properties of interneurons are altered in a way that indicates maturation of intrinsic properties, while the changes in pyramidal neuron properties in vitro are subtler. Finally, we show that all the above developmental events lead to increased network activity in the mPFC from the second to the third postnatal week.

Dimitrios Tzeranis
Univeristy of Cyprus, Cyprus

Title: Development of Novel Treatments for Central Nervous System Injuries based on Porous Collagen Scaffolds

Central Nervous System (CNS) injuries are leading causes of disabilities worldwide. Currently, there are no available treatments for preventing their consequences or inducing regeneration in injured CNS. Efforts to address the complexity of CNS response to injury have focused on developing combinatorial treatments that integrate multipotent cells, small molecules and biomaterials.
Here we describe progress on the development of implants for CNS injuries based on Porous Collagen Scaffolds (PCS)1. Despite PCS established clinical application in peripheral nerve regeneration, PCS application in CNS injuries has been minimal. We demonstrate that PCS can host and deliver mouse embryonic Neural Stem Cells (NSCs) at spinal cord injury (SCI) sites in mice and enable NSC differentiation towards both neuronal and glial lineages in vivo2. Such NSC-seeded PCS implants improved the locomotion recovery in a mice dorsal column crush SCI model3. NSC attach to PCS and grow neurosphere-like aggregates inside PCS pores in vitro. Furthermore, both primary neurons and mouse ES-derived motor neurons can attach and grow long processes on PCS struts in vitro. In order to develop injury-specific implants, we present a novel laser-based 3D microfabrication method that delivers custom mm-sized implants with complex cell-guiding μm-sized features. We have implanted such microfabricated PCS implants in sites of SCI and optic nerve crush (ONC) and demonstrate that they are well-tolerated and can deliver cells and suspensions at the injury site. Such laser-microfabricated PCS were utilized in a pilot study to deliver small- molecule analogs of neurotrophins at the site of ONC.
Our work provides diverse evidence that PCS can provide a platform for CNS implants that can deliver potent NSCs or small-molecule compounds at CNS injury sites. Furthermore, PCS implants can be engineered to match the needs of specific injuries and better control the injury microenvironment based on emerging knowledge on CNS injury elementary biology4.
References
[1] Yannas IV et al. Phil. Trans. Royal Soc. A 368: 2123-9 (2010). [2] Kadoya K. et al. Nat. Medicine 22: 479-87 (2016). [3] Kanagal SG and Muir GD. Exp. Neurol. 205: 513-524 (2007). [4] O'Shea TM. Et al. J. Clin Inv 127: 3259-70 (2017).
Acknowledgements
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SYMPOSIA

Panagiotis Sapountizis
University of Crete and IACM-FORTH, Greece

Title: How is information about stimulus location and identiy encoded and stored in the prefrontal and parietal cortices?

Previous studies have suggested a prominent role of prefrontal (PFC) and parietal (PPC) cortices in the encoding and maintenance of behaviourally relevant information in working memory. However, a direct comparison of how information about different stimulus parameters such as identity and location is encoded and stored in the two areas is missing.

We examined how identity and location of stimuli is represented in the frontal eye fields (FEF), in PFC, and the lateral intraparietal area (LIP), in PPC, during cue presentation and delay epochs of a visual search and a memory guided saccade task in two monkeys using simultaneous recordings in the two areas.

During cue presentation, spiking activity carried significant information about stimulus identity and location in both areas. However, identity information emerged earlier in LIP and was encoded more robustly compared to the FEF, whereas spatial information was encoded at similar latencies in the two areas. These results suggest hierarchical processing of identity information and parallel encoding of spatial information within the LIP-FEF circuit. In the delay period, identity information was diminished, whereas spatial information remained significant in both areas, suggesting a stronger involvement of FEF and LIP populations in maintaining spatial information in memory. We also found that distinct frequency bands in the local field potentials in the two areas were implicated in encoding and maintenance of spatial and non-spatial features with LIP showing stronger, gamma-band activity modulation and FEF showing stronger beta-band activity modulation. Finally, we found that stimulus selectivity modulated neural synchronization between the two areas.

Acknowledgements: Work supported by action “ARISTEIA II” of the Operational Program "Education and Lifelong Learning", (Action's Beneficiary: General Secretariat for Research and Technology), co-financed by the ESF and the Greek State

Katerina Kandylaki
Maastricht University, The Netherlands

Title: Why and how to employ ecologically valid tests in the neurobiology of language

Traditionally, language in the brain has been studied with tightly controlled experimental paradigms. These paradigms have been increasingly challenged for their generalisability to natural language use (Verga & Kotz, 2018; Kandylaki & Bornkessel-Schlesewsky, 2019). To increase generalisability of results, a number of groups have been exploring more ecologically valid designs (Lerner, Honey, Silbert, & Hasson, 2011; Brennan et al. 2012; Bašnáková, van Berkum, Weber, Hagoort, 2015; Kandylaki et al. 2015).
We suggest three options of how to make the study of language in the brain more ecologically valid: 1. embed tightly controlled stimuli into a rich context of stories, 2. quantify language features from the text and correlate them to the brain activity, 3. quantify regularity features from the audio signal and correlate them to the brain activity. The first option allows for traditional statistics by using an experimental and a control condition and embedding them seamlessly in the rich context. The strength of the next two options is the quantification of features and potential automation of analysis. A researcher would be able to use any text and quantify its relevant features with computational algorithms such as in Di Liberto, O'Sullivan, Lalor, 2015; Brennan & Hale, 2019; Weissbart, Kandylaki, & Reichenbach (resubmitted). Whereas in approach 2 the input of the quantifying algorithm uses text to extract the features of interest and therefore can produce one value per word or phoneme, in approach 3 the input of the (different) algorithm used the audio signal itself.
These methods and techniques have been popular in the neurobiology of language for the past decade. We see strong potential to transfer them from basic to clinical research and in a further step also clinical diagnosis and practice, as for example diagnosing language or psychiatric disorders using a pool of quantified stories and compare the brain responses to predefined expected outcomes.

1. Effects of cannabidiol (CBD) content in vaporized cannabis on tetrahydrocannabinol (THC)-induced impairment of driving and cognition

Thomas Arkell

Lambert Initiative for Cannabinoid Therapeutics, Brain and Mind Centre, University of Sydney, Australia.

BACKGROUND: As legal restrictions around recreational and medicinal cannabis are relaxed, the risks associated with driving under the influence of cannabis are of increasing community concern. Some evidence suggests that cannabidiol (CBD) may mitigate some of the adverse effects of delta-9-tetrahydrocannabinol (THC), implying that CBD enriched cannabis may differentially affect driving and cognition relative to pure THC (e.g. dronabinol), or prototypical THC-dominant chemovars.

OBJECTIVES: To test the effects of two cannabis cultivars (11% THC; <1% CBD; 11% THC; 11% CBD) and placebo (<1% THC/CBD) on simulated driving performance and cognition.

METHODS: Fourteen participants completed the study (11 males, 3 females). Participants were administered two active cannabis strains and placebo via vaporisation in a randomised and counter-balanced order over 3 sessions. Driving performance and cognition were measured at acute and post-acute intoxication phases (+0.5h, +3.5h). Plasma and saliva samples were taken before and after drug administration (-0.5h, +0.2h, +1h, +2h, +3h, +4h). Subjective drug effects and self-reported driving ability were also assessed.

RESULTS: Both active cannabis chemovars impaired driving during a car-following task and reduced performance on a Digit Symbol Substitution Task (DSST), Divided Attention Task (DAT) and Paced Auditory Serial Addition Task (PASAT). Impairment on the latter two tasks appeared to be worsened by CBD. Subjective drug effects (e.g. 'stoned') and confidence in driving ability did not vary with CBD content. There was also a significant increase in peak plasma THC concentrations following vaporization of THC/CBD equivalent cannabis relative to THC-dominant cannabis.

DISCUSSION: THC-induced driving impairment is pronounced under challenging conditions but may not be evident during monotonous and simple driving. Cannabis containing equivalent concentrations of CBD and THC is no less impairing than THC-dominant cannabis, and in some circumstances, CBD may in fact exacerbate THC-induced impairment.

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

2. Raman spectroscopy: evaluation of a non-invasive technique for the detection of topically applied ketorolac tromethamine *in vitro* and *in vivo*

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** Equal contribution*

Ocular pharmacokinetic studies investigate time- and dose dependent behavior of ophthalmic drugs. These studies are important to detect the maximum drug concentration (C_{max}), the time to reach C_{max} (T_{max}), half-life, and clearance of the drug. Based on those parameters, a dosage regimen can be created. Currently, the assessment of ocular pharmacokinetics is using tissues or fluids in a destructive test which comprises chemical pre-treatment followed by high-performance liquid chromatography (HPLC). A non-invasive pharmacokinetic assessment technique could resolve these issues. A technique that is potentially suitable for non-invasive detection of ocular pharmacokinetics is Raman spectroscopy. Raman spectroscopy identifies molecules, based on the specific inelastic scattering properties of their rotational and vibrational modes. This technique enables real-time detection of molecules without pre-processing and damaging tissue.

In this project, we evaluated the detection and quantification of ocular ketorolac tromethamine levels with confocal Raman spectroscopy after topical administration of Acular™. Confocal Raman spectroscopy and HPLC were compared in terms of sensitivity of detection. Enucleated pig eyes were treated with different concentrations of ketorolac. Hereafter, ketorolac concentrations in the aqueous humor of pig eyes were analyzed by confocal Raman spectroscopy and HPLC. Subsequently, twelve rabbits were treated with Acular™ for four weeks. At several time points, ketorolac concentrations in aqueous humor of the rabbits were measured by confocal Raman spectroscopy followed by drawing an aqueous humor sample for HPLC analysis.

In ketorolac treated pig eyes, both *ex vivo* Raman spectroscopy as well as HPLC were able to detect ketorolac in a broad concentration range. However, *in vivo* confocal Raman spectroscopy in rabbits was unable to detect ketorolac in contrast to HPLC.

To conclude, confocal Raman spectroscopy has the capacity to detect ketorolac tromethamine *in vitro*, but currently lacks sensitivity for *in vivo* detection.

3. Loss of Piccolo function in rats induces Pontocerebellar Hypoplasia type 3-like phenotypes.

Joanne Falck[1], Christine Bruns[1], Sheila Hoffmann[1], Isabelle Straub[2], Erik J. Plautz[3], Marta Orlando[4], Humaira Munawar[5], Marion Rivalan[4,5], York Winter[4,5], Zsuzsanna Izsvák[6], Dietmar Schmitz[4], F. Kent Hamra[7], Stefan Hallermann[2], Craig Garner[1*], and Frauke Ackermann[1*]

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The presynaptic active zone protein Piccolo (Pclo) is best known for its role in the formation of active zones and regulation of neurotransmitter release. Through manipulation of the *PCLO* gene, we have observed a specific effect of Piccolo loss-of-function (LOF) on the unique cerebellar mossy fibre (MF) synapse. We observe a profound impact on the anatomical, functional and behavioural level.

Analysis of Piccolo knockout ($Pclo_{gt/gt}$) brains revealed a severe reduction in brain size in comparison to wildtype ($Pclo_{wt/wt}$) counterparts, with reduced size of cerebellar, pontine and cortical regions. Formation of MF afferents to the cerebellum appear to be disrupted, as $Pclo_{gt/gt}$ MF terminals are reduced to half of size of $Pclo_{wt/wt}$. Climbing fibre innervation of the molecular layer of $Pclo_{gt/gt}$ cerebella is increased, indicating peturbation of the cerebellar network. We also observe a reduction in the α6 subunit of the $GABA_A$ receptor, expressed at the MF, which could be a homeostatic downregulation to compensate for reduced glutamatergic input from MF boutons.

On a functional level, $Pclo_{gt/gt}$ rats display impaired motor coordination, evidenced by failure in a rotarod task, despite adequate performance in tasks that reflect muscle strength and locomotion. We are currently undertaking electrophysiological recordings of MF boutons to further investigate the consequence of Piccolo LOF on on these unique synapse structures.

A mutation in the *PCLO* gene has been observed in patients with Pontocerebellar hypoplasia III (PCH III), a rare developmental disorder characterised by an abnormally small cerebellum and pons, severe developmental delay, motor deficits and seizures. As the human condition shares a number of anatomical and behavioural abnormalities with the $Pclo_{gt/gt}$ rats, we propose that the $Pclo_{gt/gt}$ mutation can be used as a model for PCH III, providing insights into how this AZ protein contributes to the formation and function of neural circuits during development.

4. "On cloud nine" or "in a funk"? conflicting effects of chronic adolescent exposure to HU-210 on tests of depressive-like behavior.

Miguel F. Ferreira[1,2], João Fonseca-Gomes[1,2], Nádia Rei[1,2], Francisco M. Mouro[1,2], Ana M. Sebastião[1,2]

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Adolescent and adult rodents are known to have differential susceptibilities to the effects of cannabinoid receptor agonists. Indeed, chronic cannabinoid exposure has been shown to induce opposite long-lasting effects, as a function of rodent age, whereby it has prodepressant- and antidepressant-like effects in adolescent and adult animals, respectively. Interestingly, while chronic cannabinoid exposure also seems to lead to short-term antidepressant-like effects in adults, there is no equivalent data regarding adolescent animals.

Here, we report two separate experiments, designed to assess the short-term affective behavioral effects of chronic adolescent HU-210 exposure. For this, two separate series of adolescent female Sprague-Dawley rats were administered twice-daily intraperitoneal injections of HU-210, following an ascending dosing schedule (PND35-37: 25µg/kg; PND38-41: 50µg/kg; PND42-45: 100µg/kg), for 11 days. Starting 24-hours following the last injection animals were tested in Open Field Test (OFT) and mFST, or the Elevated Plus Maze (EPM) and the SPT. Furthermore, samples from the hippocampus and the PFC were collected for molecular analyses.

In line with previous adult studies, HU-210-treated animals showed a marked antidepressant-like profile in the mFST – with significantly decreased immobility, and increased climbing – without alterations of locomotor activity or anxiety-like behavior in the OFT. Contrastingly, in the SPT, HU-210-treated animals showed strong decreases in sucrose preference/intake, suggesting a marked prodepressant-like impact of treatment.

In addition to having important implications for the cannabinoid literature, our results also highlight the necessity of critically evaluating the results of behavioral tests in light of their known limitations, and of using multiple tests to perform more reliable assessments. Finally, our results also raise the need for a mechanistic explanation of how a single manipulation led to the markedly contrasting effects observed, regarding depressive-like behavior.

5. **Interoceptive associations in early onset consumption of smoked cocaine**

Laura Alethia de la Fuente de la Torre

University of Buenos Aires, Buenos Aires, Argentina, Physics Department, IFIBA.

Objectives: Neurocognitive plasticity is critical for maturation throughout adolescence. However, these adaptive processes also increase vulnerability for developing addictions. The connection between plasticity and vulnerability for addictions is not fully known. Smoked cocaine (SC) is the earliest intermediate product of cocaine hydrochloride (CC) production and represents a public health problem for teenagers in developing countries. SC is highly addictive mainly due to its fast administration route, which has been linked an increased ability to sense and process body signals (interoception). However, there is scant evidence about changes during adolescence and no report has assessed interoception in SC consumers. In this study, we implement a multimodal approach (behavioral, EEG, and neuroimaging) to study differences in interoceptive performance between adolescent consumers of SC, CC and controls (CTR).

Methods: We included 25 participants that smoked (SC), 22 that insufflated cocaine (CC), and 25 matched CTR. Cocaine consumption begun between ages 14-16. We applied a heartbeat-detection (HBD) task and measured modulations of the heart-evoked potential (HEP) during interoceptive conditions. We complemented these measures with structural (MRI) and functional connectivity (fMRI) analysis of the main interoceptive hubs (insular, ACC and somatosensory cortex).

Results: HBD and HEP results showed that only SC consumers presented ongoing psychophysiological measures of enhanced interoceptive accuracy. This pattern was associated with a structural and functional tuning of interoceptive networks.

Conclusions: Our findings provide the first evidence of an association between cardiac interoception and SC consumption in adolescents. They also support models that propose hyper-interoception as a key aspect of addiction while suggesting that this enhancement may depend on specific administration routes.

6. Self-Rated Effectiveness of Microdosing With Psychedelics for Mental and Physical Health Problems Among Microdosers

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There is a growing interest in the use of low (micro) doses of psychedelic substances for health related purposes, including symptom relief for disorders like anxiety, depression, and pain. Although the focus of recent clinical trials has been on high doses of psychedelics, empirical evidence regarding the efficacy of microdosing for symptomatic relief is lacking. The present study aimed to investigate, by means of an online questionnaire, the self-rated effectiveness (SRE) of microdosing with psychedelics (MDP) for mental and physiological disorders compared to the conventional prescribed treatment and to regular doses of psychedelics.

An online questionnaire was launched on several websites and fora between March and July 2018. Respondents who had consented, were 18 years of age or older, had experience with microdosing and were diagnosed with at least one disorder ($N = 410$; 7.2%) were included in the analyses. Odds ratio were calculated to compare the SRE of MDP with conventional treatment, and regular psychedelic doses for mental and physiological diagnoses for each of the three effectiveness questions ("Did it work," "Symptom disappear," "Quality of life improved").

Odds ratio showed that SRE of MDP was significantly higher compared to that of conventional treatments for both mental and physiological diagnoses; and that these effects were specific for ADHD/ADD and anxiety disorders. In contrast, SRE of MDP was lower compared to that of higher, regular psychedelic doses for mental disorders such as anxiety and depression, while for physiological disorders no difference was shown.

This study demonstrates that SRE of MDP to alleviate symptoms of a range of mental or physiological diagnoses is higher compared to conventionally offered treatment options, and lower than regular psychedelic doses. Future RCTs in patient populations should objectively assess the effectivity claims of psychedelics, and whether these are dose related, disorder specific, and superior to conventional treatments.

7. *In-vitro* validation of systems biology derived drug-discovery to regulate molecular signature after traumatic brain injury

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Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Nearly 20 % of TBI patients die, while approximately 30% of survivors experience several health conditions such as amnesia and epileptogenesis. Existing treatments cannot prevent the progression of post-TBI sequela, hence, there is an unmet need of drugs that can halt secondary effects of TBI.

Our aim was to validate disease-modifying effects of *in-silico* discovered compounds with a predicted effect on gene networks regulated by TBI. We hypothesized that through systems-biology analysis, we would identify compounds which could address post-TBI sequela via their neuroprotective/anti-inflammatory/antioxidant properties.

Compounds were identified using our recently developed systems-biology pipeline which used TBI induced gene-expression signatures to perform LINCS-analysis. We selected compounds that have a potential in regulating post-TBI transcriptomics changes. *In-vitro* validation of chosen compounds was carried out in a neuron-BV2 microglia co-culture model, where acute neuroinflammation was induced. We assessed each compound's ability to promote neuronal viability, and to regulate tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO) which are indicators of neuroinflammation and neurotoxicity.

From 20 potential candidate compounds we validated 5 *in-vitro.* At 50 μM compound A and B decreased percentage of NO ($p<0.001$) and TNF-α ($p<0.001$) and improved neuronal viability ($p<0.001$) while compound-C was efficient at 50nM ($p<0.001$ for all experiments). At 10nM, compound-D reduced nitric oxide ($p<0.01$) and improved the neuronal viability ($p<0.01$) while compound-E showed no therapeutic effect in the co-cultures.

Our *in-silico* approach identified compounds with a potential in promoting neuronal survival and reducing neuroinflammation *in-vitro*. The most promising compounds will be validated *in-vivo*, in a clinically relevant rat model of TBI, to evaluate their capacity in regulating the modifications occurring after TBI.

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8. Psychophysiogical interaction analysis: exploring ventral striatum functional connectivity during cognitive control following drug cue exposure

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Drug cues play a central role in maintaining addiction, by eliciting craving and triggering further drug seeking and taking. We investigated the effect of drug cue exposure on the neural mechanisms of cognitive control, using a drug word Stroop task. We hypothesised that drug cue exposure would enhance appetitive processing and weaken cognitive control. 20 newly abstinent detoxified opioid dependent individuals (ODI) carried out a heroin drug word Stroop on two separate occasions whilst undergoing fMRI scanning. On one occasion, participants viewed a drug cue video[1] immediately before the Stroop task and on another occasion, they viewed a neutral cue video before the task. Following the neutral cue video, the drug word Stroop recruited regions associated with appetitive processing, including the ventral striatum (VS). Against our prediction, this activation was not enhanced by the drug cue video, but instead the VS failed to be recruited by the drug word Stroop during the drug cue session, with the difference in VS activation between sessions reaching significance (SVC $p_{FWE}<0.05$).

An explanation for this is that the ODIs are maximally motivated to maintain abstinence due to recently completing rehabilitation. Rather than triggering drug seeking and craving, the drug cue video may therefore prime inhibition of appetitive processing in the VS. To confirm this hypothesis, we conducted psychophysiological interaction (PPI) analysis investigating functional connectivity between the VS and cognitive control regions involved in the inhibition of craving. A region of interest approach was taken using DLPFC coordinates from previous craving inhibition studies[2, 3]. A cluster within this region ($k_E = 6$, SVC $p_{FWE(cluster)} = 0.044$) had increased connectivity to the VS following the drug cue compared to the neutral cue video. Our results indicate top down control over appetitive processing by the DLPFC and evidence a prefronto-stratial pathway involved in the cognitive regulation of craving[4].

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9. The desipramine-evoked CaMKII phosphorylation depends on an intact alpha1A-adrenergic receptor subtype: study in mice

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Background: Various mental disorders, including depression, are associated with abnormalities in adrenergic signaling in the brain and many antidepressant drugs act on noradrenergic system. Among the adrenergic receptors the α1 family (α1-AR) consists of the α1A, α1B and α1D subtypes. All they are Gq/11 coupled receptors, their stimulation lead to the increase of intracellular Ca2+ level and may activate the calcium/calmodulin-dependent protein kinase II (CaMKII). However, the α1-ARs subtypes differ in transduction of intracellular signaling events. Many reports suggest also their different involvement in modulation of antidepressant-like behaviors and animals' depression.
Objectives: The aim of the current study was to evaluate the effects of selective knock-out of α1A- or α1B-AR and chronic antidepressant treatment on phosphorylation at Thr286 of CaMKIIα/ß in prefrontal cortex of female mice.
Methods: Female knock-out mice devoid of α1A-AR (α1A-KO) or α1B-AR (α1B-KO) and wild type controls (WT) were chronically treated (21 days) with desipramine (20mg/kg) or saline. The protein level and phosphorylation ratio of CaMKII isoforms were analyzed in the prefrontal cortex by Western blotting.
Results: We found that both the deletion of α1A-AR and the deletion of α1B-AR did not affects the level of CaMKIIα/ß phosphorylation. However after chronic treatment with desipramine, the deletion of α1A-AR prevents the increase in phosphorylation level of CaMKIIα/ß which was observed in WT mice. This effect was not visible in the case of the α1B-AR deletion. There was no statistically significant influence of α1A-KO or α1B-KO or chronic treatment with desipramine on the total level of CaMKII protein.
Conclusions: Our results indicate different involvement of the α1A-AR and α1B-AR subtypes in the mechanism of action of the classical antidepressant drug desipramine. The α1A-AR, in contrast to the α1B-AR, appears to be necessary to obtain the proper effects of chronic despiramine treatment in prefrontal cortex of female mice.

10. Effect of acute physical exercise on associative memory and motor sequence learning via endocannabinoid signaling

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Recent studies suggest that acute physical exercise improves memory functions by increasing plasticity in the hippocampus. In animals, a single session of physical exercise has been shown to boost endocannabinoids (such as anandamide (AEA)) which are involved in hippocampal synaptic plasticity.

Here, we combined circulating AEA levels, behavioral measures and functional MRI to assess the impact of acute physical exercise (of moderate and high intensity) on associative memory and motor sequence learning in humans. We tested eighteen young, fit males in a within-subjects design across three visits (a rest visit, a moderate intensity exercise and a high intensity exercise visit). We used an associative memory task where subjects had to learn sequences of images and a serial reaction time test (SRTT) where participants had to perform finger movements which followed a hidden sequence. Both tasks were composed of 2 parts (an encoding and a test part), separated by the physical exercise or rest session. We took blood samples at each visit right before and right after the physical exercise or rest session and report differences in AEA levels from the first blood sample to the second one.

We report an increase in AEA levels as a result of acute physical exercise, both for moderate and for high intensity exercise. This increase correlates with individual hippocampal activation measures during the associative memory task, meaning that the more participants increased their AEA levels, the more they activated their hippocampus during the memory task. Further this increase also correlated with the performance of the motor sequence learning task, where the larger the AEA increase, the better participants performed. These results highlight the overarching role of the endocannabinoid system in both memory systems, and may support the role of the endocannabinoid system in hippocampal plasticity mechanisms in humans.

11. The effect of adolescent Δ_9-tetrahydrocannabinol and cannabidiol exposure on adult neurogenesis

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INTRODUCTION: Adult neurogenesis is affected by many factors, including cannabinoids, according to recent studies. While adult cannabinoid exposure increases adult neurogenesis in animal studies, adolescent exposure impairs it. This is likely due to the fast developmental changes occurring during adolescence,that affect neuroplasticity, reward neurocircuitry, cognitive function, and emotional behavior- which are disrupted by cannabinoid exposure. As a result, adolescent cannabinoid exposure may lead to neuropsychiatric disorders that are directly linked with impaired adult neurogenesis.

PURPOSE: The purpose of this study is to evaluate the effect of low, escalating doses of Δ_9-tetrahydrocannabinol (THC) on adult neurogenesis. By using a protocol developed in our lab, which attempts to simulate adolescent cannabis use, we aim to study the effect of THC exposure on adult neurogenesis, as well as the potential protective or inhibitory effect of cannabidiol (CBD).

METHODS: In this protocol, we administer low, escalating doses of THC (PND 35-37, 0.3 mg/kg, PND 38-41, 1 mg/kg, PND 41-45, 3mg/kg; i.p, twice per day) +/- CBD (dose, i.p.) to male Sprague-Dawley rats during adolescence (PND 35-45). From PND 45-60, the rats were examined weekly and on PND 60-62, they received a daily i.p. injection of BrdU (dose), in order to label the hippocampal neural stem cells during their differentiation into neuronal cells. Finally, migration, maturation and incorporation of hippocampal neural stem cells will be evaluated in adulthood (PND 85).

RESULTS: The immunohistochemistry analysis is still in process.

12. A novel mouse model of Dravet syndrome: proteomic characterization

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Introduction: Dravet syndrome is known as a rare, severe pediatric form of epilepsy with intellectual and motor disabilities. Proteomic characterization of a novel mouse model of Dravet syndrome can show alterations in protein expression involved in epileptogenesis and indicate potential new targets for treatment of the syndrome.

Methods: A novel, commercially available knock-in mouse model of Dravet syndrome, carrying the mutation in the *Scn1a*-A1783V gene, was used for seizure, behavioral and proteomic profiling. The left hippocampus was dissected from two (prior to spontaneous seizures onset) and four (following the spontaneous seizures onset) week-old male mice and analysed using LC-MS/MS with label-free quantification. Immunohistochemical staining was performed in the right brain hemisphere. ConsensusPathDB pathway tool was used for pathway enrichment analysis.

Results: Dravet mice showed an increased susceptibility for hyperthermia-induced seizures, development of spontaneous seizures, high incidence of SUDEP and hyperactivity, therefore showing an excellent face validity of this model for Dravet syndrome. Proteomic analysis of the hippocampus distinguished around 4000 proteins: 208 significantly changed in two-week-old, 881 significantly changed in four-week-old Dravet mice. Pathway analysis identified 16 and 127 significantly regulated pathways at the early and late time point, respectively. Interestingly, several regulated pathways in four-week-old mice were involved in glutamatergic, calcium and phosphatidylinositol signalling. When compared to a post-SE electrical rat model, only a small group of overlapping proteins was identified in this genetic mouse model. Differential expression of selected proteins was confirmed by immunohistochemical staining.

Conclusion: This study demonstrated marked proteome differences between Dravet and wildtype mice as well as between two- and four-week-old Dravet mice. These differences point out specific alterations in neurotransmission, which may contribute to the course of the disease and may guide future target identification.

13. Exploring modulatoin of neuroinflammation by ketamine in an animal model of depression

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Introduction: About 350 millions of people suffer from major depressive disorder (MDD) worldwide, with more than ⅔ of patients being resistant to treatment. Ketamine, a glutamatergic receptor antagonist, confers a rapid (within hours) antidepressant effect, even in treatment-resistant patients. However, mechanisms by which ketamine exerts its antidepressant effects are not fully understood. Therefore, we investigated whether ketamine's antidepressant effect is associated with modulation of neuroinflammation (microglia and macrophages activation) in the repeated social defeat (RSD) model of depression in rats.

Materials and Methods: All animal experiments were approved by the Central Authority for Scientific Procedures on Animals (CCD) of the Netherlands. Three experimental groups of 12 rats were used: control+vehicle, RSD+vehicle and RSD+ketamine. Experimental rats were submitted to a 5-day RSD protocol followed by one acute injection of ketamine (20 mg/kg). Behavior was assessed using the sucrose preference test (SPT) and open field test (OFT) for depressive-like and anxiety-like behavior. Positron emission tomography (PET)-scans were performed to measure changes in neuroinflammation, using the radiotracer 11C-PK11195 to mark TSPO protein overexpression in activated glial cells. One-way ANOVA, two-way ANOVA and Spearman were used as statistical tests.

Results: After RSD, rats showed a significant decrease in sucrose consumption and weight gain ($p<0.01$). This trend was not affected by ketamine injection.. In addition, PET imaging showed an increase in neuroinflammation in the insula and enthorhinal cortex for RSD+vehivle and RSD+ketamine, and in basal ganglia only for RSD+ketamine ($p<0.05$). There were no significant differences in PET tracer uptake between the RSD+ketamine and the RSD+vehicle group.

Discussion: Our results showed increased anhedonia due to RSD, which was not affected by ketamine injection. Additionally, RSD alone or in combination with ketamine caused neuroinflammation in the insula and basal ganglia, whereas RSD+ketamine, but not RSD alone, also induced neuroinflammation in the basal ganglia.

14. Soluble guanylate cyclase stimulator vericiguat enhances memory processes through GluA1-AMPA receptor trafficking

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Cognitive impairment is one of the main symptoms of Alzheimer's disease, which negatively impacts the quality of life of patients. Therefore, a pharmacological intervention that has memory enhancing effects would be beneficial to patient outcomes. Previous studies have implicated the importance of the intracellular cGMP-PKG signaling pathway in memory processes. This pathway is initiated through the activation of soluble guanylate cyclase (sGC) by nitric oxide (NO). sGC stimulators enhance sGC activity by directly stimulating its production while also increasing sGC sensitivity to endogenous NO. In this experiment we hypothesized that sGC stimulator vericiguat could have beneficial effects on memory functioning through enhanced cGMP-PKG signaling and subsequent increased GluA1-AMPA receptor (AMPAR) trafficking.

To evaluate the effects on long-term memory functioning in rats, different oral dosages of vericiguat were administered 30 minutes before T1 of a 24h inter-trial interval object location task (OLT) to investigate memory acquisition processes. To evaluate the effects on GluA1-AMPAR trafficking, an acute mouse hippocampal slice model was used to chemically induce long-term potentiation (chemLTP). The slices were incubated with vericiguat immediately before chemLTP induction to investigate acquisition-like processes, or 10 minutes after chemLTP induction to investigate early consolidation-like processes. GluA1 subunit dynamics were measured using western blotting.

It was found that 0.3 and 1 mg/kg vericiguat were able to significantly improve long term memory performance in the OLT. Additionally, treatment with 10 nM vericiguat increased chemLTP-induced trafficking to the membrane of a pre-existing pool of GluA1-AMPARs in acquisition-like processes only, which was found to be independent of phosphorylation of the receptor on S845.

These data suggest that vericiguat enhances memory function in rats and that the in vivo memory improvement is acquisition driven.

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15. TrkB receptor agonist as a new therapy for treatment of depression – screening platform

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Brain derived neurotrophic factor also known as BDNF, is a member of the neurotrophin family and acts as a key regulator of many neuronal processes. BDNF is a ligand for TrkB receptor which has tyrosine kinase activity. BDNF has been reported to be involved in pathogenesis of many neuropsychiatric diseases. Targeting the BDNF-TrkB pathway by the small molecular compounds may have antidepressant and procognitive effects. Here we would like to present the screening platform designed for selection of active and selective TrkB receptor agonists.

The primary screening of compound library is performed by using Microscale Thermophoresis (MST) method for identification compounds interacting with the extracellular domain of TrkB receptor. Then, selected molecules are screened *in vitro* using SN56 cell line overexpressing TrkB and the ELFI method (Enzyme-linked fixed cell immunoassay) to determine orthosteric activity and allosteric modulation of the tested compounds. Compounds are further tested in differentiated SH-SY5Y cell line model for analyzing downstream protein activation by immunoblotting. Preliminary specificity of selected compounds is assessed using SN56 TrkA cell line or by use of Trk's inhibitors (K252a). Subsequently, the ability of compounds to provoke TrkB dimerization is monitored by native electrophoresis. The presented approach has allowed to identify so far five orthosteric agonists and one positive allosteric modulator compounds.

16. Quantification of methamphethamine self-administration in *Drosophila's per* and *tim* circadian mutants

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Addiction is a complex neuropsychiatric disorder caused by repeated illicit use of addictive drugs, such as methamphetamine (METH). Psychostimulants cause changes in the brain functioning through a mechanism of drug-induced neuronal plasticity. Self-administration is one of the behavioral endophenotypes connected to addiction and serves as a measure for the rewarding effect of the drugs. Several studies have shown the role of circadian genes in the direct regulation of dopaminergic reward circuitry. That indicates the potential involvement of circadian genes in the neuronal plasticity connected to of regulation of voluntary drug consumption which contributes to addiction in general.

To improve over existing CAFE assay used to measure liquid food consumption, we developed FlyCafe, a high-throughput method using the concept of CAFE assay in combination with Drosophila Activity Monitoring system (DAMs). In this new assay for each fly we objectivly quantify: amount of psychostimulant that flies self-administer, changes in the locomotor activity during the consumption and percentage of time spent close to the food capillaries. Using FlyCafe assay we tested the voluntary METH consumption in *wild-type Drosophila* males and compared that to *period* (*per*) and *timeless* (*tim*) circadian mutants.

Our results showed distinct preference for METH over sugar-based food in *wt* flies, suggesting that METH activates motivational and reward circuits in *Drosophila's* brain. Circadian mutants showed different pattern, developing the preference for METH only on the fourth day of the self-administration, suggesting a role for circadian genes in the regulation of rewarding effects of psychostimulants.

Our findings will provide the basis for further investigation of genetic mechanisms that influence long-term neuronal plasticity induced by addictive drugs in order to uncover new therapeutic targets aimed at the treatment of substance abuse disorders.

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17. Locus impairment of DISC1 results in cognitive deficiency in rodents

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Introduction: Microglia are responsible for excessive synaptic loss in schizophrenia and Alzheimer's disease. *Disrupted-in-schizophrenia-1* (DISC1) is a gene implicated in both neuropathologies, yet its function has only been studied in neurons where it interacts with phosphodiesterase 4 (PDE4). Interestingly, both DISC1 and PDE4 are expressed in microglia. PDE4 inactivates cAMP, a second messenger needed for phagocytosis. We hypothesized that the interaction of DISC1 with PDE4 regulates microglia-mediated synaptic elimination in the hippocampus, resulting in cognitive deficits in DISC1 locus impairment (LI) mice.
Materials & methods: 10-week old DISC1 LI mice were compared to WT mice in a battery of behavioral tests (n = 15/group), including the object location task (OLT), marble burying, nestlet shredding and tube dominance test. The effect of DISC1 LI on PDE4B isoform expression in primary microglia was investigated using qPCR. Statistical significance of gene expression levels and representative outcome measures for cognition, social and instinctive behavior was evaluated using the Student's t-test.
Results: DISC1 locus impairment significantly hampered cognitive capacity and instinctive mouse behavior. In the OLT, DISC1 LI mice spent equal amounts of time on both objects, indicating premature lapse in memory and impaired hippocampal functioning. Failure to exhibit instinctive behavior, as evidenced in the nestlet shredding test, confirmed this lack of hippocampal functioning. Furthermore, the social phenotype was altered, indicating neuronal dysfunction in the prefrontal cortex. *In vitro* analysis using qPCR revealed differential expression of various isoforms of PDE4B, which constitutes the highest expressed PDE4 gene product, in microglia.
Discussion & conclusions: The differential expression of PDE4B points towards cAMP as a potential downstream effector mediating synaptic elimination. *Post mortem* analyses investigating synapses in implicated brain regions will allow to elucidate microglial contribution in the impairment. This data would implicate PDE4B as a therapeutic downstream target to negate the effects of mutations in DISC1.

18. Curcumin: Novel treatment in neonatal hypoxic-ischaemic brain injury

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Hypoxic-ischaemic encephalopathy (HIE) is a major cause of mortality and morbidity in neonates, with an estimated global incidence of 3/1000 live births. HIE brain damage is associated with an inflammatory response and oxidative stress, resulting in the activation of cell death pathways. At present, therapeutic hypothermia is the only clinically approved treatment available for HIE. This approach, however, is only partially effective. There is therefore an unmet clinical need for the development of novel therapeutic interventions for the treatment of HIE.

Curcumin is an antioxidant reactive oxygen species scavenger, with reported anti-tumour and anti-inflammatory activity. Curcumin has been shown to attenuate mitochondrial dysfunction, stabilise the cell membrane, stimulate proliferation, and reduce injury severity in adult models of spinal cord injury, cancer, and cardiovascular disease. The role of curcumin in neonatal HIE has not been widely studied due to its low bioavailability and limited aqueous solubility. The aim of this study was to investigate the effect of curcumin treatment in neonatal HIE, including time of administration and dose-dependent effects.

Our results indicate that curcumin administration prior to HIE in neonatal mice elevated cell and tissue loss, as well as glial activation compared to HI alone. However, immediate post-treatment with curcumin was significantly neuroprotective, reducing grey and white matter tissue loss, TUNEL+ cell death, microglia activation, reactive astrogliosis and iNOS oxidative stress when compared to vehicle-treated littermates. This effect was dose-dependent, with 200μg/g body weight as the optimal dose-regimen, and was maintained when curcumin treatment was delayed by 60min or 120min post-HI. Cell proliferation measurements showed no changes between curcumin and HI alone, suggesting that the protective effects of curcumin on the neonatal brain following HI are most likely due to curcumin's anti-inflammatory and antioxidant properties, as seen in the reduced glial and iNOS activity

19. The selective muscarinic type 1 receptor antagonist, biperiden, does not impair episodic novelty memory: An EEG study

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The muscarinic antagonist, scopolamine has long been used as a model of episodic memory decline, as seen in Alzheimer's disease (AD). However, due its non-selective profile it is known to impair attention and cause severe side effects. Since, memory has been largely found to rely on the muscarinic M1 subtype receptors, a more selective M1 antagonist, such as biperiden might be a better alternative. Furthermore, previous research has shown that novelty processing is impaired in AD. Therefore, we investigated the effects of 4 mg orally administered biperiden on episodic novelty memory according to a double-blind, placebo-controlled, 2-way cross-over design in a population of healthy young volunteers. Memory was tested using a three-phase novelty paradigm with abstract figures and pseudo words. The behavioral and electrophysiological effects were investigated using signal detection theory and EEG. Four evoked response potential components of interest were the N200, P300, N400 and P600. Moreover, early and late old/new effects were examined. Additionally, we measured possible side effects. According to our results biperiden did not impair novelty memory, and did not cause any severe side effects. However, compared to placebo it slowed reaction times with respect to recognition of the abstract figures but not to the pseudo words. This indicates possible impairing effects of this higher dose on attention. As such, our behavioral results do not support the application of biperiden for modeling novelty memory problems as seen in AD. The underlying electrophysiological results are being currently analyzed.

20. Circulating microRNA as potential biomarkers for psychiatric and neurodegenerative disorders

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Circulating microRNAs (cimiRNAs) are a class of non-encoding RNAs found in body fluids such as blood, cerebrospinal fluid (CSF) and tears. CimiRNAs have been implicated as promising biomarkers for central nervous system (CNS) disorders because they are actively secreted as messengers and are profoundly involved in fine-tuning of developmental and differentiation processes. Furthermore, these are attractive biomarkers because they are extremely stable, tissue enriched and can be determined in a quantitative manner. This review aims to provide a comprehensive assessment on the current progress regarding the potential value of cimiRNAs as CNS biomarkers. Within this framework five CNS disorders were explored which share a common pathological hallmark namely cognitive impairment. The CNS disorders include Major depression disorder (MDD), Bipolar disorder (BD), Schizophrenia (SZ), Alzheimer's disease (AD) and Parkinson disease (PD). The similarities and differences between altered cimiRNAs in the different disorders are presented. The miR-29 family, miR-34a-5p and miR-132-3p are further explored as common dysregulated cimiRNAs found in the CNS disorders. Furthermore, it is shown that the type of body fluid used for measuring cimiRNAs is important as inconsistencies in cimiRNAs expression directions are found when comparing CSF, blood cell-free and blood cell-bound samples.

21. Neurodevelopment and pharmaceuticals: *in vitro*, *in vivo* or organoids?

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According to recent epidemiological studies, brain-related illnesses encompassing for example depression, epilepsy and brain neoplasms will take the leading position in the global burden of disease by the 2020s. Many neurological diseases begin during embryonic and fetal life. Some of these can be caused or precipitated by drug use during pregnancy and pharmaceuticals taken by mothers may affect fetus neurodevelopment. For this reason, we need platforms for testing pharmaceuticals in the developing brain – the only way to do that is by employing adequate *in vitro* and *in vivo* models. In our work, we are aiming to approach to neurodevelopment and pharmaceutical interactions through the use of several experimental models, such as immortalized neuron-like cells and differentiated neurons derived from human iPSCs as *in vitro*, but also developing chicken embryo as *in vivo* model and human organoids of different brain regions. Based on the human iPSC, we already succeeded and recapitulated this new approach for so-called brain organoids. We managed to culture organoids by the days-in-vitro 18 and we showed that early brain development markers (such as Brna3a and FoxP1) are expressed even at this stage of growth. It's also been shown in our lab that several potential candidate genes for changing in expression might be employed for neuropharmacotoxicological analysis, e.g. pax6, mmp9, pcna. Moreover, developing chicken brain and *in ovo* as an alternative *in vivo* model was employed. The chicken egg is trustful and easy to maintain animal model, where all the events of neurodevelopment can be recapitulated under drug treatment. Based on the facts, that many different neuropathological deviations take place during intrauterine development and the last studies which show that many drugs might cause pathological changes in the developing brain – testing of the neuropharmaceuticals is still a big issue. The question is which model system to use – 2D *in vitro* or 3D organoids or *in vivo* or all of them at once?

22. In-vivo treatment with the mGluR5 negative allosteric modulator CTEP ameliorates the disease progression in the SOD1G93A mouse model of amyotrophic lateral sclerosis.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease leading to motor neurons (MNs) death and to glial cell damage. The aetiology of the disease is still poorly defined, although glutamate (Glu)-mediated excitotoxicity is assumed to represent one major cause of MN damage. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) are implicated in Glu-mediated excitotoxicity in ALS, since they are involved in prominent cellular processes and largely over-expressed during disease progression. In this context, we recently demonstrated that activation of presynaptic mGluR1 and mGluR5 produced abnormal Glu release in the SOD1G93A mouse model of ALS and that halving or abolishing their expression significantly delays the disease onset and prolongs survival, ameliorates the disease progression by postponing the decline of motor abilities, preserves the spinal cord MNs from death and reduces the astrocytes and microglia activation in SOD1G93A mice.

Due to these encouraging results, we investigated here the effects of the in vivo pharmacological treatment of SOD1G93A mice with 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy) phenyl)-1H-imidazol-4-yl) ethynyl) pyridine (CTEP), a negative allosteric modulator of mGluR5. We treated 90 days old symptomatic SOD1G93A mice with CTEP (2mg/kg/48h or 4mg/kg/24h) or vehicle, by gavage, until death. CTEP dose dependently ameliorated the clinical features in SOD1G93A mice. The lower dosage barely produced positive effects. The higher dosage significantly delayed the disease onset, increased survival and improved motor abilities in treated mice. The in vivo treatment also preserved of MNs from death, decreased the activation of astrocytes and microglia and reduced the abnormal Glu release in spinal cord. All these effects were more marked in female than in male SOD1G93A mice.

In conclusion, our previous and present results suggest that mGluR5 represents a promising target for the treatment of ALS and the CTEP in vivo effects in SOD1G93A mice support this translational perspective.

23. GABAergic neurotransmission in the human brain characterized by single- and paired-pulse TMS with EEG co-registration and pharmacological GABAA activation

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All information transmission in the brain depends on the current level of neuronal excitability. Amongst numerous neurotransmitters that maintain this dynamic state, γ-aminobutiric acid (GABA) is of particular importance, as it represents the main inhibitory neurotransmitter of the central nervous system. The present study aims to characterize the state of GABAergic neurotransmission in the human brain using a non-invasive way that will subsequently allow comparing various patient groups to healthy controls.
We combined transcranial magnetic stimulation (TMS) over the left primary motor cortex (M1) with electroencephalography (EEG) and recorded TMS evoked potentials (TEPs) before and after pharmacological activation of GABAA receptors with alprazolam. In addition, we applied paired-pulse TMS in order to explore the phenomenon of short-latency intracortical inhibition (SICI), which represents the manifestation of local inhibition mediated by GABAA receptors. 20 healthy young volunteers participated in two sessions, each consisting of a baseline recording followed by administration of alprazolam or active placebo (cetirizine) and a post-medication recording. The TMS coil was kept on the target using MRI–based neuronavigation. Three types of TMS stimuli were applied: suprathreshold single-pulse (120 % resting Motor Threshold - rMT), subthreshold single-pulse (80% rMT), and paired-pulse (80% + 120% rMT, 2.5 ms inter-stimulus interval).

Our results show that alprazolam modulates amplitudes of early TEP waves following a single suprathreshold TMS stimulus. At baseline, the paired-pulse TMS evokes substantially reduced MEPs and amplitudes of early TEP waves when compared to a suprathreshold single-pulse. The effect of SICI in TEPs is reduced following the administration of alprazolam. In MEPs, alprazolam causes an amplitude decrease after a suprathreshold stimulus, while the response to paired-pulse TMS remains unchanged. Altogether, selective effects of alprazolam vs. cetirizine are clearly observable in TEPs and MEPs, which could reflect the state of GABAergic neurotransmission.

1. The role of Lhx6 on the maturation of MGE-derived mammalian cortical interneurons.

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Inhibitory (GABA-producing, GABAergic) interneurons comprise one of the two main classes of cortical neurons, that are essential for the assembly and function of cortical neural circuits. Consistent with the critical role of inhibitory interneurons in brain function, deficits of the GABAergic neuronal network have been implicated in the etiology of several neurodevelopmental disorders, such as Autism, Epilepsy and Schizophrenia. We have recently generated a series of transgenic mice, in which the transcription factor Lhx6, that is essential for the development of Medial Ganglionic Eminence (MGE)-derived, Parvalbumin (PV) and Somatostatin (SST) expressing cortical interneurons, can be deleted in a stage and cell-type specific manner. Our preliminary results show that mice in which Lhx6 has been deleted during embryogenesis (Nkx2.1CRE dependent Lhx6 conditional KO mice), demonstrate a dramatic decrease in the number of MGE-derived interneurons, due to cell death. In addition, by performing genome wide profile analysis between Nkx2.1CRE dependent Lhx6 conditional KO and control mice, at early postnatal stages, we found that the remaining MGE-derived interneurons fail to differentiate and acquire their mature functional properties. As a result, mutant mice are characterized by severe excitation/inhibition imbalance, and develop spontaneous seizures with neonatal onset. Conversely, mice in which Lhx6 is specifically deleted at early postnatal stages (10 days after birth) from PV-expressing interneurons (PVCRE dependent Lhx6 conditional KO mice), show no defects in the number of PV+ cells, and they do not develop any seizures. We are further characterizing the cellular phenotype, as wells as, the molecular profile of these mice by performing RNA-seq. In addition, we are planning to analyze mice in which Lhx6 is deleted from both PV and SST expressing interneurons, at birth (Gad2CREERT2 dependent Lhx6 conditional KO mice). Our results will elucidate the impact of Lhx6 function in gene regulatory networks controlling distinct stage and cell-type specific processes during cortical interneuron development.

2. The role of the genome organiser protein SATB1 in brain homeostasis.

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The mammalian cortex consists mainly of principal excitatory pyramidal neurons and inhibitory interneurons, forming local microcircuits that subserve specific behaviors. The interplay between these two neuronal populations [that drive excitation (E) and inhibition (I)] are crucial in maintaining normal activity patterns in the brain. A number of neurological and psychiatric disorders have been associated with changes in E/I balance. Indeed, neural networks use several different mechanisms, globally referred to as homeostatic forms of plasticity, to maintain their firing rates at a stable level. Although activity dependent neuron-specific genetic programs associated with plasticity have recently been described, only a few key players that activate these cascades have been identified so far. Our work aims to study the role of the activity modulated transcription factor and genome organizer protein Satb1 in controlling E/I balance at the circuit level. To address this hypothesis, we have generated and currently analyse interneuron and projection neuron specific Satb1 deficient mice. Our preliminary results show that interneuron-specific deletion of *Satb1* results in a profound loss of cortical interneurons, but does not affect the early development of projection neurons. Consistent with this, mutant mice with *Satb1*-depleted interneurons exhibit behavioural and histological signs of seizure activity. Conversely, deletion of Satb1 from cortical excitatory neurons does not affect the specification or survival of both cortical neuronal types, but results in a dysregulation in the expression pattern of several activity dependent immediate early genes (IEGs), in the cortex. These findings demonstrate that SATB1 has a critical role in both inhibitory and excitatory cortical neurons to regulate activity levels in cortical networks.

3. Platelets as novel regulators of postnatal brain neural stem cells

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Pools of postnatal brain Neural Stem Cells are clustered in specialized microenvironments called stem cell niches. One such niche is located at the Subependymal Zone (SEZ) of the lateral walls of the brain's lateral ventricles. We have previously shown the specific aggregation of platelets (PLTs) within the vasculature of the niche in response to a focal demyelinating lesion in the adjacent corpus callosum (CC) and the pro-survival effects that PLT-derived factors exert on pbNSCs in vitro (Kazanis et al., 2015). Here, we extend our investigation in two ways, firstly by co-culturing SEZ-derived pbNSCs and PLTs in order to assess the effects of their direct cell-to-cell interaction and secondly by analysing the SEZ and the CC in transgenic mouse lines with altered numbers of PLTs (Nbeal2, Crlf3, JAK2V6). Our data revealed that pbNSCs co-cultured with high densities of PLTs, but in the absence of the necessary mitotic factors EGF and FGF2, retain normal levels of proliferation. Moreover, the presence of PLTs led to increased percentages of oligodendrogenic progenitor cells (OPCs) (immunopositive for Olig2) under proliferation conditions but did not result in differentiation bias after removal of growth factors. Notably, histological analysis showed deficient activation of OPCs in response to focal demyelination in the corpus callosum, with no changes in neurogenesis in Crlf3-/- mice. Finally, when co-cultures where set up using PLTs isolated form the Nbeal2-/- mice, in which α-granules are not functional, the effect on the oligodendrogenic lineage of pbNSCs was abolished. Altogether our results reveal a role of PLTs in the regulation of pbNSCs that is partly dependent on α-granules.

Kazanis I, Feichtner M, Lange S, Rotheneichner P, Hainzl S, Öller M, Schallmoser K, Rohde E, Reitsamer HA, Couillard-Despres S, Bauer HC, Franklin RJ, Aigner L, Rivera FJ (2015). Lesion-induced accumulation of platelets promotes survival of adult neural stem / progenitor cells. Exp Neurol, 269: 75-89.

4. Neurogenesis and senescence in brain stem cell populations: the role of the niche

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The processes of neurogenesis and gliogenesis continue in the mammalian postnatal brain from stem and progenitor cell populations that are located either in stem cell niches or in the parenchyma. Those clustered in niches (for example in the Subependymal Zone of the lateral walls of the lateral ventricles) retain a neurogenic capacity, but their pool becomes depleted with ageing and are prone to enter into cellular senescence both in vivo and in vitro. Those surviving throughout the parenchyma (such as the Oligodendrocyte Progenitor Cells) are unipotent, but are resistant to ageing and do not enter into senescence. Here, we report our findings showing that in cultures of postnatal brain Neural Stem Cells (pbNSCs) senescent cells are observed in higher frequency within 3D self-organized structures called neurospheres and we investigate their neurogenic versus oligodendrogenic capacity in relation to the architecture (3D versus 2D) of the microenvironment.

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5. The effects of early life experiences on dopamine receptors of adult rat basal ganglia and behavior

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Development is a dynamic process that is shaped by the combination of environmental and genetic factors. Early life experiences play a pivotal role on the final configuration of brain, which determines neurobiological systems and adult behavior. Neonatal handling (H) and maternal separation (MS) constitute two experimental animal models of early life experience. H and MS consist of a repeated brief (15 minutes) and long (3 hours) maternal separation, respectively, during the first 21 postnatal days. Both experimental models affect the Hypothalamic-Pituitary-Adrenal (HPA) axis. Neonatal handling reduces the activity of the HPA axis, while maternal separation has the opposite effect. The mesocortical and mesolimbic dopamine (DA) system are important modulators of the HPA axis. The aim of the present study was to identify the effects of early life experiences on dopamine D1 and D2 receptor levels in caudate putamen of striatum, nucleus accumbens, internal segment of globus pallidus and substantia nigra of adult rat male and female brain and to correlate these effects with adult behavior on stress and novelty. In order to determine differences in dopamine D1 and D2 receptor levels in H and MS experimental groups compared to control, western blot analysis and quantitative in vitro autoradiography using [3H]SCH23390 and [3H]raclopride ligands, respectively, were performed. The open field and novel object recognition tasks were also performed in 3-4 month-old rats. We found statistically significant reductions of D1 and D2 receptors in most basal ganglia nuclei studied of neonatally handled, but not of maternally separated animals. We also found that indices of stress in the open field task and novelty seeking were affected in experimental compared to control rats. In conclusion, it is suggested that the effects of early life experience on dopamine receptors and behavior could depend on the duration of maternal separation.
* equal contribution

6. Characterization of adult dopaminergic neurogenesis in the Substantia Nigra in wild-type mice and in the "weaver" mouse model of PD, after BNN-20 long-term administration.

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Induction of adult neurogenesis is a promising strategy for cell replacement therapy of neurodegenerative diseases. Such strategies could become crucial for the treatment of Parkinson's Disease (PD), which is mostly diagnosed in an already progressed stage, where 50-70% of the dopaminergic neurons of the Substantia Nigra pars compacta (SNpc) are lost[1].
Recent findings of both our and other research groups[2,3,4] confirm the existence of a basal rate of dopaminergic neurogenesis in the adult SNpc, significantly increased under degenerative conditions (PD models). Interestingly, our previous findings in a progressive model of PD, the "weaver" mouse, suggest that long-term administration of the synthetic microneurotrophin BNN-20 can enhance adult dopaminergic neurogenesis specifically in the SNpc, leading to partial restoration of the dopaminergic neuron number.
Herein, we present our findings on further *in vivo* and *in vitro* characterization of the adult dopaminergic neurogenesis that takes place in the SNpc of both wild-type and "weaver" mice, in control conditions or after BNN-20 administration.
Our results, using DiI tracing experiments, indicate that the largest adult brain neurogenic niche located at the subventricular zone contributes newborn dopaminergic neurons in the SNpc. Furthermore, BNN-20 appears to enhance dopaminergic neurogenesis by increasing the differentiation of neural stem cells (NSCs) into neurons, without significantly affecting their proliferation. Finally, characterization of the maturation profile of newborn dopaminergic neurons *in vivo* provides interesting information on the molecular pathway through which the adult-born neurons acquire their dopaminergic phenotype.
The aforementioned findings contribute in the general knowledge on the newfound topic of the adult midbrain dopaminergic neurogenesis, and could represent the groundwork for a future cell replacement therapeutic strategy against PD.

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7. McIdas is essential for the differentiation of multiciliated ependymal cells in mouse brain.

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Multiciliated cells are post-mitotic cells that bear on their apical surface multiple cilia that beat unidirectionally and support the directed fluid flow across an epithelium in mammals. In the brain, multiciliated cells are called ependymal cells, they line the walls of the ventricles and contribute to cerebrospinal fluid flow. Defects in ependymal cell differentiation and function have been implicated in hydrocephalus pathogenesis. Our lab research is focused on unraveling the molecular pathway that regulates the commitment and differentiation of neural progenitors towards ependymal cells in brain. Previous studies from our lab have indicated two members of Geminin superfamily, GemC1 and McIdas, as master regulators of the multiciliogenesis program. More specifically, they are important for the activation of the molecular cascade composed by transcriptional factors involved in multiciliogenesis, including p73, c-Myb and Foxj1. To investigate the role of McIdas in multiciliogenesis, we have generated mice that constitutively lack McIdas expression. McIdas knock-out mice are born in normal ratio but they display growth retardation and postnatal lethality. Moreover, these mice develop hydrocephalus as their brain ventricles were found enlarged. We have also performed immunofluorescence in coronal brain sections and found that the expression of p73 and Foxj1 in McIdas KO mice remains, indicating that progenitor cells are committed towards the ependymal lineage. However, when we examined whole mounts of lateral wall of brain lateral ventricles, we found that upon McIdas deletion, the terminal differentiation of multiciliated cells is severely disrupted, as we could not detect any multiciliated cells. In conclusion our data suggest that McIdas is not necessary for the commitment to the ependymal lineage but affects the later steps of differentiation towards multiciliated cells in the brain and its deficiency results in the development of hydrocephalus.

8. Prox1 affects axon outgrowth and neuronal maturation during central nervous system development

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Prox1 is a critical regulator of embryonic and adult neurogenesis via induction of cell cycle exit and acquisition of early neuronal identity in neural stem/progenitor cells. Paradoxically, in the majority of newly born neurons, Prox1 expression is heavily down-regulated during the transition from the state of immature to terminal differentiated neurons, indicating a functional role in inhibiting late neuronal maturation.

To test this hypothesis, we investigated whether Prox1 plays a regulatory role in the control of axon elongation and neuronal maturation. Gain-of-function analysis showed that Prox1 is sufficient to strongly inhibit neurite extension in Neuro2A and SH-SY5Y cells, either in the absence or presence of retinoic acid. Conversely, shRNA-mediated knockdown of Prox1 in Neuro2A cells induced the extension of neurites under the same conditions. Further analysis in mouse primary neuronal cultures revealed that Prox1 overexpression leads to significant reduction of axon length. To explore the molecular mechanism of this action, we performed RNA-Seq analysis in Prox1-overexpressing Neuro2A cells. In agreement with our observations, Prox1 affects many critical genes for neuronal maturation, including genes in retinoic acid, axon guidance, calcium, neurotrophin, and MAPK signaling pathways. By real time RT-qPCR analysis, we confirmed the effect of Prox1 on several genes involved in these pathways, either in Neuro2A or in primary neurons. Interestingly, Prox1 strongly inhibits many components of Ca^{+2} signaling pathway, an important mediator of axon extension and neuronal maturation such as Camkk2, Camk2b Camk4, Cacnb1 and Cacnb3. In accordance to these findings, we revealed that Prox1 is sufficient to suppress Ca^{+2} entry upon KCl-mediated depolarization and reduce CREB phosphorylation.

Collectively, these observations suggest that Prox1 may act as a potent suppressor of axon elongation and neuronal maturation via inhibiting Ca^{+2} signaling pathway.

9. Cortical and axonal defects in a novel mouse line with a genetic ablation of CNTN2+ pyramidal neurons

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Corticothalamic axons express Contactin-2 (CNTN2/TAG-1), a neuronal recognition molecule of the immunoglobulin superfamily involved in neurogenesis, neurite outgrowth and fasciculation. Although *Cntn2/Tag-1*$^{-/-}$ mice do not exhibit any obvious defects in the corticofugal system, the role of CNTN2/TAG-1+ neurons during cortical development remains elusive. We have generated a mouse line (*Tag1*$^{loxP\text{-}EGFP\text{-}loxP\text{-}DTA}$) that expresses EGFP under the *Cntn2/Tag-1* promoter and encompasses the coding sequence of Diptheria Toxin subunit A (DTA) under quiescence. We show that while the line recapitulates the expression pattern of endogenous CNTN2/TAG-1, it highlights an extended spatial and temporal expression in the forebrain, including multiple axonal tracts and neuronal populations. We established that the *Tag-1*^{EGFP} cells are widespread in the cortex and contribute to both upper and deeper layers. We crossed these mice to the *Emx1-Cre* line thus ablating the vast majority of CNTN2/TAG-1+ cortical neurons. Among the observed defects were a significantly smaller cortex, a reduction of corticothalamic axons as well as callosal and commissural defects, such as a significant reduction in the corpus callosum and the anterior commissure. An additional effect was a significant reduction of the subplate cells, which is not a direct effect of their death, but rather a proposed side effect of the widespread cortical abnormalities. Such defects are common to neurodevelopmental disorders, therefore this mouse line may serve as a useful model to study physiological and pathophysiological cortical development, as well as the mechanisms responsible for the compensation of cortical neuronal loss.

10. Effect of developmental temperature, during the embryonic stage, on the cerebellum of *Danio rerio*

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Temperature is one of the most important factors, which can have a significant impact on ectotherm organisms. Temperature variations during early developmental stages of fish could affect their later stages. We show here that temperature changes during the embryonic stage, has significant effects on the zebrafish cerebellum size. Fertilized eggs were exposed into three different developmental temperatures (TD = 24 °C, 28 °C and 32 °C) up to hatching and then all were maintained under common conditions (28 °C) till the end of metamorphosis. At the end of metamorphosis, the brain of juveniles of all treatments were removed and subjected to histological analysis. Using nissl staining, the area of three subregions of cerebellum was calculated: the anterior region (Valvula cerebelli, Va), the central region (Corpus Cerebelli, CCe) and the posterior region (Lobus Caudalis, LCa). The results showed that the area of these subregions was significantly increased in individuals that were initially exposed to 32 °C compared to those exposed to lower temperatures. Hence, our results indicate that even a short period of exposure to increased temperatures during the embryonic stage can affect later stages of zebrafish brain morphology.

11. Mirk/Dyrk1B minibrain kinase induces cell cycle exit and neuronal differentiation *in vitro* and *in vivo* and marks embryonic and adult neurogenesis

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Aim: Our studies have implicated for the first time Mirk/Dyrk1B kinase in cell cycle exit and neuronal differentiation of Neuro2A cells suggesting a similar role for neural precursor cells (NPCs). Our aim is to elucidate the role of Dyrk1B in neurogenesis applying *gain-of-function* studies *in vitro* and *in vivo*. ***Methods:*** Unilateral *in ovo* electroporation was performed in E2 chick spinal cord for Dyrk1B/GFP or GFP followed by subsequent analysis at E4 by immunohistochemistry. Dyrk1B expression was investigated in the embryonic chick and in the embryonic, postnatal and adult mouse CNS using immunohistochemistry, *in situ* hybridization and Western Blot analysis. **Results:** Forced Dyrk1B expression in E2 chick spinal cord reduces by 2.3-fold BrdU incorporation and decreases PH3 (mitosis marker), Prox1 and Sox2 (NPC markers) expression by 10, 4.9 and 1.5-fold respectively. Moreover, Dyrk1B+/GFP+ cells showed a neuronal fate phenotype, as indicated by increased Pax3 and Pax7 by 2.3 and 1.9-fold, respectively, and increased Nkx6.1, Islet-1, Doublecortin and ßIII-tubulin by 1.9, 2.1, 1.4 and 1.2-fold respectively. In E4 chick spinal cord endogenous Dyrk1B is expressed by cycling neuronal progenitors and by differentiated motor neurons. In addition Dyrk1B protein is decreased during embryonic chick CNS development. In E12.5 mouse CNS, Dyrk1B is expressed both by cycling neuronal progenitors and by differentiated neurons. Moreover, Dyrk1B is expressed in the adult mouse cortex and the hippocampus. Especially Dyrk1B is expressed all along the neuronal lineage in the adult dentate gyrus, suggesting a role in embryonic and adult neurogenesis. **Conclusions:** Mirk/Dyrk1B induces cell cycle exit and neuronal differentiation *in vitro* and *in vivo* and marks embryonic and adult neurogenesis.

12. Mechanisms of cortical interneuron development in a mouse mutant with reduced inhibition

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** Equal Contribution*

GABAergic interneurons play important roles in cortical function and their loss/ dysfunction is implicated in severe disorders (schizophrenia, epilepsy). We have demonstrated the unique and diverse roles of the RhoGTPases Rac1 and 3 in interneuron progenitors and their morphology in transgenic animals where Rac1 and Rac1/3 were ablated specifically in cortical interneurons (CINs). In the Rac1 mutant, progenitors delay their cell cycle exit resulting in a 50% decrease in CINs and an imbalance of excitation/inhibition in cortical circuits. In the Rac1/3 mutant, there are additional cytoskeletal defects resulting in an 80% decrease in CINs. Both lines die from epileptic seizures. Our data suggests that proper levels of inhibition early postnatally, is critical for the development of synaptic properties and plasticity of the prefrontal cortex (PFC). We report on the maturation of early PFC circuits in a state of reduced inhibition and on the cellular/molecular mechanisms guiding these mutant interneurons to the cortex.

In order to study local PFC circuits, we recorded extracellular local field potentials (LFPs) in brain slices of mutants and controls. In the Rac1/3 mutant, from P10 to P20, the loss of CINs leads to increase of basal synaptic transmission. In controls, the basal synaptic transmission decreases.

We also studied the migratory behavior of mutant Rac1/3 interneurons by ex vivo time-lapse imaging. Several parameters of their locomotion are decreased (velocity, frequency and amplitude of nuclear translocation). Positioning of the Golgi complex and the centrosome are also defective.

Finally, RNAseq analysis of mutant and control CIN established a list of candidate downstream effectors. Real-time PCR analysis point to a reduction in expression levels of genes coding cytoskeletal proteins, in micro-RNAs and an increase in histones.

We hope these data will contribute to the understanding of CIN function, especially since several preclinical models of CIN-based cell therapies are being established.

Acknowledgement

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13. A critical role for autophagy in oligodendrocyte maturation and myelin sheet formation

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(Macro)autophagy comprises a conserved lysosome-dependent catabolic pathway, facilitating degradation of cytoplasmic proteins and damaged organelles. Through its role in energy production and cellular homeostasis, autophagy is crucial during development as shown in many tissues and organisms, while its dysregulation has been linked to several disorders, including neurodegenerative diseases. Although a few studies implicate autophagy in CNS demyelinating pathologies, its role, particularly in oligodendrocytes, remains poorly characterized.

In our study, we aim to shed light on the significance of autophagy in CNS myelin and oligodendrocytes. We observed significant CNS hypomyelination in a Nestin-Cre; Atg5 $^{fl/fl}$ mouse line where autophagy is ablated in all CNS cells. In parallel, *in vitro* studies of Nestin-Cre; Atg5 $^{fl/fl}$ oligodendrocytes showed increased differentiation index of these cells, which, however, present morphological defects in their myelin sheet. Pharmacological inhibition of autophagy, using the highly selective autophagy kinase ULK1 inhibitor SBI-0206965, similarly increased differentiation index, and resulted in a maturation delay of myelin-producing oligodendrocytes over the three basic morphological categories that was restored by DIV8. At that time point, SBI-treated, myelin-producing oligodendrocytes showed a significantly altered morphology. We are currently examining the role of autophagy in both oligodendrocyte primary cultures as well as *in vivo* utilizing a new conditional mutant mouse line, in which autophagy is specifically ablated in the CNS myelinating glial cells after tamoxifen administration (Plp-Cre^{ERT2}; Atg5 $^{fl/fl}$). Our findings suggest that autophagy is an indispensable mechanism for oligodendrocyte maturation and myelin sheet formation.

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14. Ependymal damage in the infant human subependymal zone and after "milking the niche" in rats: a comparative analysis to assess functional consequences and clinical applications

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The method of "milking the Subependymal Zone (SEZ)" involves the controlled compromise of the ependymal layer via the cerebroventricular injection of neuraminidase, in order to allow the flow of Neural Stem and Progenitor Cells (NSPCs) that reside in the SEZ stem cell niche, in the lateral ventricle whereof they can be collected via liquid biopsies of Cerebrospinal Fluid. Here we investigate the effects of "milking" in the architecture and the function of the rat SEZ 8 months post milking using immunofluorescence analysis for ependymal cells (S100ß, ß-catenin, GFAP), proliferation (Ki67) and neurogenesis (Dcx). In addition, we use immunofluorescence analysis in order to investigate the structure and function of the human infant SEZ which is the intended target area if human SEZ were to be "milked" for clinical use. The level of maturation and the architecture of the ependyma, the presence of areas of damage and of gliosis, as well as the presence of neuroblasts have been mapped and quantified. Notably, the comparative analysis of the post-milking rat and the normally developing human SEZ revealed surprising similarities, such as the emergence of areas of ependymal damage and the flow of neuroblasts in the ventricular space. These similarities and differences are discussed in the context of basic biology and of potential future clinical applications.

15. Comparative analysis of neurogenic and oligodendrogenic activity in the subgranular zone hippocampal neural stem cell niche and the corpus callosum of lab and wild rodents

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Different populations of postnatal brain Neural Stem and Progenitor Cells (NSPCs) remain active; some within the specialized microenvironment of stem cell niches, such as the Subgranular Zone (SGZ) of the dentate gyrus, others dispersed throughout the parenchyma (for example Oligodendrocyte Progenitor Cells/ OPCs). Previous research has shown that stress and exercise can induce proliferation of NSPCs, but promising therapeutic strategies have repeatedly failed to prove clinically valuable. The aim of our study was to compare NSPC activity in the brain of "lab" and "wild" mice (Mus musculus domesticus); the former kept in controlled conditions of no stress and of limited external stimuli and the latter exposed to constant stress and physical activity due to predators, the search for food and complex intra- and interspecific interactions. The analysis was extended to include populations of “wild” mice with karyotypic variations due to the appearance of Robertsonian fusions (the joining of uniarmed chromosomes at their centromeres, leading to the formation of biarmed chromosomes and causing a reduction in the chromosome number from the typical 2n=40 down to 22), as well as the burrowing, fossorial species Microtus thomasi . We compared levels of proliferation, the appearance of progenitors of neuronal commitment (neuroblasts) and the density of oligodendrocyte lineage cells in the SGZ and the corpus callosum using immunofluorescence analysis for Ki67, Doublecortin and Olig2, respectively. Based on our results, we discuss the role of environmental (wild versus lab, burrowing), as well as of genetic (karyotype, species) factors in the activity of NSPCs.

16. Radial Glial cells can tolerate high levels of the DNA replication licensing factor Cdt1 during mid-corticogenesis

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Cortical development during embryogenesis, requires orchestration between self-renewal and differentiation processes of neural stem cell populations as well as strict regulation of the cell cycle of these populations[1]. Geminin is a known important regulator of DNA replication and cell cycle, ensuring that cells will replicate their genetic material only once per cell cycle, preventing replication stress. This is achieved by suppressing the replication licensing factor Cdt1 during the S phase of the cell cycle, thus preventing the formation of a new pre-replicative complex (Pre-RC) within the same cell cycle[2].

Current studies from our lab, highlight the essential role of Geminin in the regulation of the cell cycle of neuroepithelial cells, the primary neural stem cell population that reside in the developing cortex. Specifically, Geminin depleted neuroepithelial cells experience increased replication stress and reduced proliferation. However, Geminin depletion during mid-corticogenesis from radial glial cells (RGCs) has no equivalent effect.

To further investigate the role of Geminin-Cdt1 complex during mid-corticogenesis we performed *in vivo* overexpression of Cdt1 alone and in combination with Geminin deletion in radial glial cells via *in utero* electroporation. We examined the effects of Geminin-Cdt1 levels manipulation in respect of cell cycle progression and DNA damage. Our analysis showed that even upon aggravated conditions of Geminin deletion and Cdt1 overexpression, radial glial cells do not demonstrate cell cycle defects or replication stress-related signs. Our results propose that during mid neurogenesis, radial glial cells employ an alternative mechanism for the regulation of the replication licensing factor Cdt1 compared to neuroepithelial cells.

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17. p73 is a critical component of GemC1 signalling pathway for the transcriptional differentiation program of multiciliated ependymal cells

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Neurogenesis persists in the adult mammalian brain in two restricted germinal regions: the dentate gyrus of the hippocampus and the subventricular zone (SVZ) in the walls of the lateral ventricles. Multiciliated ependymal cells are key components of the SVZ microenvironment, as they carry multiple motile cilia on their surface to control the cerebrospinal fluid flow, thus providing multiple regulatory cues for the production of neurons within the adult brain. Dysfunction of ependymal cells has been linked with pathological conditions like hydrocephalus, despite the fact that the molecular mechanism underlying its development remain poorly characterized.

We have previously shown that GemC1 is the earliest known marker of ependymal progenitors and transcriptionally activates crucial multiciliogenesis factors, such as McIdas and Foxj1. Here we show that GemC1 regulates the transcriptional activation of p73, a transcription factor central to multiciliogenesis across different tissues, including the population of multiciliated ependymal cells in the SVZ. Moreover, we show that GemC1 co-operates with E2F5 and p73 to regulate the activation of the p73 promoter and also controls chromatin organization and epigenetic marks of p73 locus. We also provide in vivo evidence that GemC1 is not only sufficient but also essential for p73 expression in the murine brain. Finally, we show that ectopic expression of p73 in the absence of GemC1 fails to rescue the generation of multiciliated ependymal cells in vitro, suggesting that GemC1 is the most upstream factor in the differentiation of multiciliated ependymal cells. Our results highlight novel signaling cues involved in the commitment program of multiciliated ependymal cells.

18. Who gives in first? region-specific vulnerability to epileptogenesis and the role of the endogenous cortical network activity

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In order to understand –and, eventually, treat- the transition of brain networks to epileptic activity, it is essential to identify the origin of pathological discharges and how these spread to increasingly larger areas. While EEG recordings provide a sensitive assay of the brain's electrophysiological state, they lack the necessary spatial resolution to investigate these issues. To overcome this limitation, simpler ex vivo models have been developed enabling the examination of the initial state transition and subsequent pattern of spread under highly controlled conditions. However, these studies employ a large diversity of induction models, pharmacological conditions and animal ages making it practically impossible to draw wideranging conclusions and generate unifying testable hypotheses.

Here, we used simultaneous LFP recordings from mouse brain slices in order to examine the onset and pattern of epileptiform activity in 3 brain regions [hippocampus (CA3), primary motor (M1) and primary somatosensory (S1BF) cortex] under identical conditions. Experiments were performed in both young [P20] and adult animals since clinical studies have revealed a clear developmental effect on epilepsy incidence. Our study aimed to address 4 questions: a) whether different brain regions have different vulnerability to epileptogenic insults, b) what are the characteristics of epileptiform activity in each region, c) how the physiological endogenous network activity (recurring Up-states) transforms to epileptiform activity; and d) how the aforementioned are affected by maturation.

Our results revealed both regional and developmental effects in the onset/pattern of epileptiform activity. In young animals, both cortical areas developed epileptiform activity much faster than CA3. Importantly, the presence of Up-states in M1 correlated with a faster transition to epileptiform activity. Finally, CA3 developed long and stable SLEs. Taken together, these results indicate that epileptogenesis is not a uniform process, but depends on a number of variables that derive from but also affect network function.

19. Short – term plasticity in the hippocampus of a rat model of autism

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Accumulating evidence has correlated autism with pathological cortical conditions. Due to the early onset and the complex behavior of this disorder, the investigation of the underlying neurobiology in humans has been impeded. Therefore, appropriate animal models are crucial in furthering our understanding of its pathology. In this respect, the electrophysiological study of experimental models of autism provides the means to invasively explore its neurobiology based on behavior-independent measures. Considering that the base of this mental condition is the pathology of synapses in cortical circuits, it is apparent that synaptic plasticity, which plays a fundamental role in information processing, learning and memory, is profoundly affected. Synaptic plasticity, the ability of synapses to adapt their excitability, is classified in two main categories based on the sustainability of changes: long- and short- term plasticity. The aim of the current electrophysiological study was to compare *short-term* plasticity of the CA3-CA1 pathway in the *dorsal* and *ventral* hippocampus of WT and Fmr1KO during late development (adolescence-adulthood). The Fmr1KO rat is a genetic animal model of the fragile X syndrome (FXS), the most common form of inherited intellectual disability and the only identified monogenetic cause of autism in humans. In our study we used rats that belonged to the following ages: P(postnatal day)40, P50, P60 and P90 with a deviation of ± 2 days. We tested two protocols (1) an input-output test during which we applied *single* pulses at successively higher intensities: from 20μA to 260μA, in order to assess CA1 excitability, and (2) *trains* of ten pulses of the same intensity but at different inter-pulse intervals at various frequencies (1-100 Hz), in order to study short-term plasticity in Fmr1KO and WT rats along the dorsoventral axis of the developing hippocampus.

20. Interhemispheric asymmetry of GABAergic interneurons in different brain regions

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Cerebral hemispheres in humans are characterized by structural and functional asymmetry that applies to a variety of scientific theories, mainly regarding their role in emotion, memory and neurological disorders. However the issue of structural lateralization is much less studied in rodents, in spite of their extensive use as animal models of human diseases.
This study explores differences in the number of GABAergic interneurons expressing parvalbumin (PV) and associated perineuronal networks (PNNs) in adult C57BL/6J mice. Several brain areas were analyzed including the anterior cingulate cortex, the somatosensory cortex, the lateral entorhinal cortex, the basolateral anterior and posterior amygdala nuclei and the three regions of the dorsal hippocampus: the cornu ammonis 1, the cornu ammonis 3 and the dentate gyrus. PV^+ and PNN+ populations were detected by immunohistochemical staining of thick vibratome brain sections, and their number and density was quantified by stereological analysis (StereoInvestigator) on fluorescent images obtained with a confocal laser scanning microscope. The statistical analysis was carried out by the two-way repeated measures ANOVA test. Our results show that there is a statistically significant interhemispheric difference in PV^+ cells in the area of the basolateral anterior amygdala, with the left hemisphere having a higher (+17%) density of PV^+ cells; a similar tendency seen in the posterior nucleus of the amygdala does not reach significance level. The remaining regions do not exhibit interhemispheric asymmetry in either PV^+ cells or PNNs populations, highlighting the diversity of brain structures and forms of hemispheric asymmetry.

21. The tumor suppressor role of NR5A2 in nervous system malignancies

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Nervous system malignancies are characterized by rapid progression and poor survival rate. Glioblastoma multiforme is the most aggressive nervous system malignacy and despite recent advances in the provided therapy the average survival time remains low, between 12 to 15 months. These clinical observations underscore the need for novel therapeutic insights and pharmacological targets. Towards this direction, here we identify the orphan nuclear receptor NR5A2/LRH1 as a negative regulator of cancer cell proliferation and promising pharmacological target for nervous system-related tumors. In particular, by meta-analysing clinical data from TCGA and Oncomine databases, we find that high expression levels of NR5A2 are associated with favourable prognosis in patients with glioblastoma tumors. Consistently, we experimentally show that NR5A2 is sufficient to strongly suppress proliferation of both human and mouse glioblastoma (U87-MG, GL261) and neuroblastoma cells (SH-SY5Y, Neuro2A) without affecting apoptosis. The anti-proliferative effect of NR5A2 is mediated by the transcriptional induction of negative regulators of cell cycle, Cdkn1b ($p27_{kip1}$), Cdkn1a ($p21_{cip1}$) and Prox1. Interestingly, two well-established pharmacological agonists of NR5A2, DLPC and DUPC, are able to mimic the anti-proliferative action of NR5A2 in human glioblastoma cells via the induction of the same critical genes, encoding for $p27_{kip1}$, $p21_{cip1}$ and Prox1. Most importantly, treatment with DLPC inhibits glioblastoma tumor growth *in vivo* in a xenograft mouse model. These data indicate a tumor suppressor role of NR5A2 in nervous system and render this nuclear receptor a potential pharmacological target for the treatment of nervous tissue related tumors.

22. The short G1 phase of neuroepithelial cells elicits increased sensitivity to replication stress resulting to microcephaly

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Perturbations in the regulation of DNA replication during early development are associated with growth retardation and severe neurodevelopmental malformations. DNA replication is strictly regulated through the licensing of the multiple replication origins, which takes place during the G1 phase of the cell cycle. Aberrations in the licensing process impede DNA replication leading to increased DNA damage and reduced cell proliferation. Geminin is a known inhibitor of DNA replication, which secures that replication origins will be licensed only once per cell cycle. Deletion of Geminin *in vitro* causes re-replication, a source of replication stress and subsequent DNA damage-induced apoptosis. To investigate the effects of aberrant DNA replication licensing during brain development we have generated a mouse model in which Geminin is specifically inactivated from the Neuroepithelial cells, the first population of neural stem cells that reside the developing cortex.
We show here that Geminin deficient embryos exhibit a dramatic decrease in the size of the developing cortex resembling the phenotype of human microcephaly. Our results demonstrate that Neuroepithelial cells undergo replication stress upon Geminin deletion leading to increased DNA damage and gradual loss of the stem cells pool. Interestingly, when Geminin is deleted from the apical Radial Glia cells during mid-neurogenesis, we do not observe any severe brain malformations or signs of DNA damage. To shed light in the mechanism that underlies the increased sensitivity of Neuroepithelial cells to defective DNA replication we investigated whether their short G1 phase is responsible for the increased DNA damage induced by Geminin deletion. Towards that direction we established an *in vitro* system in which we can directly manipulate the length of G1 phase. We show evidence that a short G1 phase escalates the effect of Geminin deletion suggesting that an inherent feature of Neuroepithelial cells contributes to defective brain development upon replication stress.

23. GemC1 is a critical switch for neural stem cells and ependymal cells generation in the postnatal brain

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Ependymal cells of the brain form a distinct epithelium lying in close contact with the ventricles. Multiple motile cilia on their apical surface ensure cerebrospinal fluid propulsion throughout the brain. Together with the adult neural stem cells, ependymal cells constitute key components of the subventricular zone germinal niche (SVZ), where neurogenesis persists in the adult mammalian brain. During development, both ependymal and adult neural stem cells are derived from embryonic neural progenitors, called radial glial cells. However, the molecular mechanisms regulating the specification of these two cellular types remain poorly characterized. Importantly, ependymal cells malformation has been associated with disturbances of cerebrospinal fluid flow and the formation of hydrocephalus, in both mice and humans.

We have formerly shown that GemC1, member of the Geminin family, is the earliest known marker of radial glial cells, committed to the ependymal lineage. Our aim is to clarify the mechanisms regulating the fate decisions of radial glial cells and identify the impact of their dysregulation with respect to neurodegenerative diseases, like hydrocephalus. Towards this direction, we have generated GemC1 knockout mice. Our study demonstrates that GemC1 deficient mice exhibit complete lack of early committed and mature multiciliated ependymal cells in the SVZ, resulting in severe hydrocephalus. Moreover, cellular components of the niche are severely affected by the absence of GemC1 leading to impaired neurogenesis. Intriguingly, we provide evidence that GemC1-deficient cells exhibit altered cellular characteristics, thus uncovering their cell fate change towards a neural stem cell phenotype.

Our analysis suggests that GemC1 governs radial glial cells fate initiation to the ependymal lineage and the establishment of the SVZ niche. We postulate that these findings provide a better molecular insight into ependymal cells formation and will serve as an ideal model for the mechanisms underlying the pathogenesis of hydrocephalus.

24. The role of GemC1/Lynkeas in the expression of the p53 family members in the mouse telencephalon during embryogenesis

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The p53 family members (p53, p63, p73), are widely associated with tumor-suppressor roles. Mice deficient for p53 and p73 exhibit extensive brain malformations, while lack of p63 has been associated with reduced neuronal survival. Previous findings from our lab suggest that GemC1/Lynkeas regulates the transcriptional activation of p73 and direct interactions between GemC1/Lynkeas and p73 are crucial for multiciliogenesis. Furthermore, we have provided evidence that GemC1/Lynkeas is an essential factor for the generation of multiciliated cells in the mouse brain and airway epithelium; thus, it is considered to be a key factor for unveiling the mechanisms responsible for the emerging of ciliopathies.
To understand the functions of p63, p73 and p53 during brain development we have performed expression pattern analysis in the mouse telencephalon and investigated the role of GemC1/Lynkeas, using mice lacking the expression of GemC1/Lynkeas. Our preliminary data suggest that during mid embryogenesis, p53 and p73 are co-expressed in the hippocampal primordium, while p73 is also detected in earlier developmental stages, suggesting that p53's expression begins later in embryogenesis in the hippocampal primordium. GemC1 deficient embryos lack both p53's and p73's expression in the hippocampal primordium. In addition, we have observed that during embryogenesis p73 is expressed in the preoptic area of control embryos, as well as in the Cajal Retzius cells, along with p63. Cells expressing p73 are lost in GemC1 deficient embryos.
Our results highlight a distinct role of GemC1/Lynkeas regarding the p53 family members' expression patterns, suggesting additional functions in further developmental processes, besides multiciliogenesis.

25. Neuroepithelial and radial glial cells regulate differentially the initial events of dna replication during cortical development

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The cerebral cortex formation is based on the consecutive appearance of neural stem and neural progenitor cells, a procedure requiring the synchronization of self-renewal and differentiation processes with the concurrent control of cell cycle and transcription regulation. Residing the neural plate before the onset of neurogenesis, neuroepithelial cells (NECs) constitute the earliest progenitor population and proliferate through self-renewal symmetric divisions. During neurogenesis, NECs give rise to radial glial cells (RGCs) and other neural progenitors forming the ventricular and the subventricular zone. RGCs represent the main source of ventricular zone progenitors from E13,5 until the end of neurogenesis and they give rise to more committed cell types by asymmetric divisions. A distinguishing feature between NECs and RGCs is the cell cycle length, with the former to mark a shorter G1 phase [1].

According to previous studies of our lab in different transgenic mouse model, NECs and RGCs indicate differences regarding their response to DNA replication aberrations, such as exposure to replication stress. Thus, deletion of Geminin, a known DNA replication inhibitor with a key role in the cell cycle regulation, causes degeneration of NECs integrity whereas RGCs are not affected [2]. In order to further investigate the inherent differences between these diverse neural stem cells, we focus on elucidating the regulation of DNA replication initiation. We have investigated the different regulation of minichromosome maintenance (MCM)2-7 complexes loaded onto origins of DNA replication (origins' licensing) as well as the different amount of DNA replication origins that are activated (origins' firing) between NECs and RGCs in adjusted in-vitro and in-vivo systems. Preliminary results have shown that NECs tend to load more MCM2-7 onto origins of replication than RGCs.

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26. Development of stable neurotensin analogues targeting the NTS1 receptor

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Neurotensin (NT) is a peptide that plays important role in the function of the central nervous system. The involvement of NT in dopamine signaling, and the similarity of its effects to those of typical antipsychotic drugs suggest a role for NT in the pathophysiology of schizophrenia. These functions of NT are mediated mostly by activation of its type 1 receptor (NTS1), rendering NTS1 a promising therapeutic target for schizophrenia. A major drawback for the development of novel NTS1-specific drugs is their possible proteolytic degradation. NT is rapidly degraded by several proteases. Our aim is to develop novel proteolytically stable NT analogues. To accomplish this we designed and synthesized seventeen NT analogues containing modified structures of NT. The modifications of NT structure were designed such as to provide resistance of NT to proteolytic degradation. To pharmacologically evaluate these analogues we determined 1) their binding affinities for the NTS1, 2) their abilities to stimulate intracellular calcium mobilization in NTS1-expressing cells and 3) their plasma stabilities. The results from these studies revealed that three NT compounds (NT5, NT6 and NT8) interacted with relatively high affinities with the NTS1 and exhibited a remarkably higher stability than NT. Given that NT5 had the highest binding affinity among the other analogues we selected it to test its ability to stimulate intracellular calcium mobilization. As NT, the NT5 is an agonist, elevating the cytosolic calcium. In order to assess the structural determinants of binding of the novel NT analogues to the NTS1 receptor, we performed docking and molecular dynamics simulations. These findings could set the basis for the design of proteolytically stable novel NT analogues with antipsychotic-like effects.

27. Synergistic effects of early life mild adversity and chronic social defeat on rat brain microglia

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Exposure to early life stress affects the development and function of the brain and when followed by adversities in adulthood, the negative effects of stress are enhanced. Microglia has been proposed as a potential mediator of this phenomenon. In the present study, we investigated the long-term effects of mild early life stress, the consequences of a stressor in adulthood as well as their interaction on microglial and cytokine (*PPARγ, IL-1β* and *TNFα*) levels in the brain of adult male rats. As an early life stress we used a model of maternal neglect, in which the dam is present but non-accessible to the pup for 15min during postnatal days 10-13; as a stressor in adulthood we exposed animals to chronic social defeat (CSD) for 3 weeks. We determined in the hippocampus, prefrontal cortex and amygdala, the number of microglial cells by Iba-1 immunohistochemistry and the relative expression of *PPARγ, IL-1β* and *TNFα* mRNA by qPCR. Following exposure to CSD, the number of Iba-1+ cells was increased in the hippocampus and the prefrontal cortex of adult animals exposed to mild early life stress, while in the absence of CSD no such difference has been observed. Moreover, following CSD the levels of *PPARγ* mRNA were increased in the hippocampus of adult males exposed as neonates to "maternal neglect". Our finding support the notion that early life stress, even a mild one, primes microglia and enhances its reactivity to a second stressful event, later in life, in accord with the "two-hit" hypothesis.

28. The caudal cerebellar vermis is involved in the regulation of ethanol preference and forebrain dopamine transporter levels

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It has long been known that the cerebellum projects to the ventral tegmental area and the striatum, and that lesion of the cerebellar cortex affects the expression levels of dopamine receptors and transporters in a topographic manner. It is also known that the cerebellum is involved in limbic functions via the vermis of the posterior lobe. Based on the above, we studied the effects of a lesion in the vermis of the posterior lobe (i) on the manifestation of a limbic function, ethanol intake and preference, and (ii) on the expression levels of the dopamine transporter. Single-housed adult male Sprague-Dawley rats were stereotaxically injected with kainic acid or saline in the cortex of the posterior vermis. Three weeks post-surgery the rats were presented two bottles in their homecages, one containing tap water and the other containing increasing concentrations of ethanol. Ethanol intake and preference, food intake, and body weights were regularly measured for a three-week period. Six weeks post-surgery, rats were euthanized with ethanol onboard, and selected brain areas were harvested for the study of dopamine transporter protein levels. Lesion in the posterior cerebellar vermis led to a significant increase in ethanol intake and preference (85% in kainic acid-lesioned vs. 45% in sham-operated), in all ethanol concentrations tested. Ethanol intake affected dopamine transporter levels in a region-specific manner that was differentiated between sham-operated rats and rats with lesion in the posterior cerebellar vermis. Overall, the study shows that the posterior vermis of the cerebellum is involved the regulation of ethanol intake and preference along with the protein expression levels of the dopamine transporter.

29. Adversarial dictionary learning for a robust analysis and modeling of spontaneous neuronal activity

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The field of neuroscience is experiencing rapid growth in the complexity and quantity of the recorded neural activity, as advances in experimental design, measurement techniques, and computational analysis allow us unprecedented access to the dynamics of neural activity in different brain areas. One of the major goals of neuroscience is to find interpretable descriptions of what the brain represents and computes by trying to explain complex phenomena in simple terms. Considering this task from the perspective of dimensionality reduction provides an entry point into principled mathematical techniques that allows us to discover these representations directly from experimental data, a key step to developing rich yet comprehensible models for brain function. In this work, we employ two real-world binary datasets that refer to the spontaneous neuronal activity of two laboratory mice over time, and we aim to their efficient low-dimensional representation. We develop an innovative, robust to noise, dictionary-learning algorithm for the identification of patterns with synchronous activity, which is inspired from the idea of adversarial learning. We also extend this algorithm so that it can identify patterns within larger time window intervals. The results on the classification accuracy for the discrimination between the clean and the adversarial-noisy activation patterns obtained by an SVM classifier highlight the efficacy of the proposed scheme, and the visualization of the dictionary's distribution demonstrates the multifarious information that we can obtain from it.

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30. An Automated Sleep Spindles Detection Tool and its Use on Spindles Analysis and Parameterization

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Spindles along with k-complexes are bursts of oscillatory brain activity on the thalamocortical system that occur during sleep. The accurate detection and analysis of these EEG rhythms is important in sleep studies. During a typical sleep hundreds of spindles occur in various EEG electrodes and the manual detection of all these spindles by an expert is a laborious and tedious task, so the need for a reliable software tool is profound.

In this work we present such an automated software tool for the reliable detection of spindles and its use in the framework of a general software environment for spindles analysis and parameterization. The tool uses publicly available and custom spindle detection methods and analyses sequentially all channels of an EEG in order to identify the start and end of each spindle. Then the part of the signal that has been identified as a spindle is analyzed and a number of parameters is extracted so that each spindle is represented as a set of parameters and finally the whole sleep is represented as a dataset that can be further analyzed using automated machine learning techniques.

Reliable spindle detection is achieved by using the results of multiple detection methods. The parameters of each method (i.e. detection threshold) are optimized through an iterative process for any given EEG channel and then each method is applied using its optimized parameters. The results of all applied methods are combined for achieving the final spindle detection. Experimental results based on artificial EEG signals demonstrate the validity of the proposed approach and results from the analysis of real EEG signals will be presented.

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31. Reconstitution of the mouse subventricular zone neurogenic niche through 3D biofabrication.

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The understanding of the brain pathophysiology is limited as current existing models mainly focus on 2D cell culture, which lack the ability to form systems capable of studying the complex brain interactions. To this end, brain reconstitution seems to be an advanced tool for fabricating three-dimensional (3D) brain cultures, which will give rise to more sophisticated brain developmental or disease research models [1].
In the present study, we focus on the reconstitution of the adult mouse subventricular zone neurogenic niche (SVZ) located in the lateral ventricles of the brain. The SVZ niche harbours neural stem cells and sustains neuronal production into adulthood [2]. Reconstitution was evaluated using two different approaches. On the first approach, neural progenitor cells were isolated from the neonatal SVZ niche and were mixed with a bioink solution and biofabricated using a custom 3D bioprinter made in our lab. While on the second approach, neural progenitors were cultured on a decellularized subventricular zone, previously isolated from adult mice, serving as a matrix for culture and cell differentiation. In both cases, samples were immunostained and observed through confocal microscopy.
Our results show a noticeable proliferation rate and cell viability. Furthermore, our analysis suggests, that differentiation of neural progenitors is achievable in booth samples. In conclusion, our 3D culture models aim not only to elucidate brain development but also enhance the current understanding of complicated CNS pathologies.

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32. On the association of schizotypal traits with Prepulse Inhibition

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Aim: Schizotypy is considered a liability index of schizophrenia, as they share genetic, neuroanatomical and cognitive deficits. The aim of the present study was to investigate the relationship of Prepulse Inhibition (PPI) with paranoid and negative schizotypy, following a dimensional and a dichotomous approach.
Methods: Two-hundred and fifty healthy community participants were administered the Schizotypal Personality Questionnaire and were also assessed for PPI of the acoustic startle reflex. Pulses were 40msec/115dB white noise bursts, prepulses were 20msec/75dB or 85dB white noise bursts and the lead-intervals were either 30msec, 60msec or 120msec. For the dimensional approach, we examined the associations between Paranoid (ParS) and Negative (NegS) schizotypy and PPI with hierarchical stepwise regression analyses. For the dichotomous approach, we identified individuals scoring in the lower or the upper 20% of these schizotypal dimensions and we conducted between group comparisons.
Results: The fully-dimensional analysis revealed that (a) low NegS was associated with high PPI elicited with the 75dB prepulse at short intervals (all p values <0.01) and (b) low ParS was associated with high PPI elicited with the 75dB prepulse with the 120msec lead-interval and the 85dB prepulse with the 30mscec lead-interval (all p values <0.01). The dichotomous analysis revealed that both the low ParS (n=122) and the low NegS (n=128) groups had significantly higher PPI compared to their respective counterparts (all p values <0.01).
Conclusions: The present study showed that high paranoid and negative schizotypal traits are associated with lower PPI. The finding was evident irrespective of the approach (dimensional or dichotomous) employed, thus suggesting that deficient PPI is a core characteristic of paranoid and negative schizotypy.

33. Contribution of bimodal nonlinear dendritic integration of basket cells in persistent activity.

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Despite constituting about 15-20% of the total number of neurons, interneurons are known to orchestrate the activity of neuronal networks due to their mostly inhibitory signaling. New data challenge the linear point neuron dogma for dendrites by predicticting that specific morphological features along with active biophysical properties support nonlinear dendritic integration in these cells.

Our goal is to explore the effect of basket cells' nonlinear dendritic integration in persistent activity in PFC. Towards that goal, we constructed the first ball-and-stick model for Fast Spiking Basket Cells that incorporates nonlinear dendrites, based on experimental data. The model comprises of a soma, an axon and two dendrites; one exhibiting supralinear response to stimulation and the other sublinear, as well as both passive and active membrane mechanisms distributed non uniformly. In spite of its morphological simplicity, the model has been extensively validated across experimental data as to ensure its biological plausibility. We, then, implemented a biologically plausible PFC network that exhibits persistent activity, including seven pyramidal cells and two of our ball-and-stick basket cells with nonlinear dendrites, so as to dissect the role of interneuron dendritic nonlinearities in persistent activity.

All in all, to our knowledge this is the first PFC network that incorporates nonlinear active dendrites in interneurons. We focus on elucidating the contribution of the bimodal nonlinear dendritic integration of basket cells in the persistent activity of pyramidal neurons.

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34. Active dendritic integration in CA3 contributes to memory recall by increasing pattern completion efficiency

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Pattern completion is the ability of the brain to retrieve stored memories from partial or degraded recall cues. It is widely accepted that the hippocampal CA3 area, owing to its extensive recurrent circuitry, represents a key moderator of this process[1]. Although synaptic plasticity in CA3 is considered as the primary mechanism involved, no causal relationship between LTP in recurrent CA3 synapses and memory encoding has been established so far[1]. Meanwhile, recent evidence provides new insight on how active dendritic mechanisms may increase the mnemonic capacity of hippocampal networks[2]. To investigate whether or how NMDAR-mediated nonlinearities[3] contribute to pattern completion, we developed a simplified, yet biologically plausible model of the CA3 area, which consists of multicompartmental pyramidal neurons along with point inhibitory populations. We show that NMDA spikes facilitate the aggregation of temporally coherent EPSPs resulting in amplified voltage responses and increased network excitability even when partial input patterns are used. Furthermore, nonlinear integration mediates network bistability, which might serve as a threshold mechanism, ensuring reliable reactivation by a sufficient amount of temporally correlated input. Thus, we predict that NMDAR-mediated nonlinearities represent an efficient dendritic mechanism that can potentially enhance the retrieval of CA3-related memories.

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35. Improving cognitive flexibility with training on working memory in women participants and female mice

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The present study aims to determine whether working memory training can enhance cognitive flexibility in both human and mice. The human study included thirty-seven healthy women, who were divided in: a) a control (no cognitive training), b) a partially adapted (partial administration of the Letter Number Sequencing, an executive working memory task, for six consecutive days) and c) a fully adapted (administration of the entire working memory task for the same period) group. Following training, all participants were tested with another cognitive flexibility task, the Intra-Extra Dimensional Set Shift (ID/EDS). Results showed that the fully adapted group had lower response latency and made fewer attempts to complete the stages of the ID/EDS test, compared with the other two groups. In the animal study, a similar experimental design was applied (utilizing the delayed alternation task for working memory training (WMT). Female mice are divided into a naïve (remained in their home cage), a non-adaptive (learned to alternate arms, but without any delays) and an adaptive group (performed the alternation procedure with increasing delays). Following WMT, all mice underwent the Attentional Set-Shifting Task. Results showed that the adaptive group had superior performance at the Intradimentional Shift I and the Extradimentional shift stage of AST, compared to the non-adaptive group. The effect of WMT on dendritic spine morphology of prefrontal cortex and hippocampal neurons was also studied. In conclusion, our results indicate the value of WMT as a tool for general cognitive enhancement in a cross species study.

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36. Effects of sulfoximine exposure on bee cognition

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Although the disappearance of bees is now widely observed throughout the world, the multiple reasons for their losses are still poorly understood. The hypothesis of a bee cognitive impairment due to chronic exposures to multiple stressors while foraging is increasingly supported[1]. Indeed, the latest generations of insecticide such as neonicotinoids and their novel successor - the sulfoximines - are widely blamed for bee cognitive impairments because they are agonists of the acetylcholine receptor (AChR). Interfering with this major neurotransmitter impacts fundamental processes such as navigation memory[2], spatial working memory[3] and olfactory learning and memory[4], which all might explain the poor foraging efficiencies and lead to honeybee colonies desertification. However, these scientific studies were carried out based on individual performance and mainly under laboratories-based environment where the behaviour of the bees is largely affected by their immobilization. In this study, we test the impact of realistic sulfoximine exposures (similar to those that may occur after crop spraying) on free flight foragers behaviour, therefore taking into account the natural spatial complexity and social environment of the bees. The results of this study provide fundamental knowledge about the cognitive targets of sulfoximines under real conditions and aim to provide solutions to counteract their actions.

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37. RNA Stabilization and Shuttling by ELAV Proteins: A novel molecular mechanism for long-term memory formation.

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Although *de novo* transcription is known to be required for long-term memory (LTM) formation, the functional expression of these genes depends heavily on post-transcriptional gene regulation. In a two-trial training paradigm with an inter-trial interval of 45 min in *Aplysia*, Trial 1 induces TrkB signaling-dependent gene expression of *apc/ebp* at Trial 2, which is required for LTM formation. Prolonged *apc/ebp* gene expression, likewise necessary for LTM formation, requires TGF signaling during Trial 2. The *apc/ebp* transcript contains multiple AU-rich elements (AREs) in its 3' UTR, conferring an ability to bind various RNA-binding proteins including ELAV. In this study, we show that (i) Trial 2-dependent prolonged *apc/ebp* gene expression is transcription-independent and requires p38 MAPK activation downstream of TGF signaling; (ii) Treatment with TGF-1 at 45 min is sufficient for prolonged *apc/ebp* gene expression; (iii) ApELAV–*apc/ebp* mRNA interaction increases after Trial 2; (iv) A potent inhibitor of ELAV, CMLD-2, significantly reduces the expression of Trial 2-dependent *apc/ebp* mRNA and; (v) CMLD-2 during Trial 2 blocks LTM formation. Further experiments have revealed ELAV translocation from the sensory neuron nucleus following LTM training, which is likewise dependent on p38 MAPK activation downstream of TGF signaling during Trial 2. Collectively, these results elucidate a novel role for a unique form of post-transcriptional regulation: mRNA stabilization by ELAV-like proteins, as a necessary molecular step in LTM formation. Thus, our two-trial training paradigm uniquely affords us the experimental advantage of directly interrogating post-transcriptional gene regulation in future studies. Recent studies have demonstrated dysfunctional ELAV binding activity in Alzheimer's Disease brains, implicating ELAV in a role that may underlie the disease pathology. These observations suggest a model in which ELAV-mediated transcript stabilization and shuttling play a critical role in LTM formation.

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38. Challenging the point neuron dogma: FS interneurons as 2- stage integrators

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Fast Spiking (FS) basket cells (BCs) constitute one of the main types of inhibitory interneurons. A growing body of literature recognizes their importance in controlling executive functions. However, most studies have focused on their molecular and anatomical features and supported the dogma that these cells integrate inputs like linear point neurons, completely ignoring potential dendritic influences. As a result, whether a linear point neuron or a more sophisticated abstraction, like a two-stage integration, can successfully capture their synaptic integration profile, remains an open question. Towards that goal, we developed a) detailed, biologically constrained biophysical models of hippocampal and mPFC FS BCs, using anatomical reconstructions, b) 2-layer Artificial neural network abstractions (ANNs) and c) a large scale microcircuit model of 2-stage pyramidal, FS BCs and dendrite targeting interneurons. Synaptic stimulation predicted the co-existence of two distinct modes within the same tree: supralinear and sublinear. Supralinear dendrites supported local, sodiumdependent spikes and were characterized by large volume and low input resistance. Using an array of different activation patterns, we found that spatially dispersed inputs lead to higher firing rates than inputs clustered within a few dendrites. These different activation patterns result are better explained by a 2-layer ANN with non-linear hidden layers rather than a linear ANN. Finally, in order to assess the functional implications of FS BCs bimodal nonlinear dendritic integration, we trained a circuit network model to encode for a single memory. The outcomes predict that bi-modal nonlinear integration in FS BCs promotes resource savings in the encoding of new memories. Our findings challenge the current dogma, whereby interneurons are treated as linear summing devices and call for further investigation of the contribution of FS BCs in multiple executive functions such as learning and memory.

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39. Is psychosis a dysmyelination-related information-processing disorder?

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Numerous lines of evidence implicate myelin and oligodendrocyte function as critical processes affecting neuronal connectivity, which is a central abnormality in schizophrenia. Neurodevelopmental models related to dysmyelination have suggested its relation with different schizophrenia-like symptoms. Post-mortem studies in patients with schizophrenia have reported 14%–22% reduction in the density and the quantity of oligodendrocytes. Several myelin-related candidate genes have been linked oligodendrocyte and myelin dysfunction with neurocircuitry abnormalities in schizophrenia. A number of myelin gene knockout mice models exhibit schizophrenia-like behaviours, and genomic, especially GWAS, studies identified new schizophrenia loci related to oligodendrocyte genetic polymorphisms. It is known that myelin acts as electrical insulation for the ensheathed axon, which helps to preserve the amplitude and to increase the conduction velocity of the propagating axon potential. A growing body of evidence points towards the involvement of dysmyelination of the prefrontal cortex in the development of the cognitive symptoms of psychosis. Neuroimaging investigations have linked processing speed to brain anatomical connectivity, and have pointed the role of processing speed among the predictors of clinical changes in schizophrenia. The dysmyelination-induced delays in patients with psychosis may cause a discrepancy in sensory feedback mechanisms, which results in prediction error. The myelin abnormalities and the resulting conduction delays vary during the course of the multiple sclerosis and this type of cycles are possibly associated with fluctuations in conduction velocity in psychosis. It is worthy of note that the major histocompatibility complex (MHC) is responsible for the genetic overlap in both multiple sclerosis and schizophrenia. Multiple sclerosis manifests sensory and motor symptoms, and schizophrenia disordered cognition and emotion. Having in mind the interdependent relationship of oligodendrocytes and the axons they myelinate, we could suggest that both multiple sclerosis and schizophrenia may use in central nervous system a common pathway of disordered information-processing. We may suggest that interventions that preserve white matter integrity or ameliorate white matter disruption may enhance information-processing and functional outcome in psychosis.

40. Behavioral and neurochemical aspects of a zebrafish pharmacological model displaying social deficits

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Social deficits are in the core of clinical symptoms characterizing many neuropsychiatric disorders including Autism Spectrum Disorders (ASD). Zebrafish (Danio rerio), representing highly social animals emerge as a potential model organism to study normal and pathological social phenotypes. The present study aimed to develop a zebrafish model that display deficits in social behavior after sub-chronic MK-801 administration, a non-competitive antagonist of the glutamate N-methyl-D-aspartate (NMDA) and to determine the underlying changes in brain neurochemistry.

Social deficits were estimated in MK-801 treated zebrafish based on the social interaction (SI) and the Eye Contact (EC) tests, the latter introduced for the first time in zebrafish to quantify the eye contact avoidance behavior. Possible anxiety levels as well as the presence of specific behavioral stereotypies were determined using the novel tank (NTT) and the open field (OFT) tests. To determine the underlying neurochemical mechanisms, the possible modulation of glutamatergic, GABAergic and noradrenergic neurotransmission was questioned by means of Western Immunoblot analysis of GAD67, mGluR5 and beta2-adrenoceptors expression levels.

Analysis of the behavioral parameters showed that MK-801 treated zebrafish exhibited social deficits and increased anxiety levels. In agreement to studies in mammals, an imbalance between brain excitatory and inhibitory neurotransmission was found in zebrafish exhibiting social withdrawal. In addition, protein levels of beta2-adrenoceptors were increased possibly associated to the anxiety-related responses. Taken all together, our results support the importance of this pharmacological zebrafish model to study the neurobiology of social deficits, coupling brain neurochemistry to behavioral phenotypes.

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41. Modelling adult neurogenesis in dentate gyrus and its impact in pattern separation

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Hippocampus is engaged in memory processes, like episodic and spatial memory. Hippocampal Dentate Gyrus (DG) is one of the two regions where adult neurogenesis occurs and has been suggested to underlie pattern separation, i.e., the ability to formulate distinct memories of similar episodes. Principal neurons of the DG, granule cells, are considered to perform pattern separation through sparsifying and orthogonalizing their inputs. Here, we investigate the role of newborn granule cells in pattern separation using a simple computational, yet biophysically relevant, spiking network.

The DG network consists of 2,000 GCs, i.e., 1,800 developmentally-born GCs (dbGCs, > 8 weeks-old), 100 mature adult-born GCs (mabGCs, 6-8 weeks-old) and 100 immature GCs (iabGCs, 4 weeks-old), 100 GABAergic basket cells, 80 glutamatergic mossy cells, and 40 HIPP interneurons. Each neuronal type is simulated as a point neuron using the adaptive exponential integrate-and-fire model. GCs are simulated as multicompartmental point neurons, consisting of a somatic compartment connected with 12- (dbGCs) or 3-dendrites (mabGCs and iabGCs).

Three different networks were used: a network with only dbGCs, one with 50% dbGCs, 25% mabGCs and 25% iabGCs, and finally a network with equal percentages of each GC subpopulation (33.3%). Preliminary results showed that GC activity was highest in the network with equal populations of each GC category (mean ± std: 3.39 ± 0.67), followed by the 50-25-25% network, which was in turn higher than the control network. Complete lack of adult neurogenesis resulted in a network with the lowest GC population activity. These simulations indicate that as the population of abGCs grows the excitability of the DG network increases. This is because abGCs are more active than the overall GC population, irrespectively of the network's composition. Future simulations will investigate the memory capacity and pattern separation efficacy of this DG network.

42. Mechanisms of p.A53T-αSynuclein Mediated Synaptic Dysfunction

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, which is characterized of variable clinical characteristics, age of onset and course of progression. The hallmark of PD, whether sporadic or familial, is the deposition of proteinaceous inclusions, the so-called Lewy bodies, which are composed mainly of alpha-synuclein (αSyn). αSyn is the major gene linked to sporadic Parkinson's disease, however point mutations and multiplications of this gene are linked to familial forms of the disease. Of particular interest is the G209A (p.A53T) αSyn mutation that has Greek-Italian ancestry, is inherited in an autosomal dominant way and causes axonal abnormalities and distortions in synaptic connectivity. The mechanisms through which mutant αSyn affects synaptic organization remain unknown. Using cell reprogramming technologies, we have previously developed a robust induced pluripotent stem cell (iPSC)-based model of PD from patients harboring the p.A53T-αSyn mutation that faithfully simulates disease pathogenesis and uncovers novel disease-relevant phenotypes at basal conditions, including reduced synaptic connectivity (Kouroupi, G., et al., Proc Natl Acad Sci U S A, 2017). Global transcriptome analysis suggests defects in synapse formation and function and shows dysregulated expression of genes involved in synaptic signaling. Electron microscopy of p.A53T neurons indicates impaired organization of synaptic vesicle pools and microtubule disorganization. By applying artificial synapse formation assay we have studied synaptogenesis defects of p.A53T neurons. Finally, we have explored the potential therapeutic value of small molecules targeting either αSyn or synaptic signaling. In addition, complementary analysis of synaptic contacts in p.A53T transgenic mice has been performed. Altogether this work aims to gain a better insight in the events leading to synaptopathy caused by p.A53T mutation.

43. Dissecting the role of nmda receptors in place cell formation using a CA1 network model

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The ability of animals to navigate through complex environments is thought to be mediated by the hippocampus. This is supported by numerous studies reporting the existence of place cells: pyramidal neurons in the hippocampus that display spatially selective firing [1]. However, the exact mechanisms underlying place cell formation remain unknown. Motivated by the role of NMDARs in nonlinear integration of synchronous excitatory synaptic inputs, our aim is to investigate how the properties of NMDARs (i.e.,slow kinetics, synaptic strength) contribute to place cell formation.

Towards this goal, we used a biophysical computational network model of the CA1 region [2] and simulated both the direct input from EC LIII (grid-like) and the indirect intrahippocampal input from DG-CA3 (place-like), during a virtual spatial navigation task. The CA1 network model consisted of 130 excitatory pyramidal cells and a variety of 20 inhibitory local circuit interneurons, namely Axo-axonic, Basket, Bistratified,O-LM, VIP/CR+ and VIP/CCK+ cells. To examine the contribution of NMDAR kinetics and synaptic strengths during place cell formation, we modified -independently onto EC and DG-CA3 projections to pyramidal cells- these NMDAR features both above and below their control values, and assessed the effect on the resulting place cell number and information content.

We found that making the NMDAR's kinetics faster in distal apical synapses (EC input) leads to a reduction in place cell number. Moreover, increasing the conductance of NMDA receptors in proximal apical and basal dendrites (CA3 input) impairs both the number and the spatial information of place cells. Our simulations predict that physiological values of NMDAR-mediated nonlinearities onto EC projections of CA1 pyramidal cells facilitate place cell formation while the enhancement of these NMDAR nonlinearities in DG-CA3 projections have the opposite effect.

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44. Direct reprogramming of cortical astrocytes to induced-neurons using mir-124 and the neurogenic molecule ISX9: study of the molecular mechanism and *in vivo* potential therapeutic effect

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Direct neuronal reprogramming of glial cells has emerged as a promising approach for neuronal replacement using resident brain cells in order to alleviate neuronal loss due to neurodegeneration or trauma. Accordingly, recent studies demonstrate that astrocytes can be reprogrammed to induced-neurons using combinations of transcription factors (TFs), chemical cocktails or miRNAs *in vitro* and to some extent *in vivo*. However, the molecular mechanisms that drive these reprogramming processes remain largely elusive. Here, we have studied the role of the brain enriched miRNA, miR-124 and the small molecule ISX9 in inducing neuronal reprogramming of cortical astrocytes, focusing on the elucidation of the core transcriptional mechanisms that instruct the reprogramming process. Our *in vitro* data indicate that forced expression of miR-124 alone potently reprograms cortical astrocytes into immature Tuj1+ induced-neurons, while the addition of the small neurogenic molecule ISX9 significantly enhances the differentiation and maturation of induced-neurons. Molecular characterization of early-induced cells with RNA-seq analysis, real time RT-PCR and immunofluorescence has revealed that the major TFs upregulated early during the reprogramming process by miR-124 are the forebrain development TFs, Mash1 and Gsx2, while ISX9 greatly enhances the intermediate progenitor cells' TF, Tbr2, as well as the Neurogenin2/NeuroD1 transcriptional network. Interestingly, ISX9 also highly upregulates a set of genes that are implicated in midbrain and hindbrain development, leading to an expansion of the possible neuronal identities further than the forebrain. Finally, miR-124 efficiently reprograms astrocytes to induced-neurons *in vivo* in a mouse model of cortical trauma, which is further facilitated by ISX9, highlighting its *in vivo* direct reprogramming capacity and potential therapeutic value.

45. Experimental model in Multiple Sclerosis: Clinical and Neurophysiological Effects of Citrullinated MBP analogues in Wistar rats

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Multiple sclerosis (MS) is an autoimmune disease of the Central Nervous System (CNS). Although the pathology of MS remains unclear, there is evidence that T-cells recognizing encephalitogeni epitopes of myelin, play a pathogenic role in the induction of MS. Studies have shown that T-cells responses in patients are associated with the recognition of the 81-105 region of MBP. The pathogenetic role of autoimmune T-cells recognizing encephalitogenic epitopes of MBP has also been noted in Experimental Autoimmune Encephalomyelitis (EAE), the animal model of MS. EAE is induced in susceptible animals by immunodominant epitopes of the myelin sheath. Similar clinical and histopathological features to MS can be induced by immunization of myelin components.

In this study a model of EAE is developed by intraperitoneally injecting citrullinated MBP 83-99, a molecule with increased ability to induce the expression of highly toxic cytokines to CNS, on Wistar rats. The goal of this study was to investigate the ability of citrullinated MBP 83-99 to produce clinical symptoms and neurophysiological effects. We find that clinical sensory and motor symptoms developed gradually from the first month following injection of Cintroulinated-MBP 83-99 and consisted of decreased motor activity and reduced sensitivity to pain. The symptoms culminated six months later with a complete tail paralysis and a remarkable inability for motor activity. Furthermore, in vitro electrophysiological examination showed a reduction in conduction velocity of compound action potential recorded in corpus callosum in acute frontal brain slices. These observations suggest that Citroulinated-MBP 83-99 may induce a wide spectrum of behavioral and neurophysiological changes in Wistar rats, providing thus a powerful methodological tool for the study of EAE in this strain of animals.

46. Experimental Inflammatory Bowel Disease (IBD) induces innate immune memory in the brain with long-lasting effects on hippocampal neurogenesis

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Inflammatory Bowel Disease (IBD), including Crohn's disease (CD) and Ulcerative Colitis (UC), is a disease associated with dysbiosis, resulting in compromised intestinal epithelial barrier and chronic mucosal inflammation. Chronic and systemic inflammation has been associated with an increased risk for developing depression and cognitive dysfunction, often present in IBD patients. An indispensable part of the brain's innate immune system is microglia which is a major regulator of hippocampal neurogenesis, a refined and tightly regulated process of the adult brain implicated in memory, learning and mood control. Repetitive peripheral inflammation alters the function of microglia, induces memory immunity and negatively regulates hippocampal neurogenesis. Here, using a well-characterized model of experimental colitis based on the administration of dextran sodium sulfate (DSS) in the drinking water we demonstrated that hippocampal microglia was gradually "trained" and became "tolerant" in DSS colitis. Microglia tolerance in DSS colitis was accompanied by a ramified non activated morphology, increased expression of the anti-inflammatory enzyme Arginase-1 in the hippocampus and enhanced hippocampal neurogenesis. Interestingly, the "tolerant" microglia was still present in the hippocampus of mice with chronic DSS colitis and induced a neuroprotective effect on newborn neurons. However, deficits in the migration pattern of newborn neurons were present. This could impact the functional integration of newborn neurons and alter the neuronal activity of the hippocampus with debilitating effects on mood and cognition. Our findings indicate that the impact of experimental colitis in microglia and adult hippocampal neurogenesis could explain the reported cognitive and mood dysfunction in IBD patients.

47. *In vitro* reprogramming of astrocytes to ependymal cells

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Multiciliated ependymal cells constitute one of the main populations of the adult subventricular zone (SVZ) in the mammalian brain. They line the lateral ventricles and carry multiple motile cilia on their apical surface. With their coordinated movement, these cilia control the cerebrospinal fluid (CSF) flow through the ventricular system and they are crucial for the proper brain development and function. Cilia malfunction can lead to various brain disorders, like hydrocephalus.

Previous studies from our lab have highlighted two geminin family members, GemC1 and McIdas, as critical regulators of multiciliate cell fate acquisition and differentiation. GemC1/Lynkeas, as more upstream, upregulates transcription factors of multiciliogenesis, such as McIdas, Foxj1 and p73, thus governing the generation of multiciliated ependymal cells. Of note, GemC1/Lynkeas-deficient mice are devoid of multiciliated ependymal cells and develop hydrocephalus.

Astrocytes, which are one of the most abundant types of neural cells in the brain, play an important role in neurodegeneration and exhibit remarkable plasticity *in vivo,* have already been successfully reprogrammed into neural stem cells and neurons, showing potentials for therapeutic strategies for neurodegenerative diseases. Our goal is to combine these methods with our knowledge of multiciliogenesis pathway and hydrocephalus pathology in order to investigate the potential of astroglial cells to be reprogrammed towards the ependymal fate *in vitro.* For this purpose, cortical astrocytes from neonatal mice are isolated, infected with GemC1 and McIdas, through a lentriviral system and then cultured in differentiation conditions. We show that lentiviral infection of cultured astrocytes can induce a switch to the ependymal cell fate. The reprogramming efficiency is proved by the downregulation of astrocytic characteristics (GFAP, S100b) and the upregulation of ependymal markers (p73, Foxj1). The successful cell fate switch and the differentiation towards multiciliated ependymal cells in the SVZ of the brain consists a promising therapeutic approach.

48. Effect of cannabidiol on behavioral parameters in adult male mice after induction of inflammation

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Cannabidiol (CBD) is a major active component of the Cannabis plant, which, unlike tetrahydrocannabinol (THC), is devoid of euphoria-inducing properties. CBD research in animal models and humans has shown numerous therapeutic properties for brain function and protection. Broadly, CBD has demonstrated anxiolytic-like, antidepressant-like, neuroprotective, antiinflammatory and immunomodulatory benefits. The protein Concanavalin A (ConA) is a lectin found in the jack bean (Canavalia ensiformis) and was systematically studied the first time by Jones and Johns (1916). ConA has been associated with a variety of toxicological effects (like mitogenic, cytotoxic and hepatotoxic).The present study sought to investigate the effects of Cannabidiol on behavioral parameters (anxiety-like behavior and mobility) in adult mice with induction of inflammation by ConA administration. Mice were pre-treated with CBD (20 mg/kg, oral administration) for five days, and challenged with saline or ConA (20mg/kg; i.v.) on the fifth day. In particular, the behavioral analysis was assessed by using the open-field test in order to evaluate the anxiety-like behavior and mobility. During an individual 10 min task, we measured the time that mice spent in the periphery of the open field apparatus (anxiety-like index) and the number of entries in the center of the apparatus (mobility index). Behavioral studies revealed an anxiolytic-like activity and an increase of mobility after the five-day treatment of CBD. ConA intoxication has been found to increase anxiety-like behavior and reduce mobility compared to the control group. Lastly, the mice group that has been treated with CBD and ConA has shown anxiolytic-like behavior and increased mobility compared to the ConA adiministrated group.

49. Repetitive magnetic stimulation induced synaptic plasticity relies on cooperative pre- and postsynaptic activity

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Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique used in clinical practice as a diagnostic and therapeutic tool. Based on the principle of electromagnetic induction it can stimulate the brain through the intact skin and skull, and when applied repeatedly (repetitive TMS; rTMS) it induces long lasting changes in the synaptic strength. Despite its use in the clinics, the cellular and molecular mechanisms of rTMS-induced plasticity remain poorly understood. Here, we systematically compared the effects of repetitive magnetic stimulation (rMS) with classic local electric stimulation of Schaffer collateral-CA1 synapses in acute hippocampal slices and entorhino-hippocampal tissue cultures. As shown previously, 10 Hz rMS induces robust long-term potentiation (LTP) of excitatory neurotransmission. Strikingly, the same stimulation protocol induces a long-term depression (LTD) of excitatory neurotransmission when local electric stimulation is used. In a series of pharmacologic experiments using two-pathway experiments we provide evidence that a co-operative pre- and postsynaptic activation during 10 Hz rMS may transform LTD into LTP. At the molecular level we demonstrate that this effect depends on the activation of NMDA receptors, L-type voltage gated calcium channels and functional intracellular calcium stores. Finally, a computational model that is based on a voltage-dependent STDP rule with fast BCM-like metaplasticity reproduces our findings. Indeed, it is possible to predict the effects of other selected rMS protocols using the computer model. These results provide new insight on how rTMS may induce lasting changes in synaptic transmission and network excitability. They lay foundation for the development of a multi-scale computational model of rTMS-induced plasticity. Supported by the Federal Ministry of Education and Research (BMBF; CRCNS 01GQ1804A).

50. The effect of pathological developmental pathways in human brain cancer physiology

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The Promyelocytic Leukemia Protein (PML) is a cell regulator, expressed in all tissues and modifications in its expression are often related to various carcinogenic phenotypes [1]. However, the specific effects of this protein in cancer are not clear because it can have either tumor suppressing or tumor promoting effects regarding the type of the cancer [2,3]. In brain, PML participates in the physiological migration of the neural progenitor cells (NPCs) [4,5], which are also hypothesized to serve as the cell of origin of glioblastoma (GB) [6]. Therefore, we study the role of PML in GB physiology using 3D *in vitro* biological models. We are, further, considering an *ex vivo* physiological approach in order to monitor the progression of the tumor in conditions that better mimic the natural microenvironment of GB. Our findings indicate that in GB, PML inhibits tumor growth while it induces cellular migration and these two PML-driven functions are mediated by distinct cellular mechanisms.

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51. Structured connectivity exploits NMDA-non-linearities to induce diverse responses in a PFC circuit

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Prefrontal Cortex (PFC) exerts control on action selection and mediates behavioral flexibility. This is imperative during working memory (WM), when stimuli retention and integration takes place. Neurons of the PFC exhibit mixed selectivity to stimuli, yet the mechanisms that enable them to rapidly modify their response properties in a context-dependent manner remains an open question.

To answer this question we hypothesize that: a) it is possible to have neurons displaying a highly dynamic behavior in a recurrently connected network and b) rapid synaptic facilitation via NMDARs enables this behavior in a network constrained by the highly reciprocal and clustered connectivity that is prominent in PFC.

We test these hypotheses using biophysical models of PFC, which are constrained in their physiological and connectivity properties. Specifically, we predict that both NMDA nonlinearities, of L5 PFC neurons, and a structured connectivity are needed to produce highly dynamic, yet discrete network activity trajectories, thus maintaining WM information in a dynamical way while also exhibiting robustness in time.

Our results are summarized in: a) a network model that reproduces the complexity of population responses as well as the emergence of low energy activity during the WM period. b) This model can respond with different activity trajectories, in response to different stimulus applied, through a rapid, internal connectivity reconfiguration. c) This reconfiguration is mediated by dendritic nonlinearities, since eliminating them abolishes the discrimination abilities of the network. d) This reconfiguration is observed more prominently in a network connected as the PFC anatomy indicates, compared to a randomly connected network. e) We note that the aforementioned activity trajectories can in principle be attributed to dynamically recruited neuronal ensembles or to combinations of them, that respond stably in time.

52. Tracing the origins of modern neuroscience in ancient Greece

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Our understanding of the brain and how it works has undergone radical changes over the past centuries. The field of neuroscience, and its historical scientific developments, have dramatically reshaped our universal knowledge about who we are, how we think, feel and act. Furthermore, neuroscience has been interacting with our cultural landscape and redefined our understanding of who we are as individual through times.

In this project, we are interested in exploring how societies in ancient Greece, during the prehistoric period and from 7th to 2nd century B.C., evolved their understanding of the brain, based on their own structure and needs, explored the ideas of perception, "self" and feelings, among others, and came to understand the role of the brain in mediating perception, 'self', feelings, etc. We start with a discussion on Alcmaeon of Croton, who is considered the first one to attempt to study the brain. Furthermore, we will describe the work of Asklipios, Hippocrates, Plato, Aristotle, Philolaeus and Herophilus, among others.

1. Effect of curcumin in a mouse model of facial nerve axotomy

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Facial nerve axotomy model is a well-established system for studying axonal response and neuronal regeneration. This model is an experimental paradigm, allowing a systematic and detailed investigation of the reaction of neuronal and non-neuronal cells after facial axotomy. Facial nerve injury causes acute inflammatory reaction, with increased expression of cytokines. Curcumin is a natural compound with anti-inflammatory properties, therefore its application in facial axotomy model can provide neuroprotection as a result from anti-inflammatory properties. So far curcumin has not been studied in facial axotomy. In a model of sciatic nerve regeneration curcumin has shown neuroprotection due to promotion of regeneration. Thus, we expect that it would show similar effect in a model of facial nerve axotomy. We hypothesized that in a model of facial nerve axotomy curcumin will have neuroprotective effect. To test this hypothesis 40 male and female C57/Bl6 mice were subjected to facial nerve transection and treated intranasally with 200ug/g per gram body weight curcumin (experimental group) or DMSO (control group). We examined whether curcumin affects functional recovery, re-innervation, inflammation, sprouting and survival of neurons after facial nerve injury. Following the injury, we assessed both groups behaviourally for recovery of whisker hair motor performance. We also assessed microglial and astroglial activation, as well as neuronal survival and sprouting as markers of regeneration. Our results revealed that curcumin treatment significantly increased functional recovery and re-innervation, decreased microglial and astroglial activation, upregulated neuronal sprouting. As a conclusion our data suggests that curcumin has a neuroprotective effect on neuroregeneration.

2. Synchronized activity of different cortical areas in vitro and its modulation by endogenous serotonin during neuropathic pain

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Neuropathic pain is a dilapidating disorder that is extremely difficult to treat. It can lead to comorbidities like depression and anxiety. Recent evidence suggests that long term plastic changes in different cortical and subcortical brain regions might be involved in the nociceptive, emotional-affective and cognitive deficits of neuropathic pain. Several cortical areas like the somatosensory, insular and cingulate cortex show increased pyramidal cell excitability in neuropathic pain, on the other hand some areas of the mPFC show decreased excitability. However it is not well known how the dynamics of the local networks are affected by neuropathic pain in these cortical areas and how neuromodulators affect them. We used the spared nerve injury (SNI) model in mice and extracellular field potential recordings from coronal slices in vitro to record NMDA dependent spontaneous synchronized activity. We found that the frequency but not the duration of the spontaneous synchronized discharges in the hind limp primary somatosensory cortex was increased in neuropathic pain in comparison to sham operated mice. Neither frequency, nor duration of the spontaneous synchronized discharges was altered in other brain areas recorded (secondary somatosensory, anterior cingulate, agranular insular and prefrontal cortices). Application of the serotonin specific reuptake inhibitor fluoxetine caused an increase in the duration of the NMDA dependent spontaneous synchronized activity in the posterior agranular insular area in SNI compared to Sham mice. In the secondary somatosensory area, fluoxetine caused an increase in the duration of the spontaneous synchronized activity in Sham mice but a decrease in SNI mice. These results indicate cortical area specific local network alterations in neuropathic pain but also the existence of homeostatic mechanisms.

3. Chronic stress reverses the phenotypic profile of rats that overexpress human alpha-synuclein

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Aside from the long–studied causal effects of chronic stress leading to neuropsychiatric diseases, researchers are increasingly focusing on the involvement of stress axis dysfunction in neurodegenerative processes. Numerous genetic and pathological studies implicate alpha-synuclein (AS) in the manifestation of neurodegeneration in Parkinson's Disease. Notably, excess AS levels are thought to be the main determinant of pathology. We hypothesize that chronic stress may trigger a series of biochemical and behavioral changes in an animal model of increased AS burden, conducive to neurodegeneration. In this two-by-two experimental design, 9 months old male rats overexpressing human (hu)-AS BAC and wild type littermates were exposed to a chronic unpredictable stress (CRUST) paradigm. The effect of chronic stress on various behavioral facets was assessed by a comprehensive behavior test battery examining cognitive, neuropsychiatric and motor changes. Additionally, by measuring monoamines and their metabolites using high-performance liquid chromatography, we explored the neurochemical profile of the striatum and hippocampus. Finally, the biochemical profile of AS is currently being assessed in the striatum and the midbrain in addition to tyrosine hydroxylase positive dopaminergic neuron stereological counts in the substantia nigra. Our results thus far show that chronic stress affects various behavioral aspects of the hu-AS BAC rats, underlying an interaction between the genotype and exposure to stress. Specifically, locomotor tests reveal a significant reduction, solely affecting the hu-AS BAC rats that underwent the CRUST protocol. The assessment of the striatal neurochemical profile reveals the same pattern, with a reduction of dopamine seen only in stressed hu-AS BAC rats. Our initial findings suggest that the neurodegenerative phenoconversion observed in hu-AS BAC rats is triggered by exposure to chronic stress. Further studies will allow us to elucidate the underlying pathways involved in this gene-environment interaction and explore the causes of synucleinopathy pathogenesis leading to neurodegeneration.

4. The protective function of a novel neurosteroid in different glial populations *in vivo* and *in vitro*

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Neurotrophins, acting through the Trk and/or the p75NTR receptors, regulate the regenerative capacity of the nervous system via their neurogenic and neuroprotective properties. DHEA is a small endogenous neurosteroid that is able to exert potent neuroprotective effects by binding with high affinity to the neurotrophin receptors. However, DHEA is metabolized to estrogens and androgens, affecting the endocrine system and increasing the risk for hormone-dependent tumors. BNN20, an analog of DHEA, exhibits strong neuroprotective properties, which are deprived of endocrine effects. We aimed to investigate whether BNN20 exerts neuroprotective and/or restorative effects in glial populations by using *in vitro* and *in vivo* approaches.
We took advantage of the LPC-induced demyelination model in mice, a toxic model of focal demyelination. LPC is stereotactically injected in the corpus callosum. It is incorporated in the cell membrane and induces inflammation and myelin sheath phagocytosis. Oligodendrocytes degenerate soon after the injection, while remyelination follows in discrete steps. We performed immunohistochemistry to detect myelin, oligodendrocyte, microglial and astrocyte numbers in different time points during de- and remyelination in LPC-treated mice. We also used primary oligodendrocyte and microglia cultures to analyze the effect of BNN20 on isolated glial populations.
BNN20 rescues mature oligodendrocytes in the LPC mouse model. BNN20 is able to reduce myelin loss and astrocyte accumulation during demyelination. It may accelerate the process of remyelination, while it does not have an effect in oligodendrocyte precursor cells or microglia numbers. BNN20 reduces the number of M1 (pro-inflammatory) microglia and increases the number of M2 (anti-inflammatory) microglia after LPS treatment *in vitro*.
We propose that BNN20 exerts a protective effect on mature oligodendrocytes through mechanisms we are currently investigating. Our long-term goal is to explore the possibility that BNN20 may serve as a lead molecule to develop substances with potential applications in the treatment of demyelinating disorders.

5. Misfolded Proteins in the Retina

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Background: Recent research suggests that Tau is the culprit lesion along with neuroinflammation in the etiology of Alzheimer' s Disease (AD). Retina is the extention of the brain and is the most easily approachable part of the central nervous system. Detection of the pathological protein accumulations may be possible by using spectral domain optical coherescent tomography (SD-OCT) and fundus autofluorescein (FAF). There is evidence showing that retinal plaques start accumulating even earlier than the ones in the brain. Most recent Tau protein images in the brain consist of normal or reverse C-shaped paired hellical filaments.

Methods: 30 patients with PET proven AD were examined by SD-OCT and FAF. Mean age was 72. Hypo or hyperfluorescent retinal lesions were scanned by SD-OCT and C shaped neuro fibrillary tangles (NFT) were investigated in a masked fashion. The researchers agreed on the shape of the lesions. Both C-shaped (normal or reverse) filaments and thinner fibrillary structures were taken into consideration.

Results: In all the patients, NFT that exactly corresponded with the histopathologic and cryo-EM images of Tau in terms of shape and dimension were detected along with thin fibrils and lesions similar to amyloid beta. The number of the retinal filaments and other abnormal proteins was in concordance with the severity of the disease process. The advanced retinal tangles had normal or reverse paired C shapes and thin fibrils had the shape of histopathologic images seen in early developmental stages of the disease.

Conclusions: Retinal images of Tau were disclosed for the first time in live AD patients. Retinal neuroimaging is a trustable biomarker and tool for monitoring the disease.

6. The role of *Streptococcus agalactiae* surface lipoproteins in blood-brain barrier crossing

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Streptococcus agalactiae (Group B streptococcus, GBS) is among the major pathogens causing neonatal meningoencephalitis. Hence, we sought to investigate the mechanisms that GBS employs in crossing the blood-brain barrier (BBB). Preliminary experiments in a novel *Drosophila* model of BBB indicated Blr, a GBS leucine rich repeat (LRR) surface lipoprotein as important for brain entry. Here we assessed a Blr mutant GBS strain for its ability to cross the BBB and cause mortality in mice. Adult male CD-1 mice were intravenously injected with the wild type GBS strain NEM316, or the isogenic mutant *Δblr,* and their survival was monitored over one week whilst bacterial levels were determined in the blood and the brain. To identify the route of GBS brain entry, a separate group of mice was inoculated with GBS genetically marked with GFP (GFP-GBS) and their brains were processed for immunofluorescence. Our results demonstrated that the number of surviving mice in the GBS group declined gradually over one week after inoculation whereas no deaths were recorded in the *Δblr* group. Moreover, at 3 and 6 h after inoculation the brain-to-blood ratio of bacterial levels in the *Δblr* group was significantly lower than in the GBS group. By 24 h, bacteria of neither genotype were detected in the blood; nevertheless, the levels of *Δblr* in the brain were significantly lower than those of GBS. At this endpoint, GFP-GBS was detected primarily at the choroid plexuses and in brain areas adjacent to the ventricles. In conclusion, Blr lipoprotein mediates brain entry and virulence of GBS, which probably enters the brain through the epithelial "blood-cerebrospinal fluid barrier" of the choroid plexuses.

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7. Effect of NADPH Oxidase inhibitors in experimental animal models of retinopathies

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Aim: The aim of the present study was to investigate the role of NADPH oxidases (NOX) in the development of retinal pathologies, associated with excitotoxicity and diabetes, and subsequently the evaluation of NOX inhibitors as therapeutic agents.

Methods: Sprague-Dawley rats were used for the induction of the *in vivo* retinal models of A) AMPA excitotoxicity and B) streptozotocin (STZ) induced Diabetic Retinopathy (DR). (A). In order to investigate the effect of NOX inhibition against AMPA induced toxicity in the retina, rats were intravitreally administered with PBS, AMPA (42nmoles) or AMPA+VAS2870(pan-NOX inhibitor,10^{-6}-10^{-4}M), ML171(NOX1 inhibitor,10^{-5}-10^{-4}M) and GLX7013114 (NOX4 inhibitor,10^{-4}M). (B). In order to evaluate the potential protective role of NOX4 inhibition in DR, rats were intraperitoneally administered with STZ (70mg/kg) for the induction of diabetes. Two days later, rats received daily for 14days vehicle or GLX7013114 (10mg/ml, dissolved in DMSO), as eye drops (20μl/eye). Immunohistochemical studies were performed using antibodies raised against neuronal markers nitric oxide synthase(bNOS) and neurofilament (NFL), and inflammatory markers glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule-1(Iba-1).

Results: VAS2870 and ML171 (10^{-4}M) afforded neuroprotective and anti-inflammatory actions reversing the AMPA induced reduction of bNOS positive cells and attenuating the macro/microglial activation. GLX7013114 (10^{-4}M) attenuated the AMPA induced activation of macro- and microglia. In the STZ model of DR, the ocular (eye drops) administration of GLX7013114 (10mg/ml) had a minor but statistically significant effect on the diabetes-induced loss of bNOS positive cells, but fully reversed the diabetes induced reduction of NFL immunoreactivity. It also attenuated macro and microglial activation.

Conclusions: These results suggest that NOX1, NOX4 and possibly NOX2 (due to the actions of VAS2870) play a role in the pathophysiology of the retina. NOX1,2,4 inhibitors had neuroprotective and anti-inflammatory actions against retinal abnormalities caused by AMPA excitotoxicity. The novel NOX4 inhibitor,GLX7013114, may be considered as a putative therapeutic for DR due to its neuroprotective actions when administered as eye drops in the diabetic retina.

8. Increased phosphorylation at Ser-129 indicates alpha-synuclein's pathology in substantia

nigra, striatum and cortex of "weaver" mouse, a genetic model of Parkinson's disease: therapeutic prospects

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Parkinson's Disease (PD) is mainly characterized by a selective and progressive degeneration of the dopaminergic neurons of the substantia nigra (SN), leading to dopamine deficiency. Furthermore, is pathologically characterized by the gradual accumulation and aggregation of the presynaptic protein alpha-synuclein, which is also the main component of the intracytoplasmic protein inclusions called Lewy bodies (LBs), another defining feature of PD. The processes of alpha-synuclein oligomerization and fibril growth have central roles in the pathogenesis of PD and other synucleinopathies.

The "weaver" mouse (wv/wv) is a genetic model, which carries a naturally occurring point mutation in *girk2* gene, leading to excitotoxicity and, thus, to neuronal degeneration in the brain areas of SN and cerebellum. The "weaver" mouse has served as a great model, presenting significant similarities to the human PD, such as progressive degeneration of dopaminergic neurons in SN, motor difficulties (tremor, dyskinesia), cognitive impairments, neuroinflammation and oxidative stress.

Our results considering the "weaver" mouse, compared to control mice (+/+) by using Western Blot analysis demonstrate: a) increased soluble phosphorylated (Ser-129) alpha-synuclein in the Midbrain (MB), Striatum (Str) and Cortex (CX) and b) increased insoluble phosphorylated (Ser-129) alpha-synuclein at least in the CX. The above findings are supported by Immunofluorescence, which revealed increased signal of phosphorylated (Ser-129) alpha-synuclein in SN, CX and Hippocampus in the "weaver" mouse.

The phosphorylation of alpha-synuclein at Serine 129 is indicative of its pathology and 90% of the alpha-synuclein present in LBs is also phosphorylated (Ser-129). Thus, our results indicate that the "weaver" mouse develops the alpha-synuclein's pathology, as well.

Our proximate goals encompass the detection of possible aggregates by immunostaining and the further investigation of the mechanisms underlying the aggregation of alpha-synuclein in the "weaver" mouse. Moreover, we aim to explore therapeutic approaches.

9. Neurotrophin mimetic BNN27 exerts neuroprotective and neurogenic effects ameliorating cognitive impairments in the 5xFAD mouse model of Alzheimer's Disease.

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Alzheimer's disease (AD) is a progressive, age-associated neurodegenerative disorder, characterized clinically by memory decline and other behavioral disturbance. AD pathogenesis is complex, involving cholinergic neuronal loss in the basal forebrain, abnormal amyloid-β (Aβ) metabolism and aggregation, myelin and axonal failure. The hippocampus, which is firstly and predominantly affected by AD, is one of the few regions in the adult brain showing a neurogenic potential, called adult hippocampal neurogenesis (AHN). The nerve growth factor (NGF) was the first neurotrophin discovered for its stimulatory effect on survival, differentiation and growth of neurons in peripheral and central nervous system. BNN27 is a newly developed 17-spiro-steroid analog that mimics the neuroprotective effects of NGF, acting as selective activator of its receptors, TrkA and p75NTR, lacking the pharmacological limitations of neurotrophins. Based on the known beneficial effects of NGF on AD, we are investigating whether BNN27 is able to alleviate the AD–related pathology, thus providing an outstanding therapeutic potential.

Microneurotrophin BNN27 capsules were sub-dermally applied and steadily released over 60 days (10mg/kg/day) in 1.5 months old 5xFAD mice and their WT littermates. Spontaneous alternation test was used in the aforementioned mouse model to justify possible BNN27-mediated working memory amelioration, which indeed was the case. Furthermore, BNN27 treatment considerably decreased the formation of Aβ plaques within the hippocampus. We further successfully detected improvement of AHN in the BNN27-treated mice compared to their WT littermates via promotion of new neurons generation and induction of proliferation of newborn cells in the DG of the 5xFAD animals. BNN27 exerted cholinergic atrophy rescue in the basal forebrain by battling AD pathology at the soma size level in TrkA (+) cells. Nonetheless, no significant amelioration of impaired synaptic communication, myelin and axonal disruption was observed in the hippocampus after treatment of 5xFAD mice with BNN27.

10. Investigation of the effect of the synthetic cannabinoid (R)-WIN55,212-2 when administered acutely and sub chronically in the *in vivo* retinal model of AMPA excitotoxicity

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Aim: The aim of the present study was to examine the neuroprotective actions of the synthetic cannabinoid (R)-WIN55,212-2 (CB1/CB2 agonist) in the AMPA retinal model of excitotoxicity, and the effect of its subchronic administration on CB1 receptor expression and neuroprotection.
Methods: Sprague-Dawley rats were intravitreally administered (acutely) with vehicle or AMPA, in the absence or presence of (R)-WIN55,212-2 (CB1/CB2 agonist, 10^{-7}-10^{-4}M), as well as in the presence of AM251 (CB1 antagonist, 10^{-4}M) or AM630 (CB2 antagonist,10^{-4}M). The neuroprotective effect of (R,S)WIN55,212 was also examined. (R)-WIN55,212-2 was administered subchronically [25, 50 & 100μg/kg, i.p., 4 days]. Immunohistochemical studies were performed using antibodies against retinal and glial cell markers in order to assess the neuroprotective and anti-inflammatory actions of (R)-WIN55,212-2 and (R,S)WIN55,212 in the AMPA excitotoxicity model. A CB1 receptor antibody was employed to assess CB1 receptor expression.
Results: Intravitreal administration of (R)-WIN55,212-2 reversed the AMPA induced loss of bNOS expressing amacrine cells, an effect that was blocked both by AM251 and AM630. (R,S)WIN55,212 had no effect on bNOS expressing amacrine cells. (R)-WIN55,212-2 was able to reduce the activation of microglia, acting through CB2 receptors, and also to reduce macroglia activation. Subchronic administration of (R)-WIN55,212-2 reduced CB1 receptor expression, at the high dose of 100 μg/kg but not at 25 μg/kg, in both healthy and AMPA treated retinas. (R)-WIN55,212-2 (25 μg/kg) protected bNOS expressing amacrine cells, an effect that was not observed with the dose of 100 μg/kg, due to the downregulation of the CB1 receptor.
Conclusions: The results of this study suggest that (R)-WIN55,212-2 administered acutely acts on both CB1 and CB2 receptors in the retina protecting neurons, macro and microglia against AMPA excitotoxicity. In contrast subchronic administration of (R)-WIN55,212-2 at high doses causes downregulation of the CB1 receptor and abolishes the neuroprotection.

11. The endocannabinoid 2-arachidonoylglycerol and ABHD6/MAGL enzyme inhibitors display neuroprotective and anti-inflammatory actions in the *in vivo* retinal model of AMPA excitotoxicity

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Aim: The aim of the present study was to investigate a) the potential neuroprotective effects of the endocannabinoid 2-arachidonoylglycerol (2-AG) and novel inhibitors of its metabolic enzymesα/ß-hydrolase domain-containing 6 (ABHD6) and monoacylglycerol lipase (MAGL) against AMPA excitotoxicity in rat retina and b) the mechanisms involved in the neuroprotection.
Methods:Sprague-Dawley rats, wild type and Akt2-/- C57BL/6 mice were intravitreally administered with phosphate-buffered saline or (RS)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid hydrobromide (AMPA), in the absence or presence of 2-AG, as well as in the presence ofAM251 or AM630 [cannabinoid 1 and 2 receptor (CB1/2R) antagonist, respectively] or Wortmannin (Phosphoinositide 3-Kinase/Akt inhibitor). In a different group of animals, AMPA was co injected intravitreally with AM12100 (ABHD6 inhibitor) or AM11920 (dual ABHD6/MAGL inhibitor). Immunoreactivity studies were performed using rat retinal (brain nitric oxide synthetase, bNOS), microglia (Iba-1) and macroglia (GFAP) markers, and TUNEL staining in WT mice. Real time PCR was employed to assess CB1/CB2 expression in the retina.
Results: Intravitreal administration of 2-AG blocked the AMPA induced loss of bNOS amacrine cells in a dose dependent manner.AM251, AM630 and wortmannin attenuated its neuroprotective actions. 2-AG also reduced the number of TUNEL+ positive cells, induced Akt phosphorylation and failed to protect retinas of Akt2-/- mice. Real time PCR confirmed CB2 expression in the retina. AM12100 and AM11920 were also able to reduce the loss of bNOS amacrine cells, with the greatest reduction provided by AM11920 (dual-inhibitor). AM11920 attenuated the AMPA induced activation of both microglia and macroglia.AM12100 and 2-AG were able to reduce microglia activation.
Conclusions: These data suggest that 2-AG exerts neuroprotective and anti-inflammatory actionsin the AMPA model of excitotoxicity in rat retina mainly via activation of CB1R/PI3/Akt and CB2Rs, respectively.In addition, it appears that the dual inhibitor AM11920 has the pharmacological profile of a novel therapeutic for retinal disease.

12. Disruption of SVZ niche integrity and ectopic recruitment of neuroblasts in non-neurogenic regions following brain chemical lesion

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Aims: It is largely accepted that life-long neurogenesis presents a spatially restricted distribution, thus brain regions beyond the subventricular zone (SVZ) and dentate gyrus of the hippocampus are considered "non-neurogenic". Yet, recent studies proposed that under pathological conditions, a latent neuroblastic potential of the glial cells of non-neurogenic regions can be activated resulting in the genesis of new neurons. Aim of our study is to investigate whether disturbance of the strictly organized adult mouse SVZ and depletion of neurogenesis in the area activates astroglial cells of neighboring non-neurogenic parenchyma to acquire a neural progenitor potential.

Methods & Results: Our data indicate that brain chemical lesion induced by multiple stereotaxic intraventricular injections of the mito-toxic agent arabinoside-C is leading to disrupted cytoarchitecture of ependymal zone, SVZ niche integrity and neurogenesis. Moreover it triggers doublecortin+ (DCX+) neuroblasts ectopic presence in the adjacent non-neurogenic subcortical parenchyma, the majority of which cluster inside myelinated white matter tracts. Characterization of the spatio-temporal distribution of ectopic neuroblasts has been performed at multiple time points following the chemical lesions (4, 15 days and 6 weeks), . Further to elucidate the origin of the ectopic neuroblasts we applied viral fate-mapping approaches targeting the lineages of SVZ cells.

Conclusions: Our studies are on-going to decipher the origin and lineage of neural progenitors and investigate whether parenchymal neuroblasts originated in the non-neurogenic striatal area or migrated from activated neurogenic zones.

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13. Oligodendroglial and neuronal exosomes: potential culprits in synucleinopathies?

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Synucleinopathies such as Parkinson's disease (PD) and Multiple system atrophy (MSA) are neurodegenerative diseases characterized by the presence of distinctive neuronal or glial cytoplasmic inclusions within neurons and oligodendrocytes, respectively. The major constituent of such inclusions is the neuronal protein alpha-synuclein (aSyn). Since mature oligodendrocytes do not normally express detectable levels of aSyn, a prevailing hypothesis is that aSyn is entering oligodendrocytes following its release by neurons. Secreted neuronal aSyn, either free or associated with exosomes, can be uptaken by neighboring recipient cells, thus suggesting a possible mechanism for cell-to-cell transmission of aSyn pathology.

In the current study, we investigate the uptake and the potential seeding capacity of exosome-associated aSyn, in the presence/absence of haSyn Pre-formed fibrils (PFFs) as seeds for PD and MSA-like pathology, in rat oligodendroglial cells stably overexpressing the human oligodendroglial phosphoprotein TPPP/p25α, human MAPT/tau and/or human aSyn (and control cells) and in primary mouse oligodendroglial and neuronal cultures. As sources of exosome-associated aSyn we utilize brain-derived exosomes isolated from mice expressing the endogenous wild-type protein (WT-aSyn), transgenic mice expressing the haSyn in oligodendrocytes (PLP-aSyn) or the PD-linked hA53T-aSyn in neurons (hA53T-aSyn) and mice knock-out for aSyn (KO-aSyn), as well as exosomes secreted from the above oligodendroglial cell lines.

Our results indicate that both brain- and oligodendrocyte-derived exosomes contain aSyn, TPPP/p25α and MAPT/tau protein cargo, thus suggesting their possible intercellular transfer between neurons and oligodendrocytes and pinpointing a potential role of exosomes in the spread of aSyn pathology in synucleinopathies. Further studies will enable us to decipher the role of TPPP/p25α and MAPT/tau in the release of aSyn-containing exosomes, in an attempt to unveil their potential use as therapeutic tools in synucleinopathies as well as biomarkers of disease state.

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14. NR5A2 protects neuronal cells from oxidative stress-induced apoptosis

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Oxidative stress is a common underlying factor in many neuropathological conditions and brain-related diseases. Identification of new molecular pathways with neuroprotective action against oxidative stress-induced apoptosis may offer useful insights for novel therapeutic approaches. To this end, here we identify orphan nuclear receptor NR5A2 as a critical protective factor in neuronal cells. In particular, we provide evidence that adenoviral-mediated overexpression of NR5A2 significantly decreases apoptosis and promotes survival of H_2O_2-treated SH-SY5Y cells and *ex vivo* cultured murine cortical neurons. Most importantly, dilauroyl-phosphatidylcholine (DLPC), a well-established pharmacological agonist of NR5A2, is sufficient to recapitulate its neuroprotective action in primary neuronal cells. On the other hand, Cre-induced NR5A2 ablation from primary neurons, isolated from NR5A2$^{floxed/floxed}$ mice, enhances apoptosis after H_2O_2 treatment. In agreement, genetic deletion of NR5A2 gene from the nervous system of mouse embryo leads to a massive induction of neuronal apoptosis. By exploring the molecular mechanism of NR5A2 neuroprotective function, we identified key anti-apoptotic genes, such as Bcl-xl and Bcl-2 as well as PI3K/AKT pathway, as potential downstream targets. Future analyses will include ChIP and transcriptional assays to test the direct binding of NR5A2 on these targets. Collectively, our observations render NR5A2 as a critical neuroprotective factor and promising drug target for brain-related diseases.

15. A high content screen in a patient-derived model of Parkinson's disease identifies a kinase inhibitor as a candidate therapeutic

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Disease-modifying therapies remain an important unmet need for neurodegenerative diseases, including Parkinson's disease (PD). The aim of this study was to use induced pluripotent stem cell-derived neurons generated from patients harboring the p.A53T α-synuclein mutation in order to identify small molecules with potential therapeutic value for PD. Our search focused on kinases, since they are attractive clinical targets for treatment of various disorders, including degenerative diseases. We have previously established a robust model of p.A53T-neurons that captures PD pathogenic processes, including protein aggregation, compromised neuritic growth, fragmented axons and disrupted synaptic connectivity. p.A53T neuronal cultures were adapted to miniature 384-well plates for screening of a small kinase inhibitor library for drugs that would enhance tyrosine hydroxylase (TH) expression, an enzyme that characterizes dopaminergic neurons which are lost in PD. Following high content imaging and quantitative immunofluorescence analysis, we identified a kinase inhibitor, BX795 that consistently increased TH immunofluorescence. BX795 also alleviated significantly the pathological phenotypes of p.A53T-neurons by reducing the neurodegeneration index and limiting protein aggregate formation. To determine the molecular pathways and biological processes associated with the neuroprotective function of BX795, we applied quantitative mass spectrometry. Systematic profiling of protein expression changes upon treatment with the kinase inhibitor revealed significant downregulation of a cohort of 187 proteins that are abnormally upregulated in p.A53T-neurons. Enrichment analysis using DAVID software showed that these proteins associated predominantly with mRNA translation, protein catabolism and transport. Further experimental investigation suggests that BX795 acts through mTOR, a key pathway in the regulation of mRNA translation, protein synthesis and autophagy. Current efforts aim to define further the disease-modifying effects of BX795 and delineate the molecular mechanisms that instigate amelioration of pathology in p.A53T-neurons after BX795 treatment.

16. Identifying novel neurotrophin analogs: from structural interventions to biological selectivity to promote neuroprotection and neurogenesis.

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Neurotrophins (NGF, BDNF, NT3, NT4/5) are secreted growth factors that exert neuroprotective effects by promoting neurogenesis and preventing neuronal death. They act by selectively binding to their respective high-affinity, pro-survival receptors TrkA, TrkB and TrkC. Additionally, all mature neurotrophins and their pro-isoforms bind to p75NTR death receptor, resulting to the activation of pro-apoptotic signal transduction cascades. While these molecules have been shown to slow or prevent neurodegeneration, their reduced bioavailability and inability to penetrate the blood-brain-barrier limit their use as potential therapeutics. Our lab has previously shown that neurosteroid DHEA activates NGF receptors exerting neuroprotective and anti-apoptotic effects. Based on that finding, we have synthesized C17-spiroepoxy steroid derivatives of DHEA, named BNNs, that lack the endocrine side effects of the parent molecule. We have recently showed that BNN27 binds to and activates TrkA and p75NTR leading to neuroprotection.

In continuation of these findings, two sources of synthetic compounds are now screened to identify candidates of interest. The first source is a library of DHEA derivatives, designed to have increased neuroprotective action and neurotrophin receptor selectivity. The second source is a series of TrkB agonists, developed via *in silico* analysis to obtain non-peptide, small molecules with high potency and specificity. Neurotrophin mimetics were tested on neurotrophin-dependent, TrkA/TrkB/p75 expressing, cell lines, investigating receptors activation and downstream signaling, as well as cell survival. Based on biological evaluation of the compounds, we redesign structural changes and perform specific mutagenesis assays, in order to further improve their selectivity and neuroprotective actions.

The compounds that show greater survival rates and selective activation of the receptor will be further investigated for their pharmacological properties against neurodegenerative diseases, such as Alzheimer's Disease. We will test whether these molecules can ameliorate the detrimental effects of the toxic Amyloid-beta and therefore prevent neuronal cell death, behavioral defects and enhance neurogenesis.

17. Knockdown of amyloid precursor protein suppresses neuronal cell differentiation and signaling

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Amyloid precursor protein (APP) plays a critical role in Alzheimer's disease, mainly because it is the source of the toxic Aß peptides. However, the role of the full-length APP in neuronal function has not yet been entirely elucidated, although accumulating evidence supports its involvement in synaptic function and membrane plasticity. In the present work we investigated the role of APP in nerve cell differentiation and signaling, using the neuroblastoma cell line SH-SY5Y, as well as the SH-SY5Y/APP- cell line, in which APP expression was downregulated using shRNA. We found that, in the absence of APP, the cells could not differentiate and contained significantly lower levels of the essential for synaptic transmission proteins pCaMKII, pCREB and pERKs. Furthermore, the PI3K/Akt signaling pathway was over-activated, and could not be stimulated after treatment with neurotrophic or growth factors, such as insulin or nerve growth factor. Under stress, and particularly after glucose deprivation or endoplasmic reticulum (ER) calcium depletion after treatment with thapsigargin, the pAkt levels were significantly increased only in SH-SY5Y cells. More importantly, basal levels of the STIM1 protein, an ER Ca^{2+} sensor which translocates upon Ca^{2+} store depletion, were considerably higher in SH-SY5Y/APP- cells, indicating a different dynamic response in calcium mobilization. In this context, using the live calcium imaging technique, we observed that SH-SY5Y/APP- cells were unable to respond to agonists, such as bradykinin or carbachol. Lastly, transfection of SH-SY5Y/APP- cells with a vector which encodes for the intracellular carboxy-terminal domain of APP was able to partially restore the phenotype. Collectively, these results confirm the importance of APP-mediated signaling in nerve cell differentiation and synaptic function and further support the hypothesis that disturbance of calcium homeostasis contributes to the pathogenesis of Alzheimer's disease.

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18. A new player in CNS axon regeneration

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Contactin-2 (CNTN2) is a cell adhesion molecule of the immunoglobulin superfamily that is highly regulated during development in the CNS and PNS. In adulthood, it is expressed by neurons as well as Schwann cells and oligodendrocytes. It plays a critical role in the clustering of VGKCs at the juxtaparanodal area of myelinated fibers. *Cntn2*$^{-/-}$ mice show significant hypomyelination in the CNS. This defect arises from reduced maturation capacity of oligodendrocytes. In the optic nerve in particular, CNTN2 absence from the mutants results in a reduction of the number of axons as well as in a redistribution of axon diameters.

Previous studies have implicated CNTN2 in axon regeneration but its role remains largely undiscovered. Our aim is to uncover a possible role of this protein in the optic nerve crush (ONC) model in mice, which is widely used for studying CNS injury.

Young adult *WT* and *Cntn2*$^{-/-}$ mice were subjected to ONC and neuronal survival in the retina was examined 5 days later, showing no significant effect of CNTN2 absence. When regeneration was tested at 2-weeks post injury, mutant animals displayed a significantly higher number of regenerating axons, suggesting that CNTN2 negatively regulates this process. We excluded that the observed phenotype is a result of different myelin levels or of the expression of glial CNTN2, taking advantage of two transgenic mouse lines expressing low and high levels of glial CNTN2 exclusively (not axonal), showing restored myelin levels. Our analysis proposes that axonal CNTN2 is the one that negatively regulates axon regeneration by regulating Akt phosphorylation.

Our data so far propose an important role of axonal CNTN2 in regeneration after trauma in the CNS and its mechanism of action is currently under investigation. Surprisingly, slightly lower myelin levels and glial CNTN2 expression levels do not affect this process.

19. Effects of the endocannabinoid 2-arachidonoyl-glycerol when administered intraperitoneally or as eye drops on retinal neuroprotection and CB1 cannabinoid receptor downregulation

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Aim: The endocannabinoid anandamide and the synthetic cannabinoids R-(+)-methanandamide and HU-210, induced the downregulation of the CB1 receptor (CB1R), which led to the lack of neuroprotection, after subchronic/chronic intraperitoneal (i.p) administration (Papadogkonaki et al., 2019), despite their acute neuroprotective effects to retinal neurons (Kokona & Thermos, 2015). The aim of this study was to investigate a) the effect of the subchronic administration of 2-arachidonoyl-glycerol (2-AG) on CB1R expression in control retina and in the AMPA excitotoxicity model and b) the time-dependent neuroprotective actions of 2-AG when administered as eye drops.
Methods: Sprague-Dawley rats were administered 2-AG (25, 50, 100μg/kg, i.p, 4 days) or by eye drops (1%, 2x/day for 2 days or 1x/day for 4 or 8 days). Immunohistochemical studies and western blot analysis were performed to examine CB1R, bNOS and Iba-1 immunoreactivity, Akt or ERK1/2 phosphorylation.
Results: Subchronic(4d) administration of 2-AG (i.p) induced attenuation of CB1R expression both in control retina and in the AMPA excitotoxicity model and failed to protect bNOS-expressing amacrine cells. It increased Akt- but had no effect on ERK1/2-phosphorylation. Eye drop administration of 2-AG for 2/4 days protected bNOS-expressing amacrine cells against AMPA excitotoxicity. The 8-days treatment did not provide neuroprotection. CB1R expression was not affected by 2-AG in any of the time points of the topical administration. AMPA excitotoxicity induced activation of microglial cells after 2 days, whereas this effect was abolished after 4 days. Administration of 2-AG (2 days) attenuated the number of Iba-1 positive cells.
Conclusion: These results provide new evidence regarding the actions of 2-AG when administered via two different routes, namely intraperitoneally and as eye drops, regarding CB1R downregulation and neuroprotection in retina. Further studies are essential to decipher the importance of 2-AG as a putative neuroprotective and anti-inflammatory agent in retinal disease.

20. Direct reprogramming of cortical astrocytes to induced-neurons using mir-124 and ISX9: study of the enhancement of a latent midbrain dopaminergic program by Shh and Fgf8

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Replacement of lost neurons due to trauma, neurodegeneration or stroke by directly reprogramming resident glial cells into induced-neurons holds great promise as an alternative therapeutic approach to brain transplantation. To this end, reprogramming of astrocytes to induced-neurons has been well studied during the last years both *in vitro* and *in vivo* using combinations of transcription factors (TFs), chemical cocktails and miRNAs. However, the underlying mechanisms that dictate the reprogramming process have been poorly addressed so far. We have investigated the role of the brain enriched miRNA, miR-124 and the small neurogenic molecule ISX9 in inducing neuronal reprogramming of cortical astrocytes, focusing on the characterization of the transcriptional networks that instruct the reprogramming process. Molecular characterization of early induced-neurons with RNA-seq has revealed that the major TFs upregulated early during the reprogramming process are implicated in forebrain development, such as Neurogenin2, NeuroD1, Tbr2 and Gsx2. However, the transcriptomic analysis also revealed a set of highly upregulated genes that are related to midbrain development such as Lmx1b, En1, Gli1 and Foxa1, proposing the existence of an alternative route the induced-neuronal cells are potent to undergo, other than obtaining a forebrain neuronal identity. Here, we have studied the role of Shh and Fgf8 in reinforcing the latent midbrain dopaminergic program of reprogrammed cells by real time RT-PCR and immunofluorescence analysis. Our results indicate that the addition of Shh along with Fgf8 enhances the maturation and morphological complexity of induced-neurons, while it represses the cortical fate, as revealed by the downregulation of cortical markers, such as Tbr1.

21. A pyrimidine analogue is a selective CRF1 receptor antagonist

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Anxiety and depression are serious neuropsychiatric diseases affecting a large percentage of the population. Corticotropin releasing factor (CRF) plays a pivotal role in anxiety and depression mostly through its interaction with the type 1 CRF receptor (CRF1R). The important role of CRF and CRF1R in the pathophysiology of anxiety and depression in conjunction with the disadvantages of the currently prescribed drugs rendered the CRF1R a significant target for antidepressant and anxiolytic therapy. So far many non-peptide CRF1R-selective antagonists displayed antidepressant and anxiolytic properties in preclinical studies, without, however, any of them being available for clinical use. In an effort to develop novel small non-peptide CRF1R-selective antagonists, we have synthesized and pharmacologically characterized a series small of substituted pyrimidine analogues. Analogue 3, which has a N,N-bis(methoxyethyl)amino group at position 6 and a methyl in the alkythiol group at position 5, bind to CRF1R, with the highest affinity. In this study we further determined the pharmacological properties of analogue 3 by testing its ability to antagonize CRF, as well as, its CRF1R-selcetivity. The results of this study suggest that analogue 3 is a potent CRF1R-selective antagonist, blocking the ability of sauvagine, a CRF-related peptide, to stimulate 1) the proliferation of macrophages and 2) the cAMP accumulation in HEK 293 cells via activation of CRF1R but not via the type 2 CRF receptor (CRF2R). Construction of molecular models of CRF1R allowed us to examine the interactions of this receptor with analogue 3 and the structurally different antalarmin, which is a prototype CRF1R-selective non-peptide antagonist. The results of this study will advance the development of novel CRF1R-selective non-peptide molecules, which will provide extremely useful tools for the determination of the role of CRF1R in anxiety and depression and a therapeutic potential for the treatment of these disorders.

22. Histological and immunohistochemical characteristics of human glioblastoma

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Basic histology has been the guide for diagnosis of intracranial gliomas. Histopathologic and immunohistochemical input has also contributed to the prognosis of malignant gliomas.This study investigated the immunohistochemical existence of substrates that can contribute to the pathogenesis of the specific malignancy. Some routine histological stain was also performed.

Tissue samples were taken from human gliomas located in different cerebral sites. Following the immersion of the samples into formalin, routine histological methods were used for the identification of the tumor, along with immunofluoresence for bNOS expression.

Nitric oxide synthetase (NOS) has been found in glioblastoma cell line (Hokari et al., 1994) and has been induced in primary cortical astrocyte cultures (Hewett and Hewett, 2012). We found bNOS (1:2000, Sigma) expression into malignant cells located in the microenvironment of glioma. Their origin, possibly asctrocytic has to be evaluated. Other substrates, including parvalbumin and/or calretinin will be also assessed.

The localization of the above-mentioned substrates along with new molecular observations (e.g. presence of mutant IDH1) will contribute to the knowledge of cellular interactions of glioma microenvironment.

Stemmer-Rachamimov AO, Louis DN., Curr Opin Oncol. 1997 May;9(3):230-4.

Hewett JA, Hewett SJ., Methods Mol Biol. 2012;814:251-63

23. Animal models in neuroscience: the paradigm of Alzheimer's disease and schizophrenia

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Animal models are pivotal for both neurodegenerative and neuropsychiatric/neurodevelopmental diseases' research. It is crucial for scientists to choose the proper animal model in order to conduct the desired research.
This review will present the existing animal models for one major neurodegenerative disease, Alzheimer's and another neurodevelopmental disorder, schizophrenia.
More specifically, following a short presentation of the diseases, the review will present all the animal models for these two ailments, their benefits and pitfalls, their contribution in the decipher of their pathogenesis, along with the possible therapeutic outcomes.
Special interest of the review is the gender of the experimental animals used in the particular research and the extrapolation of their results into the treatment of the diseases in humans.
The conclusions drawn from the studies used in the review provide useful information for better understanding other neurodegenerative and neuropsychiatric/neurodevelopmental disorders.

24. Impairment of chaperone-mediated autophagy in rats is accompanied by aberrant induction of macroautophagy in the degenerating nigrostriatal axonal terminals

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Aims: Aim of the current study was to assess the contribution of macroautophagy to the dopaminergic axonal degeneration that precedes nigral cell death, evoked by inhibition of the Chaperone-mediated autophagy (CMA) pathway.
Methods: In order to inhibit CMA, we have stereotaxically injected adeno-associated viruses expressing shRNAs targeting LAMP2A receptor or scrambled shRNAs in the rat substantia nigra (SN). At 2 and 3 weeks post-injection, we examined indices of macroautophagy induction and the formation of autophagic vacuoles (AVs) in the nigrostriatal axis by Confocal and Electron Microscopy. The integrity of the nigrostriatal projections and the astro- and micro-gliosis in both striatum and SN at these time-points were also assessed by Confocal Microcscopy.
Results LAMP2A down-regulation was accompanied by abnormal accumulation of AVs at synaptic nerve terminals, prior to dopaminergic degeneration at 3 weeks post-injection. At this early time point, the levels of Bassoon, a negative regulator of autophagy and a marker for the active synaptic zone, were decreased, whereas levels of ULK-1 were increased. Increased astro- and micro-gliosis was observed in both SN and striatum.
Conclusion Our data suggest that uncontrolled induction of macroautophagy may, at least in part, be responsible for the nigrostriatal terminal degeneration that occurs early in this model, well before cell soma degeneration. Further, our results provide the first *in vivo* evidence that ULK-1 is a CMA substrate and may act as a link between CMA and macroautophagy. Therefore, down-regulation of macroautophagy may represent a promising target to reverse the damage and rescue the deteriorating dopaminergic neurons.

25. Induction of LRRK2 dimerization and kinase activity by extracellular stressors in models of idiopathic PD via phosphorylation of LRRK2.

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LRRK2 kinase activity plays a critical role in the induction of neuronal death in cases of familial PD involving mutations in the gene encoding the LRRK2 protein; and more recent evidence implicates LRRK2 activity in the more common form of idiopathic PD. Auto-phosphorylated LRRK2, at Ser1292, is increased in dopamine neurons of the ventral midbrain in post-mortem tissue from idiopathic PD patients. Additionally, increased phosphorylation of its substrate Rab10 is also detected in this neuronal population. Both indices of LRRK2 kinase activation are also elevated in cellular models employing extracellular stressors, such as fibrillar alpha-synuclein or mitochondrial toxins. LRRK2 kinase activity is tightly linked to its dimerization. We are employing a method to label and purify dimeric LRRK2 from cells treated with such extracellular stressors to determine if dimerization is affected under such conditions or the intrinsic kinase activity of dimeric LRRK2 itself. We find that in cells exposed to pathogenic alpha-synuclein species, the intrinsic kinase activity of purified wild type LRRK2 dimers is actually increased compared to dimers from un-treated cells. Interestingly, this alteration of the intrinsic activity of wild type LRRK2 appears to be mediated at least in part by phosphorylation of LRRK2 itself. When isolated dimers from treated cells were first treated with a non-specific phosphatase, the increased in vitro kinase activity was no longer observed. To determine which specific residues are phosphorylated in the hyper-active wild type LRRK2 dimers, we created a series of phospho-mutants, substituting the specific phospho-residues with Ala. We will determine if the inability to auto-phosphorylate LRRK2 at specific site(s) impacts the induction of kinase activity following exposure to various extracellular stressors. A better understanding of the regulation of LRRK2 kinase activity in multiple models of PD will aid in our development of novel therapeutic molecules that inhibit LRRK2 activation.

26. Exploring the pro-inflammatory capacity of the plasma cytokinome of Parkinson's disease patients with or without a LRRK2 mutation

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LRRK2 is linked to both familial and idiopathic Parkinson's disease (PD) and is highly expressed in immune cells, such as macrophages, monocytes and neutrophils. Inflammation is a hallmark of PD and it has been demonstrated that cytokines are overexpressed in PD patients. The large number and the diverse nature of cytokine and metabolite molecules present in serum interacts with specific populations of blood cells and modulate their function. LRRK2 is thought to play a key role in the innate immune response against pathogens and toxins and its mutant active form may promote a constant neuroinflammatory phenotype. Furthermore, LRRK2 expression in monocytes can be induced by several pro-inflammatory mediators. As a result, the LRRK2-mediated inflammatory response may directly contribute to PD development during prodromal stages of the disease.

All the above led us to hypothesize that the cytokinome present in PD patients with or without activating LRRK2 mutations can induce the activation of LRRK2 in healthy control immune cells. To test this hypothesis, we treated peripheral blood mononuclear cells (PBMCs) isolated from a healthy donor with plasma from 20 individuals falling into four different groups: Healthy individuals without a LRRK2 mutation, iPD patients, and LRRK2 mutation carriers with & without PD. Following overnight exposure to plasma, the cells were subjected to Fourier-transform infrared spectroscopy (FTIR) spectroscopy to identify certain metabolic changes in activation. Parallel treated cells were kept for analysis of changes in LRRK2 activation; and a full cytokine panel was quantified from parallel aliquots of plasma. LRRK2 function will be assessed using several in-house ELISA-based assays, including quantification of LRRK2 levels, phosphorylation and in vitro kinase activity. Our analyses will determine if specific inflammatory mediators present in plasma from certain disease-groups can induce biochemical changes in peripheral blood cells, as well as changes in the levels or activity of LRRK2.

27. LRRK2 dependent regulation of TLR4 trafficking in activated macrophages.

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The activation of immune cells, such as macrophages and microglia, by extracellular ligands of Toll-like receptors (TLR) 2 and 4, such as pathogenic species of alpha-synuclein, can contribute to the propagation of neurodegenerative diseases such as Parkinson's disease (PD). Mutations in the *LRRK2* gene are the most common genetic cause of PD, however LRRK2 plays a critical role in the more common sporadic form as well. It is highly expressed in macrophages, microglia, and specific monocyte populations, and contributes to their activation and release of inflammatory mediators. A critical component of this regulation involves coordination of trafficking of the TLR2 and TLR4 receptors. Several years ago it was reported that the Rab GTPase Rab10 participates in the recycling of TLR4, in a manner depending on its GTPase activity. When macrophages are stimulated with the TLR4 ligand LPS, a transient decrease in plasma membrane expression is observed, and this is absent in cells down-regulated for TLR4. Since that time, Rab10 was identified as a major phospho-substrate of LRRK2. The goal of this study was to examine if the role of Rab10 in TLR4 recycling is dependent upon phosphorylation by LRRK2. We treated RAW macrophages with LPS or fibrillar alpha-synuclein and examined the induction of LRRK2, the plasma membrane localization of TLR4, and the phosphorylation of Rab10. We find a transient increase in auto-phosphorylation of LRRK2, accompanied by a more persistent phosphorylation of Rab10. In similarly treated cells, inhibition of the kinase activity of LRRK2 blocked the recycling of TLR4. We will perform similar studies in cells deficient in LRRK2 or over-expressing a phospho-mutant form of Rab10. Our goal is to more fully characterize this signaling axis and the specific role of LRRK2 activity in this cellular activity.

28. The activities of LRRK2 and GCase are positively correlated in immune cells in a manner dependent upon LRRK2 kinase activity.

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Mutations in LRRK2 are the most frequent genetic cause of Parkinson's disease (PD), and occur in sporadic cases as well. Similarly, mutations in the *GBA1* gene, encoding the lysosomal glucocerebrosidase enzyme, are the most common risk factor for PD. In purified peripheral blood mononuclear cells (PBMCs) from idiopathic PD patients or those carrying the A53T mutation in alpha-synuclein, GCase activity is unchanged; however, in PD patients harbouring a mutation in *GBA1* GCase activity is significantly lowered. In contrast, in purified peripheral blood monocytes from idiopathic PD patients, GCase activity is reduced. LRRK2 plays a critical role in many cellular activities, primarily through its phosphorylation of multiple members of the Rab GTPase family, and in particular in the regulation of the lysosomal degradation. It was previously shown, in a novel dried blood spot assay, that GCase activity was actually *increased* in carriers of the G2019S – kinase hyperactive – LRRK2 mutation; whereas, in differentiated iPS-derived neurons from a carrier of a different LRRK2 mutation, GCase activity is reduced. The goal of this study was to explore more directly the link between the kinase activity of LRRK2 and GCase activity. We isolated PBMCs from idiopathic PD patients, as well as both healthy and affected carriers of the G2019S LRRK2 mutation, and measured GCase activity. Similar to the previously reported findings from whole blood, in isolated PBMCs from affected G2019S carriers, GCase activity was increased, and positively correlated with LRRK2 kinase activity. In multiple cellular models we have verified that kinase activity of LRRK2 is correlated with GCase activity. In RAW macrophages, GCase activity in LRRK2 deficient cells is significantly reduced. We are currently further examining this link in macrophages following different activators; however taken all together the evidence suggests that LRRK2 activity regulates GCase activity in a cell type specific way.

29. Study of dementia associated genes in the Cretan Aging Cohort

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Introduction: Phenotypes of Alzheimer's disease (AD), Frontotemporal Dementia (FTD) and related dementias are frequently overlapping, hindering accurate premortem diagnosis. To tackle this issue, imaging and biological markers are increasingly used, often with limited success. Among these biological markers, genetic testing is offering a promising approach for accurate diagnosis.
Aims: Our aim here was to assess the value of genetic testing in dementia diagnosis by screening known AD and FTD genes in a cohort of dementia patients, residing on the island of Crete, Greece.
Methods: We genotyped 100 participants of the Cretan Aging Cohort suffering from dementia (95 of whom clinically diagnosed with AD) using Whole Exome Sequencing (WES). In these patients, we studied common AD- and FTD- associated genes (*APP*, *PSEN1*, *PSEN2*, *APOE*, *C9ORF72*, *CHCHD10, CHMP2B, FUS*, *GRN*, *MAPT*, *SQSTM1*, *TARDBP, UBQLN2, VCP*). As a comparator, we used WES data from 20 patients with mild cognitive impairment (MCI) and 81 cognitively normal controls.
Results: We identified several variants of potential clinical significance in the *APP*, *PSEN1*, *PSEN2, APOE, MAPT, GRN and TARDBP* genes. Importantly, the *TARDBP* p.Ile383Val pathogenic variant was detected in two of the patients with dementia that had been clinically diagnosed with AD (an 82 year old woman and an 80 year old man, respectively). The same variant has been found (through another research program) in a third Cretan patient with the typical FTD/ALS (amyotrophic lateral sclerosis) phenotype. As expected, the *APOE* ε4 allele was more common in dementia patients (24.0%) than in cognitively intact controls (7.4%; p=0.006).
Conclusions: Our analysis revealed variants of potential clinical importance in several dementia associated genes, including the FTD-associated *TARDBP* p.Ile383Val pathogenic variant in two patients initially diagnosed with dementia of the AD type. This highlights the importance of genetic testing as a biomarker in dementia diagnostics.

30. Study of Tau phosphorylation

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Alzheimer's disease is a progressive neurodegenerative disease, mostly affecting the elderly. This condition is characterized by amyloid plaques and neurofibrillary tangles consisting of hyperphosphorylated protein tau. Tau, which is found in abundance in neurons, is associated with microtubules, promoting their polymerization and function. Hyperphosphorylation of tau leads to neurofibrillary tangles and changes the subcellular distribution of the protein. Tau pathology, as well as Alzheimer's disease, are correlated to cerebral blood hypoperfusion, which limits the delivery of oxygen and glucose to the brain. The present study examines the effects of oxygen and glucose deprivation and age on the phosphorylation and subcellular localization of tau. Acute brain slices of C57BL/6 mice were perfused using artificial cerebrospinal fluid and were treated with either hypoxia or hypoglycemia. Our results indicate that, in total brain extracts, oxygen deprivation causes a reduction in total tau levels, as well as dephosphorylation at sites pSer356, pSer262. On the contrary, phosphorylation of tau at site pSer404 was increased. Glucose deprivation appears to lead in a decrease of total tau levels and dephosphorylation in two sites examined (pSer356, pSer262), but did not affect the third site (pSer404). In order to investigate the subcellular localization of tau, cytoskeletal elements, synaptosomes and soluble fractions were isolated, under hypoxic and hypoglycemic conditions. For studying the effect of age, we compared total brain extracts and cytoskeletal elements of aged mice to those of young adult mice. It was found that in total brain extracts, the levels of total tau, as well as phosphorylation at sites pSer356 and pSer262, were augmented in younger mice. Therefore, it can be concluded that tau phosphorylation can be affected by stress or age.

31. Neuroprotective effect of metabotropic glutamate receptor agonist in rotenone model of Parkinson's disease

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Recently, a number of publications have appeared linking the development of Parkinson's disease (PD) with constant contact with pesticides in general, and with the mitochondrial complex I inhibitor rotenone, in particular. Last decades it has been shown that rotenone appears to reproduce the neurochemical, neuropathological and behavioral feature of PD in rats. These findings have led to the assumption that PD may be accompanied by disturbances of other neurotransmitter systems of brain also. The aim of this work was to study effects of antagonist of metabotropic glutamate receptor of 5th subtype (mGluR5) on the development of catalepsy as well as nitric oxide (NO) generation in brain areas of rats subjected to chronic exposure to rotenone. Rotenone at a dose of 1.5 mg/kg, i. p. was injected to male Sprague-Dawley rats daily during 60 days. Oil was injected as vehicle to the control rats (1 ml/kg). NO generation was directly measured in cortex and striatum of rat brain using Electron Paramagnetic Resonance spectroscopy. On the 1st day and 10th day following rotenone administration there were no increases in NO levels. NO generation was enhanced in the rat striatum after 20 days of treatment with rotenone . Rats demonstrated akinesia and rigidity in the catalepsy test on days 30 and 60. Thus, in our experiments it has been shown that long lasting exposure to rotenone at low dose induced catalepsy as well as enhance of NO production in the rat striatum. Antagonist of mGluR5 MPEP decreased catalepsy caused by chronic rotenone treatment as well as partially prevented rotenone-induced stimulation of NO generation. The results of our work might form the basis for designing novel strategies for neuroprotection against PD disease, this socially important pathology.

32. Effects of BNN27 on experimental models of acute retinal degeneration and injury

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Vitreoretinal diseases can cause vision loss at varying degrees and ultimately, irreversible blindness. Intensifying retinal cell survival in the injured retina could be remarkably favorable for many retinopathies with a degenerative component. BNN27, a blood brain barrier permeable, C17-spiroepoxy derivative of dehydroepiandrosterone, has shown neuroprotective activity through interaction with neurotrophins' receptors, TrkA and p75^{NTR}. In this study, we administered systemically BNN27 in two murine models of acute retinal degeneration/injury; retinal detachment (RD) that results in photoreceptor cell death and NMDA-mediated, excitotoxicity-induced retinal injury that leads to cell death primarily of the retinal ganglion cells (RGCs) and the inner retina. In experimental RD, TUNEL+ photoreceptors were drastically decreased after a single administration of BNN27 while macrophage/microglia infiltration, as well as, two markers of gliosis were significantly increased 24 hours post injury. However, single or multiple administration of different doses of BNN27 were not able to maintain the overall survival of photoreceptors 7 days post RD. Additionally, BNN27 did not induce activation/phosphorylation of TrkA^{Y490} in the detached retina although the mRNA levels of the receptor were increased in the detached photoreceptors. In NMDA-induced retinal injury, BNN27 did not alter RGCs' death or death of the inner retina although it significantly reduced cell death of the photoreceptors in all three doses that were tested. Furthermore, it did not induce changes in inflammatory cell infiltration or in retinal gliosis. Moreover, similarly to RD injury, BNN27 did not induce activation/phosphorylation of TrkA^{Y490} in the NMDA-injured retina. Taken all together, BNN27 can reduce cell death in the injured retina but cannot demonstrate a clear neuroprotective role either in RD or in NMDA-mediated retinal excitotoxicity. Furthermore, BNN27 did not exert its effects through TrkA signalling, so future studies are needed in order to elucidate the molecular mechanism of BNN27 in these models of acute retinal damage.

33. Mind the mitochondria: mitochondrial targeting to deal with stress

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Stress is a risk factor for most chronic conditions, and stress-related disorders are the leading cause of disability worldwide. However, the success of therapeutic intervention is suboptimal, due to our limited understanding of the underlying molecular mechanisms. Mitochondria have recently arisen as key players in regulating stress responses. Here, we show mitochondria-related changes after chronic stress. To assess potential stress-relieving effects of mitochondrial targeting, we are characterizing new custom-synthesized, mitochondria-targeted antioxidants. We focus on a synthetic mitochondria-targeted antioxidant, based on hydroxytyrosol, a phytochemical with antioxidant properties. We have optimized dosages for in vitro and in vivo administration and explored the impact in mitochondrial function, oxidative stress and physiology in human cell lines and chronically treated mice. Pharmacological manipulation of mitochondrial function has the potential to alleviate stress-related symptoms and pave the way for identifying novel pharmacological targets and personalized treatments for psychiatric disorders.

34. Interplay of hippocampus, amygdala and medial prefrontal cortex in the stress response and behavior: insights from a mouse model of maternal separation

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It is well established that Early-Life Stress affects the physiological function of the Hypothalamus-Pituitary-Adrenal (HPA) axis, thus challenging the neuroendocrine response to any future stressful stimuli, and possibly the homeostasis of the organism. Adverse early-life effects may act via epigenetic mechanisms, altering the gene expression of important molecules that are related to stress tolerance. Glucocorticoid and Mineralocorticoid receptors (GR, MR) are key mediators of the stress response, regulating the expression of their target genes. In addition, a balanced activation of GR and MR is necessary for the HPA axis response and maintenance of homeostasis. Following a protocol of maternal separation and early weaning (MSEW) we have examined mouse stress responses by behavioral tests, namely Open Field (O.F.T) and Elevated Plus Maze (E.P.M). Furthermore, we have estimated the average mRNA levels of genes associated with HPA function (Nr3c1, Nr3c2, Tsc22d3) as well as of neuroplasticity mediators (Bdnf) in three stress-related brain regions, namely hippocampus, amygdala and medial prefrontal cortex. Our observations indicate that individual differences regarding the stress response should be taken into account when assessing behavioral traits underlying stress-susceptibility, as the interplay amongst genetic background and epigenetic modifications can differentially influence gene expression in different brain areas, thus altering the neural circuits that mediate the adaptive responses to stress, and generating stress-vulnerable phenotypes.

35. Mechanical stimulation of carotid baroreceptors reduces cerebral blood flow

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The arterial baroreceptors are part of a medullary reflex, which through compensatory adjustments of the heart and vasculature maintains blood pressure within a limited range. Baroreceptor stimulation has also been reported to inhibit higher brain areas. This study investigated effects of experimental baroreceptor stimulation on cerebral blood flow. Carotid baroreceptors were stimulated by neck suction (-50 mmHg) in 15 healthy subjects (8 trials of 10 s duration); neck pressure (+8 mmHg) was used as somatosensory control condition. Blood flow velocities in middle and anterior cerebral arteries (MCA and ACA) of both hemispheres were assessed by functional transcranial Doppler sonography (fTCD). Heart rate and blood pressure were also recorded. Neck suction led to reductions in bilateral MCA and ACA flow velocities. This accompanied the expected heart rate, systolic and diastolic blood pressure decreases; the blood pressure decrease correlated positively with the ACA flow velocity decline. The reduction of cerebral blood flow during baroreceptor stimulation may reflect reduction of activity and hence reduce requirement for blood flow in structures like the insula, cingulate and medial prefrontal cortex. These structures are closely linked to the cardiovascular centers in the medulla and are located in the MCA and ACA perfusion territories. As indicated by the correlations between blood pressure and ACA flow velocities, blood pressure decline may further contribute to the cerebral blood flow reduction during baroreceptor stimulation. Central nervous inhibition arising from the baroreceptors may prevent cortical overarousal and limit pain and negative affect during periods of stress-related blood pressure increase.

36. Effects of chronic cannabinoid exposure during adolescence and adulthood on the reward-facilitating effects of cocaine in adult rats

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Marijuana is by far the most commonly abused illegal substance in the world. According to research findings, chronic exposure to cannabis and synthetic cannabinoids during adolescence increases the risk of using other addictive substances in adulthood. Along these lines, the purpose of the present study was to determine the effects of chronic administration of the cannabinoid receptor agonist WIN 55,212-2 during adolescence and adulthood on the reward-facilitating effects of cocaine in adult rats. Male Sprague-Dawley rats were divided into two groups and received chronic WIN 55,212-2 (0, 0.1, 1 mg/kg, i.p.) injections, one group in adolescence (postnatal day 30-51) and the other in adulthood (postnatal day 65-86). Rats were then assessed for potential alterations of cocaine's (0, 2.5, & 10mg/kg, i.p.) reward-facilitating effects by using the curve-shift variant of the intracranial self-stimulation (ICSS) procedure. Rats that were exposed to the 1 mg/kg WIN 55,212-2 dose in adolescence showed increased baseline ICSS threshold compared to the control group and the group that received the 0.1 mg/kg dose of WIN 55,212-2. Moreover, the group of 1 mg/kg of WIN 55,212-2 showed decreased baseline asymptotic rate of responding, compared to the control group both in adolescence and adulthood. Administration of cocaine (2.5, and 10 mg/kg, i.p.) to drug-naïve rats induced a dose-dependent decrease of ICSS threshold in both experimental groups. Furthermore, the reward-facilitating effect of 2.5 mg of cocaine was increased in rats that were exposed to 1 and 0.1 mg/kg WIN 55,212-2 in both adolescence (greater effect) and adulthood (minor effect), compared to the control group. Overall, the present results reveal that chronic cannabinoid exposure can increase the reward-facilitating effects of other addictive substances, such as cocaine, which may explain some cases where cannabis is a gateway drug for other, more harmful, addictive substances.

37. Psychotic–like behaviour, in a rat model of Parkinson's disease based on human alpha-synuclein overexpression

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Parkinson's Disease (PD), in spite of being known as a motor-impairing neurodegenerative disease, has numerous non motor symptoms. Parkinson's disease Psychosis (PDP) is the most challenging non-motor symptom (50% incidence), affecting both the prognosis and the progression of the disease and dramatically reducing patients' quality of life. PDP is commonly perceived as a complication of dopamine therapy used to treat motor symptoms. However some evidence indicates that PDP may precede motor symptoms and manifest in the absence of PD medications. In addition, there are no safe therapeutic options and no specific animal models available to study this condition.
Herein, we used in-house bred humanized alpha-synuclein BAC transgenic rats created by Nuber et at (2013). We initially evaluated locomotor activity in an open field, prepulse inhibition, striatal, cortical and olfactory dopamine levels with HPLC and electrochemical detection and markers of dopaminergic activity with Western immunoblotting. Locomotor activity was assessed following pharmacological (haloperidol - typical antipsychotic -, clozapine - atypical antipsychotic -, pimavanserin - atypical antipsychotic for PDP -, D-amphetamine, SCH 23390 - D1 receptor antagonist - and ropinirole - D2 receptor agonist), or viral (stereotactic injection of human alpha-synuclein silencing microRNA in the ventral tegmental area) manipulations.
We show that compared to their wild type littermates, huAS BAC rats exhibit increased striatal dopamine levels and locomotor hyperactivity from the early age of 3mo and a prepulse inhibition deficit at 12 mo. Their hyperactive phenotype is reversed following the administration of haloperidol, clozapine, SCH 23390 and downregulation of human alpha-synuclein along the nigrostriatal/mesolimbic tract
These data support a connection between aberrant human alpha-synuclein expression and a psychosis-like phenotype in huAS BAC rats. Fascinatingly this in vivo data may have analogies to PD, where recent findings suggest that a premotor hyperdopaminergic state may occur.

38. From adolescent to aged: the effect of aging on anxiety-like behavior and acetylcholinesterase activity in female mice

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Age–related changes have been associated with behavioral manifestations and cholinergic system alternations. The aims of the present study were to investigate anxiety-like behavior in 4 age groups of female mice as they get older and determine the acetylcholinesterase (AChE) activity in specific brain regions (cerebral cortex, striatum and hippocampus). The mice were divided into the following groups: a) adolescent [1-month-old (m.o.)], b) adult (3-4 m.o.), c) middle-aged (11-12 m.o.) and d) aged (15-16 m.o.).Anxiety-like behavior was assessed by using two behavioral tests, the open field and the elevated plus maze test. These tests are based on the animals' fear/anxiety for the unknown environment (open field and height), despite their tendency to explore it. The results showed that the adolescent group appeared to be highly anxious in comparison to adult group. Moreover, middle-aged and aged groups were more stressed than the adult group. The AChE activity was determined in both salt-soluble (SS-AChE) and detergent-soluble (DS-AChE) in the three brain regions, by using Ellman's colorimetric method in all ages. The results exhibited both SS- and DS-AChE activity in adolescent, middle-aged and aged group to be significantly lower than the adult group, in all three brain regions. Moreover, correlation tests were performed between the two behavioral tests and the AChE activity (SS and DS) and no significant correlation was occurred between them. Conclusively, the comparison between adolescent group and adult group, revealed an initial anxiety reduction, that was followed by an age-dependent anxiogenesis. This increase was followed by a reduction of AChE activity in both SS and DS fractions in all three brain regions studied.

39. Cannabidiol affects subanesthetic ketamine – induced pattern on motor activity and specific neurobiological alterations

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Introduction: Cannabidiol (CBD) is a non-addictive compound of cannabis which exerts its actions mainly through the endocannabinoid system. Ketamine is a non-competitive NMDA receptor antagonist, known for its anesthetic properties. Ketamine also displays prominent central actions when administered in subanesthetic doses, including the psychotogenic action.
Aim: The purpose of this study was to investigate the modulatory role of CBD pretreatment on ketamine –induced behavioural effects and specific alterations in neurobiological parameters related to the endocannabinoid and the glutamatergic system.
Methods: Adult male Sprague-Dawley rats received low doses of CBD i.p. and 20 minutes later, they were injected with a subanesthetic dose of ketamine or saline. Open field motor activity was recorded for a one hour registration period. Protein expression of specific neurobiological indices related to endocannabinoid function, glutamatergic status and neuroplasticity were assessed, in specific rat brain regions.
Results: CBD treatment at low doses did not alter motor activity, while higher doses induced a mild depressant effect. Interestingly, the low CBD doses did not affect ketamine–induced profile, while higher doses enhanced ketamine–induced motoric effects in terms of horizontal and vertical activity. Protein expression analysis following CBD and ketamine treatment revealed a neurobiological profile which accompanied the aforementioned behavioural changes.
Conclusion: CBD modulated ketamine–induced effects. These findings will potentially contribute to the understanding of the pharmacological profile of CBD, an agent with antipsychotic potential, and of the functional interplay between endocannabinoid and glutamatergic systems.

Keywords:
Cannabidiol, ketamine, rats, motor activity, behavior, glutamate, neurobiological indices

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40. Sexual dimorphic effects of restraint stress in the limbic system: The role of corticoptropin releasing factor receptors

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Prefrontal cortex (PFC) is a brain region implicated in modulating and controlling cognitive functions and behaviour. Serious deficits in PFC function are associated with fundamental elements of schizophrenia, while a combination of genetic and environmental factors contributes to the manifestation of symptoms that exhibit intense sex differentiation. Stressful environmental stimuli directly affect brain function and may be related to the cause of the disorder. Stress response involves the release of corticotropin-releasing factor (CRF) from the hypothalamus and appears to exhibit significant differences between female and male individuals. Aim of this study, was to investigate the effect of CRF on physiology of PFC, namely on long-term synaptic potentiation (LTP), in adult female and male mice as well as in female and male mice of the MAM neurodevelopmental model of schizophrenia. Electrophysiological recordings were performed in layer II of PFC in brain slices in the presence or absence of a-Helical CRF9-41, a CRF receptor antagonist, as well as in brain slices of female MAM and control mice that had undergone chronic administration of the CRFR1 antagonist, antalarmin. The presence of a-Helical CRF9-41 reduced the rate of potentiation of synapses in male mice, while causing an increasing tendency in females. Variations were observed in synaptic potentiation between the two sexes. Specifically, it appears that LTP is diminished in male MAM-mice compared to control mice, as opposed to female-MAM mice, that showed no differences. Chronic administration of antalarmin on control female mice significantly increased the levels of synaptic potentiation, while in MAM-female mice it prevented the emergence of synaptic plasticity. Activation of CRF receptors appears to have a sexually dimorphic effect on PFC, which may support the observed differences between sexes in response to stress and in the manifestation of stress-related disorders, such as schizophrenia.

41. Effects of the administration of neurotrophin BNN-20 in neurogenesis in the «weaver» mouse model of Parkinson's disease.

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The "weaver" model is the only genetic model that mimics the neuropathology of Parkinson's disease. Homozygous "weaver" mice display abnormalities in the cerebellum, hippocampus and in spermatogenesis, as well as spontaneous neurodegeneration of dopaminergic neurons in the pars compacta of the substantia nigra (SNpc). Synthetic micro-neurotrophin BNN-20 exhibits pleiotropic neuroprotective activity on the dopaminergic neurons of the "weaver" SNpc, not only by significantly inhibiting but also by reversing neurodegeneration. The purpose of this study was to investigate the beneficial effects of BNN-20 on the generation of new dopaminergic neurons in the SNpc of the «weaver» mouse and their origin. Immunohistochemical staining was performed in the SNpc in order to quantify mature dopaminergic neurons (TH+) as well as the population of FOXA2+ (immature and mature) dopaminergic-lineage neurons as a way to assess the activation of developmental neurogenic pathways. The subependymal zone (SEZ) of the ventricular system is one of the major neurogenic areas of the adult brain and the effect of BNN-20 on SEZ neurogenesis was assessed by immunohistochemical quantification of neural progenitor cells (Dcx+) and of cell proliferation (PCNA / Ki67+). Finally, a protocol for labeling SEZ neural stem cells and their progeny via the intracerebroventricular injection of the lipophilic dye DiI was evaluated. This protocol was used in order to investigate the potential contribution of SEZ-derived cells in neurogenesis in SNpc.

42. The effect of eye and head position on reading speed in a simulation of prosthetic vision

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Inherently automatic and critically important in accurately perceiving our external world, visual scanning has been one of the greatest challenges for artificial vision, splitting the field into two broad segments of devices, those which adapt stimulation delivered to the visual system based on gaze position, so that the focal point can be steered with shifts in eye position, and those that do not, and are usually steered by head motions. Because the early visual pathway and the first stages of cortical processing are retinotopically organized, we hypothesized that prostheses which respond to normal gaze changes, including shifts in eye position, would perform better on perceptual tasks than those that are only head-steered. To test this hypothesis, the present study rapidly and non-invasively switched focal compensation modes, by employing a simulation of artificial vision from a thalamic visual prosthesis. Normal, sighted subjects ($n = 23$) read simple sentences under conditions of Full-gaze compensation (including eye position), and Head-only viewing (that ignored eye position) in a simulation with 2000 phosphenes. Results showed that participants were immediately able to read under Full-gaze compensation at an equivalent visual acuity of logMAR 1.0, but were nearly unable to perform the task under Head-only compensation despite interventional coaching to hold their eyes still and only move their heads. Eye movements under the Head-only condition were highly interfering, as the phosphenes did not respond in an intuitive way. For the largest text tested, logMAR 1.3, the Full-gaze condition provided about 50% of normal reading speed with 100% accuracy, whereas the Head-only condition provided less than 5% of normal speed with below 15% accuracy. We conclude that gaze-compensated prosthesis designs are likely to produce substantially better outcomes for implant recipients than alternatives that are not sensitive to eye movements. Supported by Fulbright Foundation, Haseotis Foundation, Wood Foundation.

43. Interactions between ventrolateral prefrontal cortex and visual area V4 during cued visual attention and working memory

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Selective attention is central to cognition and adaptive behavior. Attention can be allocated to specific locations or features (e.g. color) based on external instructions or internal goals maintained in working memory. To study the neural mechanisms of attentional guidance, we conducted simultaneous, extracellular electrophysiological recordings from ventrolateral prefrontal cortex (vlPFC) and visual area V4 of the non-human primate. We investigated how information about the location and color of the behaviorally relevant stimulus is represented in neural activity of these areas during the cue, delay and visual array presentation periods of a covert attention task. The instruction about where or what to attend was encoded by the majority of vlPFC neurons early on, in the cue period and information about the future target was maintained throughout the delay period. Unlike vlPFC, only a minority of V4 neurons encoded the feature or the location of the future target in the delay period. Furthermore, during visual array presentation, spatial attention signals emerged earlier in vlPFC. These results suggest that vlPFC is a source of signals that bias activity in V4 in favor of behaviorally relevant stimuli. Synchronization of activity between vlPFC and V4 was enhanced in theta frequencies during the cue and delay epochs whereas enhanced synchronization in the beta and gamma range with spatial attention was prominent during the presentation of the visual array. Directed influences in the vlPFC-V4 network estimated by Granger causality analysis revealed that across areas interactions in the theta frequency band originated from vlPFC, whereas interactions in the beta and gamma frequency bands originated from V4. These findings suggest fundamental differences in frequency-specific communication principles that involve the prefrontal cortex compared to those previously proposed for visual areas.

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44. Potential users of visual prosthesis: their views on the process and expected outcomes

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Patient views toward visual prosthesis implantation, testing, along with perceived risks and benefits about neuro-technology remain an unexplored field, and what is known mostly concerns cortical devices [1, 2]. With three prosthetic devices approved for clinical trials and use [3, 4, 5], data directly from affected individuals could maximize benefits as early as the enrolment phase. We, thus, investigated blind individuals' perspectives in Greece about implanted vision restoration devices, in a pilot questionnaire study. This study was on perceived benefits and risks, desired visual outcome, motivation and advice-seeking. It is part of a larger project to combine qualitative and quantitative data from potential patients. One of our most important findings is that the average assessment of perceived dangers in artificial vision is higher than for perceived benefits. We, also, found that benefits for the thalamic approach are deemed to be greater than those for the retinal approach; however, for dangers, the cortical approach is considered to be the most dangerous of all, followed by the thalamic and then retinal approaches. We also found that the particular causes of blindness, residence and age of onset may affect the assessment of the different approaches, primarily for the retinal benefits and the thalamic dangers. This study contributes to theoretical understanding of visually impaired individuals' views, and can maximize benefits for both patients and clinical investigation and treatment through creating a psychosocial protocol that refines the selection of candidates [6] for a neuro-prosthetic device.

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45. Passive learning in simulated artificial vision: a proposed study

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Simulations of artificial vision are often used in visual prostheses research in order to guide device design, with normal-sighted subjects performing psychophysical tasks (e.g. recognizing, grasping, placing, wayfinding [1-3]) to give insight on capabilities. As we have previously reported for the task of reading [4], daily use of a simulated device over a period of 40 sessions improved population-level effective acuity by a factor of logMAR 0.3 (equivalent to a doubling of visual acuity). However, our previous research, along with the rest of the field, overlooks the likely more frequent post-implant experience of passive activities. To address this gap, we now therefore seek to investigate visual perceptual learning through longitudinal passive experience by tracking the artificial vision acuity of normally-sighted subjects after they watch videos through a simulated prosthesis, hypothesizing that a similar level of improvement will be found for the same total amount of exposure as in our previous study with an active task. We will create a virtual reality setup that simulates how vision would appear through a visual prosthesis device with 500 phosphenes (bright percepts elicited upon stimulation in areas across the visual pathway). On a daily basis, subjects will watch single half-hour episodes of classic American television. Periodically, we will measure their visual acuity through the simulation with a standardized reading task (MNREAD) similar to the one used in our previous report. Given that perceptual learning is found to transfer across retinal locations, improves discrimination in neighbouring tasks and transfers across modalities, we expect to find similar results of strong improvement with experience, which will be critically important to design rehabilitation strategies for all eventual recipients.

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46. Precision pupillometry with spatially selective stimulus

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We here present a light stimulus source that can advance pupillometry to a higher level of control and precision, as current pupillometers lack spatial selectivity in illumination. The developed light source is based on a laser light beam having a cross section consisting of discrete pixels, allowing for an arbitrary pattern of illuminated pixels. Both the illumination pattern and the photon number per pixel per unit time are computer controlled, offering simple and unsupervised scanning of these parameters.
Moreover, infrared light exactly superimposed on the stimulus pattern can be used for acquiring exact information on the illumination geometry on the pupil. We present pupillometry measurements demonstrating the potential of this source to offer a wealth of information on vision and brain function.

47. The principle of inverse effectiveness and its interaction with the temporal rule of multisensory integration

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A fundamental principle of multisensory integration (MI) is that of inverse effectiveness (IE; maximum multisensory gain is attained when the unisensory inputs evoke weak neuronal responses). Previous behavioural IE investigations have yielded conflicting findings regarding the levels of noise that lead to maximum multisensory gain and are mostly limited to speech stimuli with artificial auditory degradations. We examined the behavioral validation of IE using naturalistic degradation in both sensory streams of audiovisual speech. Participants were asked to identify three syllables (/ba/, /fa/, /tha/) presented visually, aurally, or audiovisually at different levels of noise/noise combinations. Analyses showed that according to the: a) Contrast index: gain was minimized for maximal degradations of the auditory stream independent of visual noise only for /ba/; b) Absolute Difference (in %) index: gain was maximized for high auditory and low visual noise combinations for /ba/, /fa/, while in /tha/ for low noise in both streams; and c) MI and Absolute Difference indices: gain was minimized for auditory stream of the highest noise only for /ba/. Therefore, IE was only validated for specific indices and stimuli. Thus, our findings along with previous studies place the extension of IE from neurons to behavior in question. Utilizing the high/low gain multisensory combinations of Exp.1, we examined the interaction of IE with the temporal rule of MI (i.e., synchronous or close in time stimulation has higher integration likelihood). In Exp.2, the audiovisual speech stimuli were presented at different stimulus onset asynchronies and participants completed temporal order judgments. Analyses revealed a higher asynchrony tolerance for high gain as compared to low gain audiovisual pairs. Overall, these findings suggest that the magnitude of multisensory gain and width of the temporal window of integration interact as a function of the effectiveness levels of the auditory and visual streams of the speech event.

48. The role of neuronal DEG/ENAC ion channel family members in organismal stress responses

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Organisms receive and process external information to adapt their behavior to an ever-changing environment. The molecular mechanisms that underlie neuronal integration of sensory information towards motor adaptation are not fully understood. Dopamine signaling is involved in several forms of behavioral plasticity, in reward processing and in the control of motor output. In *C. elegans*, the functionality of dopamine pathway can be easily assessed by monitoring a specific locomotory response to environmental food availability cues, termed basal slowing. By implementing molecular genetic manipulation technics and behavioral assays we identified three degenerin ion channel proteins to participate in sensory integration through modulation of the dopaminergic pathway. Utilizing advanced imaging technics, we found that degenerins DEL-2, DEL-3 and DEL-4 are expressed in mechanosensory, chemosensory and motor neurons. These ion channel proteins modulate basal slowing response and respond to gustatory stimuli. They affect neurotransmission efficiency of dopaminergic and motor neurons, even though they do not adopt a synaptic localization pattern. Moreover, they act through the dopamine receptors, DOP-1, DOP-2 and DOP-3, mainly DOP-2. We also demonstrated that PKC-1, but not PKC-2, plays a crucial role downstream of these receptors. Furthermore, degenerin effects are largely influenced by stress conditions, such as heat and starvation. Notably, the stress response transcription factors DAF-16/FOXO and SKN-1/Nrf couple degenerin ion channel function to environmental conditions and behavioral output. Our results provide new insights into how sodium channels participate in the mechanism of neuronal integration.