27TH MEETHING OF THE HELLENIC SOCIETY FOR NEUROSCIENCE

DRAFT ABSTRACT BOOK
(final version will be uploaded after the meeting)

ORAL & POSTER PRESENTATIONS

ORAL PRESENTATIONS

STUDY OF THE NEUROPROTECTIVE EFFECTS OF TNFR1 AND TNFR2 *IN VIVO* IN TRANSGENIC MICE WITH CONDITIONAL GENE TARGETING IN NEURONS, USING EXPERIMENTAL MODELS OF MULTIPLE SCLEROSIS

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TNF is a pro-inflammatory cytokine that is up-regulated in the CNS in neurodegenerative disorders like Multiple Sclerosis. However while non-selective TNF inhibitors are effective treatments for autoimmune diseases, they exacerbate disease in MS. TNF is produced in a transmembrane form (tmTNF), which acts through the TNF receptors I and II (TNFR1 and TNFR2) and a soluble form (solTNF), which acts only through TNFR1. The two forms of TNF exert opposing beneficial and deleterious effects in the CNS, implying differential receptor functioning and different roles in neurodegenerative diseases. The aim of this study was to conclusively examine the complex and controversial roles of neuronal TNF and TNF receptors in CNS pathology. To achieve this, conditionally gene-targeted mice in which TNFR1 or TNFR2 are selectively deleted from glutamatergic neurons (nTNFR1KO and nTNFR2KO mice respectively) were developed by the Cre/loxP system (mice with conditional TNFR1, TNFR2 alleles kindly provided by Prof. G. Kollias). First the mice were characterized for tissue specificity of the TNFR1 and TNFR2 deletions. Receptor depletions were observed only in glutamatergic neurons and not other tissues while mice carrying the loxP-flanked TNFR1 (TNFR1f/f) and TNFR2 (TNFR2f/f) alleles expressed normal levels of both receptors and were used as controls. Mice were also characterized in levels of receptor expression by qRT-PCR using RNA from cortical neuron culture and tissues. Next, two experimental MS models, experimental autoimmune encephalomyelitis (EAE) and cuprizone-induced demyelination (CPZ) were examined to see whether neuronal depletion of TNFR1 or TNFR2 alters the clinical symptoms or development of neuropathology. EAE was induced by immunizing mice with MOG35-55. nTNFR1KO mice showed a trend towards delayed EAE onset compared to TNFR1f/f, suggesting that TNFR1 might play a role in promoting EAE onset, while nTNFR2KO mice showed markedly reduced incidence of EAE compared with TNFR2f/f, but animals that showed symptoms developed full-blown EAE. In the cuprizone model, disease is scored by neuropathology and immunohistochemistry in the corpus callosum. In this model we studied demyelination and remyelination (LFB, CNPase immunostaining), neurodegeneration (APP immunostaining) and microgliosis (Iba1 immunostaining). Similar to EAE, nTNFR1KO mice were significantly protected from demyelination and inflammation compared with controls, further supporting a deleterious role for neuronal TNFR1 in demyelinating disease. Surprisingly neuronal TNFR2, which is strongly neuroprotective *in vitro*, had no effect on the development of MS-type disease *in vivo*. Overall, selective inhibitors of TNFR1, and its ligand solTNF, rather than TNFR2 agonists might provide neuroprotective effects in CNS demyelinating diseases.

SEX SIMILARITIES AND DIFFERENCES IN THE MAM MODEL OF SCHIZOPHRENIA IN MICE

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The goal of our study was the establishment of the neurodevelopmental MAM model of schizophrenia in mice and the study of sex differences in their 'schizotypic-like' phenotype. Pregnant mice were injected with 26mg/kg of Methylazoxy-methanol acetate (MAM) or saline, on GD 16 or 17. Male and female offspring (MAM- or saline-treated) were examined during adulthood (> 90-days old) to establish schizotypic-like characteristics. Additionally, for MAM16 treated mice cognitive function was assessed with the contextual fear-conditioning paradigm, long-term potentiation (LTP) by recording field excitatory postsynaptic potentials (fEPSPs) in Hippocampus (HPC) and Prefrontal cortex (PFC) and trait anxiety was measured using the elevated plus maze (EPM) task. The results of the schizotypic-like phenotype showed that female MAM-treated mice exhibited enhanced hyperlocomotion and stereotypic behavior compared to saline-treated mice, after acute administration of MK-801, significant reduction of the horizontal length of HPC, a trend towards thinning of PFC and reduced Parvalbumin (PV) expression in the PFC, but not in dorsal HPC [1]. Male MAM- treated mice, showed decreased Prepulse inhibition of the acoustic startle reflex (PPI), statistically significant thinning of the PFC, a trend towards reduced HPC size, as well as decreased PV expression in the PFC compared to saline-treated mice. Upon examining the cognitive function, both male and female MAM-treated mice displayed impaired contextual fear memory, suggesting a possible deficit in the underlying neurophysiological mechanisms of HPC. Indeed, the fEPSPs recorded in the CA3-CA1 synapses of HPC revealed decreased expression of LTP, for both sexes, whereas only male mice showed impaired LTP expression in PFC. In addition, EPM task revealed high levels of trait anxiety in female MAM-treated mice, but decreased levels in male MAM-treated mice. Our results demonstrate that both male and female mice, prenatally treated with MAM on GD16, display several core schizophrenia-like deficits, suggesting that the MAM model could be used as a neurodevelopmental model of schizophrenia in mice. We further reveal cognitive deficits and trait anxiety alterations in both male and female mice prenatally exposed to MAM.

Reference

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MODULATION OF APOPTOSIS CONTROLS INHIBITORY INTERNEURON NUMBER IN THE CORTEX

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Cortical networks are composed of excitatory projection neurons and inhibitory interneurons. Finding the right balance between the two is important for controlling overall cortical excitation and network dynamics. However, it is unclear how the correct number of cortical interneurons (CIs) is established in the mammalian forebrain. CIs are generated in excess from basal forebrain progenitors and their final numbers are adjusted via an intrinsically determined program of apoptosis that takes place during an early postnatal window. Systematic gene expression analysis, genetic cell-lineage tracing and phenotypic characterisation of mouse mutants have demonstrated that CI subtypes are specified by distinct region-specific transcriptional programmes operating within progenitor domains of the subpallium. *Lhx6* encodes a LIM homeodomain transcription factor which is specifically expressed by MGE-derived precursors and their derivative CIs expressing somatostatin (Sst) and parvalbumin (Pv).Here we have combined phenotypic analysis, genetic lineage tracing, cell transplantation and chemogenetic activation to query the specific responses of CI sub-lineages to the removal of *Lhx6* activity. We find that *Lhx6* is required to maintain the normal complement of MGE-derived CIs and that reduction of this inhibitory neuronal subpopulation in *Lhx6* mutants results in a surprising increase of *Lhx6*-independent CGE-derived CIs and re-balancing of the total number of CIs. The compensatory increase of CGE-derived CIs is due to a reduction in apoptosis, which can be modulated cell-autonomously by

neuronal excitability during a critical postnatal period. Our results provide fundamental insight into the mechanisms that match the size of CI populations to the physiological requirements of cortical circuits and pave the way for understanding the impact of neuronal activity on cell transplantation-based therapeutic targeting.

REPETITIVE MAGNETIC STIMULATION RESTORES SYNAPTIC EXCITATION/INHIBITION BALANCE OF CULTURED RAT CA1 PYRAMIDAL NEURONS IN A MATERNAL IMMUNE ACTIVATION MODEL OF SCHIZOPHRENIA

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Activation of the maternal immune system during gestation associates with an increased risk for the development of neuropsychiatric disorders in the offspring, such as autism and schizophrenia. However, diagnostic and therapeutic approaches to detect and reverse alterations in brain function in the offspring during a pre-symptomatic stage are currently missing. Here, we prepared enthorhino-hippocampal slice cultures from litters of the well-established polyinosinic–polycytidylic acid [Poly(I:C)] MIA model of schizophrenia(i) to study synaptic excitation/inhibition balance and (ii) to test for the effects of repetitive magnetic stimulation (rMS), which is a non-invasive brain stimulation technique used in clinical practice. Electrophysiological recordings disclose increased synaptic inhibition in CA1 pyramidal neurons of MIA cultures whereas excitatory neurotransmission remains unaffected. This synaptic phenotype is also present in slice cultures prepared from cross-fostered animals, hence confirming that gestational infection triggers increased inhibition. Strikingly, a 10 Hz rMS protocol restores synaptic excitation/inhibition balance in the MIA group without affecting neurotransmission in the controls. These results provide first experimental evidence that rMS is capable of reversing alterations in synaptic excitation/inhibition imbalances under pathological conditions, which may be of considerable clinical relevance in the context of brain diseases associated with alterations in cortical excitability.

GEMC1/LYNKEAS GOVERNS THE COMMITMENT AND GENERATION OF MULTICILIATED EPENDYMAL CELLS IN THE BRAIN

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Neurogenesis persists throughout adulthood in two restricted germinal regions: the subgranular zone (SGZ) of the hippocampal dentate gyrus 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 cilia on their apical surface to propel the cerebrospinal fluid (CSF), thus providing multiple regulatory cues for the generation of neurons within the adult brain. Ependymal cells are generated from radial glial cells (RGs) shortly after birth, however their specification has been established earlier during embryogenesis. Ependymal malfunction has been linked to hydrocephalus, one of the most common neurodevelopmental defects which is characterized by the excessive accumulation of CSF in the brain ventricles. However, the causes of hydrocephalus and the pathways controlling the generation of ependymal cells remain poorly characterized. Findings from our laboratory reveal that GemC1/Lynkeas, a member of the Geminin family, is the earliest known marker of RGs committed to the ependymal cell lineage in the developing mouse brain[1]. Interestingly, RGs upon GemC1/Lynkeas overexpression lose their neural stem cells characteristics and prematurely differentiate into multiciliated ependymal cells. Moreover, GemC1/Lynkeas transcriptionally activates key multiciliate factors, through co-operation with E2F family members, like McIdas and Foxj1[2]. To further characterize the molecular pathway governing the generation of ependymal cells, we generated mice that lack GemC1/Lynkeas expression. These mice exhibit postnatal lethality and severe hydrocephalus. Lack of GemC1/Lynkeas abolishes the physiological formation of the SVZ, which is entirely devoid of multiciliated ependymal cells. GemC1/Lynkeas deficiency also blockskey transcriptional regulators of multiciliogenesis already since embryogenesis. Finally, whole genome transcriptomics analysis of the SVZ revealed that the ablation of GemC1/Lynkeas disrupts the transcriptional program of ependymal cell generation. Summarizing, our study identifies GemC1/Lynkeas as a key component of the molecular cascade regulating not only the proper generation of ependymal cells in the adult brain but also the commitment towards the ependymal lineage. Finally, we propose GemC1/Lynkeas as a novel candidate for the identification of mutations involved in hydrocephalus.

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FUNCTIONAL MAPPING OF THE CA1 HIPPOCAMPAL REGION WITH OPTOGENETICS

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Disentangling neuronal heterogeneity and mapping out functional connectivity is an enormous task as, even in a single brain region, individual neurons differ greatly in their morphology, connectivity and electrophysiological properties. Towards this end, optogenetics offer a powerful approach through the application of genetically encoded voltage indicators (GEVIs). ArcLight, a GEVI that gives one of the largest optical signals, was expressed via viral injections in the hippocampus of transgenic Cre-mice, in either CaMK2a-positive excitatory cells or PV-positive inhibitory cells. Excitatory and inhibitory fluorescent signals were observed at rest and in response to electrical stimulation of the Schaffer collateral axons in CA1 with fast (1kHz) optical recordings in slice preparations. Pharmacological blocking of the $GABA_A$ receptors was contrasted to control conditions. Pre-processing of the data involved reforming video strings through filtering, converting data to comparable scales, and finally removing trends. Data processing comprised elements from complex network analysis such as network construction and topology evaluation and analysis through various network measures and through the structure of minimal spanning trees. Finally, spectral clustering was performed and then hierarchical clustering of the sub-graphs was created, in order to understand their structure. Our results indicate that pyramidal neurons receive inhibition in stratum oriens following SC activation and signal transduction in stratum radiatum. The phenomenon displays features of lateral inhibition. Fast recordings enabled us to recognize a signal "escape route" from the hippocampus within the first 20msec. Pharmacological blocking of $GABA_A$ receptors electrically homogenizes the network resulting in a characteristic decrease of latencies between recognized functional groups. It also increases the overall activation of CaMK2a–positive neurons with a more prominent effect in stratum radiatum. Preliminary analysis of PV-positive interneuron activity reveals $GABA_A$ independent inhibition following SC activation. Finally, blocking inhibition affects the organization of connectivity skeleton after the application of the electrical stimulus. Combining the compelling advantages of genetic encoding and targeted expression and those of optical recordings provide a methodology of unprecedented power to study functional connectivity in the brain.

CHOLINERGIC MODULATION OF HIGHER OLFACTORY CIRCUITS IN ADULT ZEBRAFISH

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Cholinergic neurons project to a wide range of targets and adjust neuronal circuit function through multiple mechanisms in a concerted fashion. In olfactory cortex, it has been proposed that cholinergic input occurs during salient events and triggers the formation of olfactory memories. Models predict that cholinergic input changes the network from a non-plastic "recall" state into a plastic "learning" state by enabling synaptic plasticity at intra-cortical synapses. As a consequence, odor-evoked activity patterns occurring during episodes of cholinergic inputs should become stabilized and possibly enhanced in amplitude. In order to examine effects of cholinergic modulation on odor representations in higher olfactory brain areas we generated BAC transgenic fish expressing channelrhodopsin-2 (ChR2) under the control of the promoter for choline acetyltransferase 1 (ChAT1). Odor-evoked activity patterns were measured in the posterior part of the dorsal pallium (Dp), the zebrafish homolog of the mammalian olfactory cortex, by multiphoton calcium imaging in an ex-vivo preparation of the intact brain. We found that Dp receives diffuse innervation by cholinergic fibers.Optogenetic stimulation of cholinergic inputs alone produced sparse and weak responses of Dp neurons. Pairing of odor stimulation with optogenetic activation of cholinergic stimulation had, on average, only small effects on the mean amplitude of odor responses. However, after pairing, correlations between activity patterns evoked by repeated applications of the paired odor were enhanced, indicating that activity patterns became more stable. This effect was long-lasting. These results support the hypothesis that cholinergic modulation promotes the formation of olfactory memory in Dp and provides a starting point to investigate the underlying mechanisms.

DROSOPHILA TAU REGULATES HABITUATION & LONG-TERM MEMORY

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Fly models of microtubule-associated Tau-related disorders rely on ectopic expression of human transgenes. So far however, very few studies have addressed the role of the endogenous dtau proteins in the normal function of the adult nervous system. Although a recent study has revealed that the absence of dtau has no major functional impact on flies, the effects of its removal on adult behavioral plasticity, if any, were not investigated. Here, we examined the distribution of dtau in the adult fly brain using immunohistochemistry and revealed that the protein is mainly distributed in the adult fly nervous system, and particularly exhibits specific staining within the Mushroom Body (MB), the major structure involved in associative and non-associative behaviours in flies. Interestingly, dtau appears conspicuously present MB lobes as well as in the calycal area. By attenuating dtau expression through the use of either two different *dtau* mutant alleles or a dtau shRNA construct specifically in adulthood using the GAL80ts system, we demonstrate that dtau down-regulation specifically impairs foot-shock habituation, a form of non-associative behaviour, and enhances associative long-term (LTM) olfactory memory. Importantly, associative 3 min short-term (STM, "learning") and Anaesthesia-Resistant Memory (ARM) are not affected. Moreover, concentrating our efforts on defining the minimal subset of neurons where attenuation of dtau results in resistance to shock habituation and LTM enhancement, we found that both phenotypes specifically depend on the MB intrinsic neurons. Finally, molecular analyses using proteomic approaches illuminate its role as a regulator of proteins involved in synaptic plasticity and protein translation. In conclusion, our results strongly suggest a novel role of dtau in behavioral plasticity in flies.

A TALE OF TWO TREES: MODELING APICAL AND BASAL TREE CONTRIBUTION TO L2/3 V1 PYRAMIDAL CELL ORIENTATION SELECTIVITY

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Pyramidal neurons, a mainstay of cortical regions, receive a plethora of inputs from various areas. Afferent synapses are received by either the apical or basal dendritic trees, which are morphologically distinct. Both trees differentially contribute to the somatic response, although their exact functional roles remain unclear. Inputs to apical dendrites are integrated en-masse at the apical trunk and propagate to the soma. Basal dendrites, on the other hand, branch out from the soma, with inputs being integrated semi-independently. Thus, these trees define distinct anatomical and possibly functional sub-units. To assess the latter, we modeled the complex response pattern of the L2/3 V1 pyramidal neuron to spatially tuned synaptic input. Our goal was to elucidate the contribution of each tree to the response pattern of the neuron, namely its orientation tuning curve. Towards this goal, we created a morphologically detailed computational model of one such cell in the NEURON simulation environment. The model was validated using electrophysiological data recorded *in vitro* and *in vivo*. We investigated the role of dendritic integration at the basal and apical trees, and its contribution in shaping cell responses. Results show that somatic action potentials are generated only when input coincides bilaterally, as unilateral stimuli are unable to evoke an adequate response at the soma. In addition, given equal synaptic drive, the responses of the neuron appear to be initiated by the apical tree, as its dendritic spiking activity temporally precedes somatic spike-like activity. Finally, basal tree activity, in the form of either depolarization or spiking, is essential for producing somatic activity, albeit occurring in close temporal proximity to the somatic spike-like events. This model provides evidence for distinct computations taking place in the basal and apical trees of the neuron.

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BEHAVIORAL AND NEUROCHEMICAL PROFILE OF ALPHA-SYNUCLEIN TRANSGENIC RATS: INSIGHTS INTO PARKINSON'S DISEASE PATHOPHYSIOLOGY

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α-Synuclein (a-syn) is a presynaptic neuronal protein linked genetically and neuropathologically to Parkinson's disease (PD). PD is associated with severe motor symptoms, however, they are often accompanied and even preceded bynon-motor symptoms (NMS) such as olfactory deficits, cognitive decline, anxiety and depression that significantly influence the quality of life of patients. Furthermore, beyond nigrostriatal dopaminergic dysfunction, there is a widespread deregulation of other neurotransmitters. The aim of this study was to evaluate the progression of NMS in male and female a-syn BAC transgenic rats, generously provided to us by Olaf Riess, Tubingen University(Nuber et al. 2013),using a behavioral test battery [open field (locomotor activity), elevated plus maze (anxiety), buried pellet test (olfaction), prepulse inhibition (sensorimotor gating), Morris water maze (learning & memory), forced swim test and sucrose preference (depressive-related behaviors)] and neurochemical analysis (catecholamines, glutamate and GABA) with HPLC and electrochemical detection at 3, 6, 9, 12 and 18 months. BAC males exhibit reduced food consumption and body weight, increased locomotor activity and reduced anxiety starting at 3 months. Furthermore, both males and females exhibit an early onset olfactory deficit

and depressive-like phenotype at 3 months, a spatial learning deficit at 6 months and sensorimotor impairment at 12 months. Neurochemical correlates indicatedopaminergic dysfunction in the striatum and glutamatergic and GABAergic dysfunction in the amygdala and hippocampus, possibly related to observed locomotor hyperactivity, reduced anxiety and cognitive impairment, respectively. a-syn BAC rats provide a valuable tool to evaluate a-syn's role in the pathogenesis of NMS in PD.

LONGITUDINAL LEARNING: EXPERIENCE-DRIVEN CHANGES IN PERFORMANCE ON A SIMULATED PROSTHETIC VISION READING TASK

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In simulations of artificial vision, various psychophysical tasks have been administered for examining performance and its development through training over different periods of time. Practice-related learning effects have been found with eccentric reading for human subjects and with letter recognition tasks for non-human primates. However, the time course of learning to adapt to phosphene vision in a more complex task such as reading, has yet to be addressed in humans. Here, as part of the development of a thalamic visual prosthesis, we show the beneficial aspects of training to read under a simulation of artificial sight, over a period of at least eight weeks and with the use of a gaze contingent architecture. Specifically, we investigated how normal, sighted subjects adapt to phosphene vision by examining the learning process of reading simple sentences out loud in 20-minute sessions on a daily or a near-daily basis in a task based on the MNREAD visual assessment. Eight subjects practiced reading novel sentences presented on a LCD monitor and updated with a real-time gaze contingent mechanism that reveals more detail at the point of regard, to simulate a thalamic visual prosthesis. Sentences were presented at five font sizes (logMAR 1.0-1.4) through three center-weighted phosphene patterns (2000, 1000, 500 phosphenes) or a control condition with text in unadulterated form. Reading accuracy (fraction of words read correctly) and reading speed (number of correctly read words per minute) was measured across sessions. We found that through training, reading speed exhibited an increase equivalent to doubling of phosphene count. Most strikingly, the hardest condition (smallest font size through 500 phosphenes), while initially illegible, proved highly usable after training. Consistent with neuroplastic changes being function-related, we found that gaps in the daily training plan lead to slower learning on reading speed, but, surprisingly, not reading accuracy. Taken together, our results are promising for the clinical design of a thalamic prosthesis and essential for designing post-implant rehabilitation strategies. Our findings suggest that a pattern with 500 phosphenes (130 in central vision) can be indeed usable for reading, an important part of daily living.

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***EX VIVO* ANALYSIS OF CORTICAL NETWORKS IN NORMAL AND PATHOLOGICAL BRAIN STATES**

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The mammalian cerebral cortex is a highly complex yet modular structure that exhibits distinct modes of activation, reflecting the state and processing capabilities of the brain. Spontaneous network activity during quiescent states is a ubiquitous characteristic of cortical networks and has an active role in information processing: through its effect on neuronal conductances and membrane potential it provides a mechanism for modification of neuronal excitability. In this way it is thought to provide the neuronal context within which external signals are processed and interpreted. Among the many patterns of spontaneous cortical activity that have been reported, recurring epochs of self-maintained depolarizations (Up states) are unique in several respects: they are present throughout life, in all mammalian species and cortical areas examined; they are observed *in vivo*, during quiescent states, but also *in vitro*, in acute and even organotypic slices, indicating they are a robust phenomenon and that mechanisms are in place to actively maintain them. Importantly, spontaneous Up states are intrinsic to the cortex and occur naturally, as a result of the recurrent connectivity and the membrane and synaptic properties of its constituent elements, and as such they are thought to represent the *default activity of cortical networks*. In our lab we have developed the technology to record this activity in mouse brain slices throughout the lifespan and under diverse experimental conditions. We find that spontaneous Up states are systematically modified by age, cortical region and sex, and can therefore serve as an index of the functional maturation and differentiation of the cerebral cortex. By combining recordings from wildtype and knockout animals with pharmacological manipulations, we also reveal a clear and previously disputed effect of nicotinic signaling in cortical synchronization. Ongoing experiments focus on the neuronal communication within local microcircuits using data from multi electrode arrays and graph theory analysis to investigate how functional connectivity patterns are altered during different states of endogenous cortical activity.

EFFECTS OF WORKING MEMORY TRAINING ON COGNITIVE FLEXIBILITY IN BOTH MAN AND MOUSE

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The present study aims to investigate the effect of working memory training (WMT) on cognitive flexibility in both humans and mice. The human study included forty healthy male participants, who were divided in: a) control group (no cognitive training), b) partially adapted group (administration of an executive working memory task, the letter number sequence (LNS), up to the strings with three digits, for six consecutive days) and c) fully adapted group (for six consecutive days participants were administered the entire LNS test). Following training, all participants were tested in another cognitive flexibility task, the ID/EDS. Results showed that the fully adapted group had improved performance on the ID/EDS test, since they made fewer errors and fewer attempts to complete the stages of the test, compared with both the partially adapted *(all p values <0.005)* and the control *(all p values <0.05)* groups, who did not differ between each other *(all p values >0.2)*. Using a similar experimental design, the animal study examined the effect of WMT (utilizing the delayed alternation task in the T- maze) on cognitive flexibility. Male (B6) mice (8 months of age) are divided into the naïve (mice remained in their home cage), the non-adaptive (mice learned to alternate arms but without any delays) and the adaptive group (mice performed the alternation procedure with increasing delays). WMT lasted for 9 days, and 2 days later, all mice undergo the Attentional Set - Shifting Task (AST), a test of cognitive flexibility. Our preliminary results show that the adaptive group had a better performance at specific stages of the test (the reversal stages and the extradimentional shift), compared to the non- adaptive group. In conclusion, our results show that working memory training improves the adaptation to new rules, in a cross species study.

POSTER PRESENTATIONS

CHAPERONE MEDIATED AUTOPHAGY REGULATES NOTCH SIGNALING AND MODULATES NEURAL STEM CELL DIFFERENTIATION DURING DEVELOPMENT

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The last few years autophagy have been associated with the ability of Neural Stem/Progenitor Cells (NSCs) to generate the enormous complexity of mammalian brain. The main autophagic systems are macroautophagy and Chaperone Mediated Autophagy (CMA). Although macroautophagy has been strongly linked to nervous system development and CMA dysregulation is correlated to many brain-related diseases and disorders, nothing is known about its physiological role in brain organogenesis. Accordingly, the aim of this study was to examine the involvement of CMA in neural differentiation during development. Specifically, we investigated the role of lysosomal receptor and rate limiting step of CMA, LAMP-2A. Thus, here, we showed that CMA is highly active in NSCs and that LAMP-2A and Hsc70, basic components of CMA, are strongly expressed in ex vivo cultured NSCs. Interestingly, LAMP-2A is preferentially expressed in early post mitotic neurons as compared to nascent astroglial cells. This trend of LAMP-2A expression was also confirmed in different developmental stages in vivo. Most importantly, shRNA-mediated knockdown of LAMP-2A strongly impaired the ability of NSCs to produce neurons in vitro and induced their potential to generate astrocytes, whereas LAMP-2A overexpression led to the opposite effects. Mechanistically, we showed that LAMP-2A is involved in NSC fate decisions, by interfering with Notch1 signaling pathway. Forced expression of LAMP-2A in NSCs reduced the levels of Notch1 Inracellular Domain (NICD) as well as expression of downstream effector genes, Hes1 and Hes5. These data imply that NICD may be a direct target of CMA. In agreement, we identified a CMA recognition motif on the NICD peptide sequence (KFERQ like motif). Targeted mutagenesis of this motif reduced the capacity of CMA to degrade NICD and significantly induced NICD-mediated transactivation of Hes5 promoter. Collectively, our data suggest a key role for LAMP-2A and consequently of CMA in the regulation of neuronal fate acquisition.

HIGH CONTENT SCREENING ANALYSIS IN AN INDUCED PLURIPOTENT STEM CELL-BASED MODEL OF FAMILIAL PARKINSON'S DISEASE REVEALS A KINASE INHIBITOR THAT ENHANCES THE SURVIVAL AND/OR DIFFERENTIATION OF DOPAMINERGIC NEURONS

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Induced pluripotent stem cells (iPSCs) hold great promise in Biomedical Research for the development and study of *in vitro* cellular models for neurological/ neurodegenerative diseases, currently incurable, with prospective medical applications. Human iPSC technology combined with high content screening and quantitative image analysis, enabled us to create and analyze a cellular model of familial Parkinson's disease using fibroblasts from patients harboring the autosomal dominant G209A mutation in the alpha-synuclein gene SNCA, which results in the p.A53T pathological alpha-synuclein protein (αSyn). The purpose of this study is to identify small molecules that selectively enhance the survival and/or differentiation of dopaminergic (DA) neurons as a new therapeutic approach. Since kinases are attractive targets for the treatment of various diseases, including degenerative diseases of the brain, we chose to test a library of kinase inhibitors. Human iPSC differentiation to mature neurons was adapted to miniature 384-well plates. Using this platform, we screened a kinase inhibitor library for enhancement of the dopaminergic phenotype as determined by immunofluorescence for the enzyme tyrosine hydroxylase (TH), which is involved in the biosynthetic pathway of dopamine. Following quantitative image analysis, we identified a TBK1/PDK1 kinase inhibitor that may be involved in the survival and differentiation of DA neurons. Inhibition of PDK1 kinase in models of Alzheimer's disease attenuates memory deficits while TBK1 kinase is involved in the immune response controlling the expression of several pro-inflammatory cytokines. We investigate the possibility that these kinases may be potential PD targets.

ENDOGENOUS CORTICAL ACTIVITY IS RESISTANT TO CHRONIC STRESS INDUCED BY ORAL CORTICOSTERONE ADMINISTRATION

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Introduction: The detrimental effects of chronic stress have been extensively examined by assessing animal behaviour, as well as neuronal microanatomy and molecular markers. However, the effect of stress on network function has remained largely unexplored. In the present study, we have investigated the impact of chronic corticosterone administration on endogenous cortical activity. Oral administration of corticosterone (CORT) induces anxiety-like behaviour and anhedonia in mice (Gourley & Taylor, 2009; Lee et al. 2010) and has been proposed as a model of metabolic syndrome (Cassano et al. 2012), or depression (Gourley et al. 2008). It has also been connected with stress-related memory deficits (Klug et al. 2012). Network function was assessed by ex vivo recordings of spontaneously recurring Up and Down states. This activity pattern, characteristic of quiescent states such as slow wave sleep and anesthesia, is also preserved in brain slices, and is considered the default activity of the cerebral cortex, reflecting endogenous connectivity and synaptic dynamics. As such it is an optimal preparation with which to assess the effects of chronic stress on brain network function. CORT (100 μg/ml) was supplied in the animals' drinking water for 2 weeks (P28-P43) and their weight was checked daily. At the end of the 2-week period mice were tested on the Elevated Plus Maze (EPM) task, and on the next day, the brains were extracted and cut into 400μm slices. Following at least 1h equilibration time, spontaneous network activity was monitored through local field potential (LFP) recordings. A second group of animals was submitted to identical CORT treatment, followed by a 4-week washout period during which they received regular tap water. At the end of week 4, these mice were also submitted to the EPM task and their brains prepared for electrophysiology. Animals receiving CORT exhibited reduced weight gain, although they consumed more food and liquid. They covered less distance in the EPM and spent more time in closed arms than controls. However, analysis of network activity revealed no significant differences in any electrophysiological parameter. After washout, the body weight, adrenal weight and adipose tissue were similar for both CORT-treated and control animals, as were the EPM and LFP recordings data. The physiological and behavioral assessment of CORT treated animals, compared to age-matched controls, indicated the treatment was effective in inducing a full-blown stress response. Nevertheless, the network activity parameters remained similar in the two groups, suggesting that the endogenous cortical dynamics are fairly resistant to changes induced by manipulations of the stress hormones.

MANNAN-CONJUGATED MYELIN PEPTIDES INDUCE T CELL ANERGY AND TREAT MULTIPLE SCLEROSIS-TYPE DISEASE IN HUMANIZED DR2 MICE

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Antigen presenting cells play a critical role in the regulation of immune responses. Our previous studies show that myelin peptide antigens conjugated with an oxidized form of the polysaccharidemannan (OM) induce antigen-specific anergy in Th1 and Th17 cells and prevent and treat CNS inflammation and demyelination in a variety of multiple sclerosis (MS) models (Tseveleki et al., 2015,*Exp.Neurol.* 267:254-267). Here we investigated whether OM-conjugated myelin oligodendrocyte glycoprotein 35-55 peptide (OM-MOG) presented exclusively in the context of the MS candidate susceptibility gene HLA-DR2 is sufficient to induceT cell tolerance and protect mice against experimental autoimmune encephalomyelitis (EAE). Dendritic cells (DC) from wild type C57BL/6 (B6) mice orDR2 transgenic mice expressingHLA-DR2 in the absence of murine MHC Class II genes were loaded with MOG, OM-MOG or PBS and co-cultured with murine splenocytesin an *in vitro* antigen presentation assay. OM-MOG-loaded B6 and DR2 DC stimulated reduced T cell proliferation responses and production of IL-2, IL-2 receptor (CD25) and effector cytokines by $CD4^+$ T cellscompared to MOG. These defects were reversed by the addition of IL-2 to the culture medium, in accordance with a T cell anergy model. OM-MOG completely protected DR2 mice against the clinical and neuropathological features of MOG-induced EAE when administered in prophylactic or therapeutic protocols. These results strongly support the use of OM-conjugated myelin peptides for re-introducing peptide-specific T cell tolerance and the treatment human autoimmune diseases such as MS.

POST-TRANSLATIONAL REGULATION OF THE NEUROGENIC TRANSCRIPTION FACTOR SOX11 MODULATES NEURONAL MORPHOGENESIS

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Sox11 is a key transcription factor (TF) that belongs to the SoxC (SRY-related HMG-box C) family, which is critical in the regulation of embryonic and adult neurogenesis. Loss of Sox11/ Sox4 in the neural stem cells (NSCs) of adult mice, for example, abolishes their neuronal differentiation. In contrast prolonged Sox11 expression in the NSCs of adult mice causes delayed maturation, delayed spine development and reduced morphological complexity. Sox11 has in addition gathered major attention following the recent discovery that mutationsin Sox11 cause Coffin- Siris Syndrome, a rare genetic disorder associated with developmental delay. Overall, these findings underline the necessity to precisely regulate Sox11's activity. Here, we found through Mass Spectrometry that Sox11 has at least 10 phosphorylated Serines. Using phosphomimetic/non-phosphorylatable mutants we characterized the functional relevance of Sox11's p-Serines. We found that 3 of the p-Serines, located around the HMG-BOX, are essential for Sox11's transcriptional activity and that one of those serines, i.e., ser133, is phosphorylated by Protein Kinase A (PKA). In vivo, both ser133 mutants were able to prevent loss-of-SoxC induced cell death of developing neurons. Interestingly the Ser133 mutants showed differential impact on dendritic morphogenesis of SoxC-deficient neurons. These data show that Sox11's phosphorylation by PKA modulates dendritic development potentially through altering Sox11's transcriptional activity on distinct target genes.

THE INFLUENCE OF GENES ASSOCIATED WITH THE ENDOCRINE SYSTEM AND SOCIAL ENVIRONMENT TO STUDY GENETIC IN PSYCHIATRY AND THE RISK OF DEPRESSION AND BIPOLAR DISORDER.

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The main aim of the study was analysis polymorphisms of genes: *FKBP4*, *BAG1*, *GLCCL1*, *DUSP1*, *SRD5A1*, *STIP1* associated with the regulation of stress axis in major depressive disorder (MDD) and bipolar disorder (BDD). Interactions between genes and the environment (stress in life, work) affect the homeostasis of the human body, interfering the proper functioning of the brain and lead to mental disorders. The study was performed on a Polish samples of 739 unrelated patients from Wielkopolska region with mood disorders diagnosed with either major depressive disorder or bipolar disorder according to DSM-IV diagnostic criteria (American Psychiatric Association 1994) and control group. Molecular analysis: The DNA samples for genetic analysis have been obtained by isolation from peripheral blood with used of salting out method then spectrophotometer Nanodrop. Determination of genetic polymorphisms of selected genes have been made using sets of primers and probes by TaqMan SNP Genotyping Assays was performed in a AbiPrism7900HT system (Applied Biosystems). Statistical analysis was done with use of statistical packages STATISTICA v. 9.0, and GraphPad InStat v. 3.06 calculator. The obtained results suggest that stress axis activation (via environmental risk factor – psychosocial stress) together with the genetic susceptibility affect MDD risk and emphasize the important role of gene-environment interaction in development of this disorder. Example in the analysis of *STIP1* polymorphisms with presence of psychosocial stress factor at the first episode an association of AA genotype and A allele of rs4980524 (p= 0.05 and p=0.02, respectively) was observed when comparing MDD patients with and without this clinical phenotype. However, no such differences were observed with the same phenotype in BD patients. Special variants of *STIP1* were associated with major depressive disorder as well as with a clinical phenotype which is stress factor before the first episode of illness thus confirming the well-established relationship between the HPA axis regulation, which mediates the stress response, and development of mood disorders, particularly in women.

INVESTIGATING MOLECULAR COMPONENTS OF ANESTHESIA RESISTANT MEMORY IN *DROSOPHILA*

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Memories in *Drosophila* can be distinguished in many different types; Short Term Memory (STM), Middle Term Memory (MTM), Anesthesia Resistant Memory (ARM) and Long Term Memory (LTM). While STM lasts for about 3 minutes, MTM 2-4 hours and LTM lasts from 24 hours to days or even weeks, ARM overlaps with MTM and LTM, being detectable from 3 to 24 hours after training the flies [1]. Interestingly, although ARM is considered to be a part of long-lasting memories due to its 24-hr maintenance, it is independent of *de novo* protein synthesis [2].Only a few genes are known to be involved in ARM formation, including *radish* [3]and *drk* [4]. However, the molecular pathways leading to ARM formation remain unknown. Thus, we used a proteomics approach and tissue limited tagged transgene expression to identify protein interactors of Drk within the Mushroom Bodies (MBs) - the center for olfactory learning and memory in *Drosophila*-, to define novel molecules with potential roles in ARM. We will report results from this study whose broad aims are to provide further insight into the molecular pathways involved in ARM.

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ROLE OF HYPOTHALAMIC NNOS-DERIVED NO SIGNALING IN THE CONTROL OF THE GNRH DRIVEN MATURATION OF THE REPRODUCTIVE AXIS

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The onset of puberty and the regulation of fertility in mammals are governed by a complex neural network, primarily in the hypothalamus, that converges onto the gonadotropin-releasing hormone (GnRH)-producing neurons, the master regulators of gonadotropin secretion and postnatal gonadal growth and function. For the GnRH neurons to exert their pivotal role in the establishment of a fertile phenotype, their activity and neurosecretory capacity needs to be precisely regulated by upstream pathways. As early as in the 1990's nitric oxide (NO) was presented as a key molecule in the preovulatory GnRH/LH surge, while results from different groups have suggested the interaction of neuronal nitric oxide synthase (nNOS or NOS1) -containing neurons with the GnRH system (Bellefontaine et al., 2011; Chachlaki et al., 2017). Even though NO has now been long recognized as a key player in the central hormonal regulation of ovulation during adulthood, no one had considered the possibility that it could act in an earlier stage as the master regulator of GnRH neurons before puberty, hence participating in the actual maturation of the neuroendocrine axis. Challenging this idea we identified for the first time a series of mutations on the *Nos1* human gene in patients with Constitutive hypogonadotropic hypogonadism (CHH), as well as in probands related to Constitutional delay of growth and puberty (CDGP). This exciting finding not only highlights a key role of nNOS in the establishment of a fertile phenotype, but most importantly, paves the way for a better understanding of conditions of human idiopathic infertility (Boehm et al., 2015). To this aim, we present evidence supporting a novel role of hypothalamic NO signaling in the regulation of the GnRH neuronal population during a crucial window of the infantile period known as minipuberty. During minipuberty, NO controls the transcriptional activation of the *Gnrh* gene (Messina et al., 2016), as well as the GnRH neuron firing activity and the release of the GnRH peptide, coordinating the events leading to the sexual maturation and the acquisition of a fertile phenotype. We are hopeful that our results will expand our understanding of how the neuroendocrine axis is regulated during postnatal development, and will possibly provide opportunities for therapeutic strategies against debilitating conditions.

GENETIC SCREEN OF *DROSOPHILA MELANOGASTER* TO IDENTIFY GENES IMPLICATED IN PREMATURE FOOTSHOCK HABITUATION

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To survive in an environment providing a huge amount of information, animals have developed a form of behavioral plasticity that allows them to ignore repetitive or prolonged non-reinforced stimuli called *habitiuation*. Habituation is a normal process that occurs after an animal has been exposed to a repeated stimulus and allows greater ability to discriminate salient information, such as food, or danger. Premature habituation, is a deficiency, where the animal, diminishes its response to a stimulus much faster than expected. Defective habituation has been linked to attention deficits and schizophrenia. Because the molecular mechanisms that govern normal habituation, which are likely disturbed in ADD and schizophrenia, are still largely unknown, we aim to gain an understanding by identification and characterization of genes expressed within the neurons we have determined essential for the process. For this reason, we used the powerful genetic tool, *Drosophila melanogaster* together with an assay to assess habituation to mechanosensory stimuli, which in our case is low voltage electric footshock. Therefore, we started a genetic screen, to identify the largest possible number of genes that when mutated result in premature habituation. This will provide a first collection of genes whose function is essential within a particular neuronal type to promote normal habituation. For this screen, we mainly used the Minos transposon technology. In particular, we used MiMIC insertions (Minos Mediated Integration Cassette) and some MiET1 insertions. We will present the initial results of our screen. Also, based on previous laboratory data, blocking neurotransmission from the mushroom bodies results in premature habituation to footshock stimuli, so an additional goal is to focus on two phosphatases Ptp61F and CSW, expressed in the MBs, which were among the identified genes from the screen, for further analysis using behavioral and pharmacological approaches, such as RNAi experiments and tests for reversal of the deficit by *Methylphenidate (Ritalin)*, the compound that is used both to diagnose and treat ADD/ADHD. Our long-term aim is to establish Drosophila as a promising model to understand complex neurological diseases such as ADD/ADHD.

PROTECTION AGAINST EAE BY MANNAN-CONJUGATED MYELIN PEPTIDES INVOLVES T CELL ANERGY CHARACTERIZED BY REDUCED ANTIGEN-SPECIFIC PROLIFERATION AND BUT NOT ALTERED MIGRATION OF T CELLS TO THE CNS

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Multiple sclerosis (MS) is a chronic, inflammatory, neurodegenerative disease of the CNS in which the immune system plays a prominent role, resulting in demyelination, axonal degeneration and progressive neurological disability. Experimental autoimmune encephalomyelitis (EAE) is a powerful tool for studying immunopathogenesis in MS that can be induced in animals by immunization with myelin antigens. Recently we showed that myelin peptides conjugated to an oxidized form of the yeast polysaccharide mannan (OM) induces peptide-specific peripheral T cell tolerance and protection against EAE when administered prophylactically or therapeutically in mice. Our own unpublished data show that tolerance in splenocytes and lymph node cells is characterized by reduced peptide-specific proliferation responses, and IL-2, and IL-2 receptor (CD25) production by CD4+ T cells, defects reversed by incubation with IL-2 in accordance with a T cell anergy model. In this study we investigated whether OM-MOG35-55 peptide (OM-MOG) administration modulates disease by affecting the ability of T cells to migrate into the CNS or by increasing their sensitivity to activation-induced cell death. We generated chimeric mice using combinations of CD45.2 (wild type C57BL/6) and CD45.1 (congenic C57BL/6) mice with or without the 2D2 MOG_{35-55}-specific TCR transgene in the transferred donor immune cells. CFSE-labeled splenocytes and lymph node cells isolated from donor mice that had been previously tolerized or not by vaccination with OM-MOG or PBS, respectively, were adoptively transferred into recipient mice with ongoing MOG-EAE. Isolation of CFSE-labeled cells from tissues of the EAE mice 5-7 days after transfer revealed that OM-MOG-tolerized cells showed equal proliferation and unimpaired migration into secondary

lymphoid and CNS tissues compared to non-tolerized cells, albeit with a small delay in reaching the CNS tissues (meninges and parenchyma). We next tested whether the T cells isolated from OM-MOG tolerized mice showed increased susceptibility to apoptosis that might be associated with their anergic phenotype and inability to induce EAE. Our results show that OM-MOG tolerized T cells do not show increased apoptosis after activation induced cell death assay, compared to their controls. These results indicate that OM-MOG imposes T cell tolerance either through direct interaction with MOG-specific T cells or through induction of an independent population of immune regulatory cells.

TARGETED DELETION OF GEMC1/LYNKEAS LEADS TO DEFECT IN ADULT NEUROGENESIS

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Cerebrospinal fluid (CSF) keeps the brain tissue buoyant and acts as the vehicle for delivering nutrients to the brain. Hydrocephalus of the brain occurs when generation or circulation of CSF is not correctly regulated.A significant percentage of hydrocephalus is caused from inherited genetic abnormalities which affect the development of multicilated ependymal cells. These cells contain hundreds of cilia that move in a coordinated manner. The specification of radial glial cells to ependyma lineage is initiated during late embryogenesis, while the generation of ependymal cells occurs during the first postnatal days. We have previously shown that GemC1/Lynkeas, is a master regulator of ependymal cell lineage which regulate the expression of key transcription factors for ependymal cell differentiation such as c-Myb and Foxj1. Subventricular zone (SVZ) is localized at the external wall of the lateral ventricles. Type-B1 cells, retains stem cells properties in the postnatal period and generate highly proliferative cells termed type-C cells that give rise to neuroblasts. Neuroblasts migrate toward the olfactory bulbs forming the rostral migratory stream where they differentiate into neurons. We have shown that mice lacking Lynkeas expression develop severe hydrocephalus and dying postnatally. We would like to investigate the effects of hydrocephalus in the composition and function of SVZ. We have examined the generation of type-C and neuroblasts in the knock out mice. We have shown that in the absence of GemC1, Ascl1+ cells were reduced. Neuroblasts in mice that lack GemC1, do not form RMS and remain concentrated in the SVZ. Our results suggest that hydrocephalus affects subependymal zone cells and ongoing neurogenesis in adult mice.

***IN VITRO* AND *IN VIVO* MODELS FOR NON-SYNDROMIC AUTISM SPECTRUM DISORDER: VALIDITY FEATURES**

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Autism Spectrum Disorder (ASD) is a behaviourally defined neurodevelopmental condition. The core ASD symptoms are social reciprocity along with communication deficits and interest restriction, accompanied by repetitivebehaviours. Multiple genes and environmental factors have been associated with ASD but still the aetiology is not fully understood. This project explores the existing in vitro and in vivo models that have been used to investigate the neurobiology of non-syndromic ASD (ASD not accompanied by dysmorphic or medical conditions). A comprehensive literature review of the PubMed database has been conducted to summarise the ASD genetic and environmental models. Construct (aetiology), face (phenotype) and predictive (treatment) validity are discussed providing a more robust platform for investigating ASD. In conclusion, all the in vitro models have a priori strong construct validity since they have been extracted by human tissues derived from individuals with ASD; mutations in *SHANK3* and *TRPC6*genes in induced pluripotent stem cell (iPSCs) models show robust face and predictive validity. The evaluation of the in vivo non-syndromic ASD models allowed cross species comparisons. The *Cntnap2* knockout (KO) zebrafish[1], zebra finch songbirds[2] and rodent models showed strong validity features in all domains. The Valproic Acid (VPA) model showed high validity features across the zebrafish, rodent and non-human primate (NHP) species[3]. Evidently, the way forward is to combine not only genetic and environmental models (e.g. double-hit experiments) but also in vitro and in vivo models in order to gain a more holistic overview of the ASD aetiology and its possible pharmacological treatment.

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NOVEL PSYCHOACTIVE SUBSTANCE (NPS) AWARENESS, USE AND HEALTH PERCEPTION FOLLOWING THE UK PSYCHOACTIVE SUBSTANCES ACT 2016

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The risk of potential harms prompted the UK government to introduce The Psychoactive Substances Act (PSA) in 2016 banning the production, supply, importation and exportation of NPS[1]. Here we investigate UK NPS awareness, use and knowledge of potential associated health risks prior to and following The 2016 PSA. The Bristol Online Survey was in English, advertised on the drug forum Bluelight and social media and University email between 7 January and 7 February 2015 (168 responses) and 9 March to 18 September 2017 (726 responses). UK country of residence responses were extracted for analysis. Similar trends in UK NPS use, motivations and risk perception in 2015 and 2017 are seen despite the difference in number of survey responses and the recent 'illegal' status of NPS in the UK, although a 15% increase in UK NPS awareness is noted in 2017 (Table 1). The main motivation for UK NPS use remains to be the influence of friends and the preferred drug combination is NPS + alcohol. Most NPS were sourced from friends (39%), a dealer (39%) or via the internet (73%)(despite their illegal status). The effect of gender, age, sexual orientation, education level and employment status on NPS awareness and use ($p<0.05$, Chi squared test) will be presented. Despite the recent introduction of The UK 2016 PSA our 2017 survey indicates that UK NPS use (27%) remains higher than the rest of Europe (8%)[2] and higher than UK use in 2015 (17%). UK user and non-user perception of NPS safety has so far not altered, indicating a need for enhanced targeted prevention interventions, alongside regulation.

Table 1. Survey responses

	1 month Survey 2015 Number responses (%)	**6 month Survey 2017 Number responses (%)**

Total responses	168	726
UK responses ***Male*** ***Female***	98 (58%) *34 (35%)* *64 (65%)*	500 (69%) *197 (39%)* *303 (61%)*
UK NPS Awareness ***Male*** ***Female***	71 (72%) *26 (76%)* *45 (70%)*	434 (87%) ▲ *176 (89%)* ▲ *258 (85%)* ▲
Main reason for non-NPS use: 'I believe NPS are highly risky for health'	46 (57%)	240 (66%)
UK NPS Use ***Male*** ***Female***	17 (17%) *8 (24%)* *9 (14%)*	134 (27%)▲ *95 (48%)* ▲ *39 (13%)*
UK NPS User Risk perception (% Don't know, No risk or Low risk)	6 (35%)	63 (47%)
Main Motivation of UK NPS Use: • **Friends take them** • **Give me a good high**	14 (82%) 9 (52%)	80 (60%) 91 (68%)
Main UK NPS-drug combination: • **Alcohol** • **Cannabis**	15 (88%) 11 (65%)	84 (63%) 77 (57%)

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CHRONIC ORAL METHYLPHENIDATE INTAKE AFFECTS WHITE MATTER MORPHOLOGY AND NMDA RECEPTOR DENSITY IN NORMAL RATS

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Methylphenidate (MP) is a widely used psychomotor stimulant labeled for treatment of ADHD. Due to widespread use of MP in adults and children, it is essential that all treatment-induced effects be analyzed. In this study, we tested MPs potential to induce neuromorphological abnormalities and to affect excitatory neurotransmission. Adolescent male rats drank either water or MP through an 8-hour limited access, which involved the presentation of a lower MP dosage for the first hour followed by a more concentrated solution for the remaining 7 hours. Two MP-treatment groups were included in the study, a low-dose (4 mg/kg / 10 mg/kg) and a high-dose (30 mg/kg/60mg/kg). This treatment protocol is clinically relevant, since its pharmacokinetic profile is similar to that of clinical MP doses.After 3 months of treatment, rats were split in two groups. In one group, the rats were euthanized and brains were processed either for *in vitro* structural magnetic resonance imaging (MRI) or for [^{3}H]MK-801 *in vitro* NMDA receptor autoradiography. The other group went through a 4-week period of abstinence, at the end of which the rats were euthanized and the brains were processed for [^{3}H]MK-801 *in vitro*NMDA receptor autoradiography. Chronic oral MP intake, with either dose, led to a significant decrease in the volume of the posterior corpus callosum/external capsule, 6-8 mm caudal to the anterior commissure, in a region that includes the cingulum bundle, the dorsal hippocampal commissure, and the external capsule. The cingulum bundle is a set of white matter association fibers that connects retrosplenial and cingulate cortices with the dorsolateral prefrontal and orbitofrontal cortices, with regions of the parietal and temporal cortices, and with the entorhinal and perirhinal cortices and the subiculum. The dorsal hippocampal commissure contains interhemispheric fibers distributed to the entorhinal cortex, the pre- and parasubiluculum, and the retrosplenial cortex. Structural changes in these white matter tracts predict functional alterations in the brain regions that are connected through them. Chronic oral high-dose MP intake led to significantly lower NMDA receptor density in prelimbic/infralimbic, insular, orbital, sensory, motor, piriform, and rhinal cortices, while leaving cingulate and retrosplenial cortices unaffected. These effects were reversible, as they were not observed after a 4-week abstinence period. Overall, the results suggest that chronic oral MP treatment in normal animals may lead to structural/functional changes in the projections from cingulate and retrosplenial cortices to frontal, parietal, and limbic areas of the cerebral cortex.

FUNCTIONAL VULNERABILITY OF A PLASTIC NEURAL CIRCUIT DURING DROSOPHILA PREMATURE DEATH

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Prolonged desiccation or/and starvation causes dizziness and hallucinations while water and food supply in starved individuals prior to collapse of major physiological systems results in full restoration of normal brain activity. Are all neurons and circuits equally affected by the stressor? We addressed this question in the brain of the genetic model organism *Drosophila melanogaster*. To identify physiological state transitions and pre-death symptoms we conducted longitudinal assessments of motor function in young, 5 days old, flies prematurely dying by desiccation or/and starvation. We found that flies exhibit phases of normal, spontaneous locomotor activity (e.g. foraging) intermingled with phases of rest and of uncontrolled, generalized pre-death hyperactivity. Pre-death functional collapse and the pathological traits observed during the terminal stage of live (the last 2 hours) are reminiscent to aged individuals undergoing natural death. We further demonstrated that loss of climbing ability in starved individuals coincides with the loss of visually induced escape response (LIER). In accordance to age-dependent death, during premature death LIER loss is caused by signal transmission failure through the plastic, but not the hard-wired components, of the central nervous escape circuit. Remarkably, loss of circuit function can be restored by feeding the starved animals, suggesting that plastic circuit components were not irreversibly damaged. Thus the young, prematurely dying drosophila brain of drosophila, offers the possibility to genetically or environmentally target key molecules to delay functional collapse or accelerate functional recovery of the most vulnerable circuit elements.

LATE LIFE MORBIDITY COMPRESSION IN DROSOPHILA

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According to the James Fries'[1] compression morbidity hypothesis, if in humans the onset of disability could be delayed, then disability could be compressed into a shorter period before death. In support of this hypothesis, large national surveys showed that disability decline rates exceed the observed decline in mortality. Nevertheless, the majority of older persons are disabled in the last year of life. Apart from compressing the period of morbidity in a continuously increased human lifespan is equally important to reduce the number of disabled people and the severity of disability. To avoid pathology related to chronic and neurodegenerative diseases, a hallmark of mammalian systems, we examined the compression morbidity hypothesis in the genetic model organism *Drosophila melanogaster*. We have previously

demonstrated that pre-death disability traits in flies present remarkable similarities to those in rodents and humans. In this study we have performed environmental manipulations to extend lifespan in flies and ask whether life prolonging dietary interventions have positive or negative effects on late age pathophysiology. Particularly, we raised wild-type (Oregon-R) larvae in a diet with 2:1 carbohydrate/protein ratio with or without the addition of the antioxidants curcumin and Super fruit. After emergence, adults were separated into two triplets (control, Super Fruit, Curcumin groups) of males and females, the one continued to be fed in the 2:1 C:P ratio diet and the other in a 8:1 C:P ratio diet. The physical status of flies was examined once/day from the age of 60 days old until death. We found that high C;P ratio diet increased lifespan by 20.% in both sexes. Both antioxidants increased lifespan in males and females fed in low C:P ratio diet, but had no effect in the lifespan of males and even decreased the lifespan of females, both fed in high C:P ratio diet. No significant difference was found in the average duration of disability or its severity trajectories among the cohorts. However, the percentage of individuals without disability in the last day of life was increased and the severity of disability reduced in flies raised in high C:P ratio diet. In contrast, flies raised with antioxidants in high C:P ratio diet exhibited increased disability and pathological signs of late-onset diabetes. In conclusion, dietary manipulations that increase lifespan in flies can improve or worsen late life quality for the individual and the cohort but disability duration and course of disability severity can not be compressed.

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INCREASED PHOSPHORYLATION AT SER126 INDICATES ALPHA-SYNUCLEIN'S PATHOLOGYIN SUSTANTIA NIGRA OF THE "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 characterized pathologically by the gradual accumulation and aggregation of the presynaptic protein alpha-synuclein (α-syn). The processes of α-syn 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 a) progressive degeneration of dopaminergic neurons in SN, b) motor difficulties (tremor, dyskinesia) c) cognitive impairments, d) neuroinflammation and e) oxidative stress. Our preliminary results by using Western Blot analysis, suggest that the "weaver" mouse develops the alpha-synuclein's pathology. In particular, our results, compared to control mice (+/+), demonstrate a) increased phosphorylated (Ser129) alpha-synuclein in SN and Striatum (Str) which is indicative of the formation of alpha-synuclein oligomers, fibrils and aggregates, and b) reduced levels of alpha-synuclein in SN and Str, which complies with a previous study in Str and implies the existence of aggregated alpha-synuclein. Our proximate goals encompass the confirmation of our preliminary results by immunostaining and the investigation of the mechanisms underlying the aggregation of alpha-synuclein. Moreover, we aim to explore therapeutic approaches by a) treating the aggregates through targeted adenoviral vector delivery of KLK6 (human kallikrein 6), a serine protease capable of cleaving alpha-synuclein in the CNS and b) examining the impact of the BNN-20 administration in alpha-synuclein's pathology; BNN-20 is a micro-neurotrophin -chemical analogue of endogenous dehydroepiandrosterone (DHEA)- which exhibits strong anti-oxidant and anti-inflammatory effects.

CANONICAL TGFβ SUPERFAMILY SIGNALING IN THE CONTEXT OF THE MPTP INDUCED PARKINSONISM

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Aberrant activation of the canonical TGFβ Superfamily signaling system has been implicated in several immuno-inflammatory neurological disorders, including Parkinson's disease (PD), however, due to the complex, context and cell-type specific mode-of-action of this system, its precise role in PD pathophysiology is still inadequately characterized. To address this issue we generated double transgenic reporter mice expressing Green (GFP) and Red (RFP) Fluorescent Proteins under the control of well characterized BMP- and TGFβ-responsive elements, respectively, and subjected them to the well-established neurotoxin MPTP thus, simulating a parkinsonian phenotype. Remarkably, acute MPTP intoxication led to the activation of both reporters along the nigrostriatal pathway, specifically in astrocytes, visualizing robust *in situ* activation of both BMP and TGFβ pathways in a PD-like environment. Interestingly, activation of the GFP reporter was dominant within the degenerating *striatum*, whereas RFP expression dominated in the *substantia nigra* (*SNpc*) of the MPTP-treated animals. To assess the potential neuroprotective/neurotoxic role of BMP-signaling in the context of MPTP-induced pathology, we performed a series of unilateral stereotactic injections of rAAVs overexpressing human Noggin (natural BMP-antagonist) or human BMP2 (potent BMP-signaling activator) in the right *SNpc* prior to neurotoxin treatment. Preliminary analysis revealed that animals overexpressing Noggin exhibited a trend for improved performance in specific behavioral tasks. Overall, our on-going studies implicate the TGFβ-superfamily signaling in PD-related neuropathology and may pave the way for a better understanding of the underlying pathogenic mechanisms; thus providing insights towards novel therapeutic strategies.

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CHOLINERGIC EFFECTS ON HIPPOCAMPAL SYNCHRONOUS DISCHARGES OF YOUNG RATS EXPOSED IN ETHANOL *IN UTERO*

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Fetal Alcohol Syndrome (FAS) results from fetal exposure to alcohol and is associated with several long-term neurological abnormalities of the offspring, including increased susceptibility to seizures. Ethanol affects inhibitory ($GABA_A$) and excitatory (NMDA) neurotransmission, but it is unclear how these changes affect neuronal network synchronization; the cholinergic role in these conditions is also unclear. The aim of this study was to investigate the effects of the prenatal ethanol exposure on the generation and modulation of synchronous interictal-like epileptiform discharges (IEDs) *in vitro* recorded from the CA1 area of temporal hippocampal slices. Female Sprague-Dawley rats were given a solution of 15% v/v ethanol as their only source of drinking water before and throughout gestation and up to postnatal day (PD) 15 of the offspring. The latter were sacrificed at PD 21-35 to conduct extracellular electrophysiological recordings (FAS slices), with controls from same age normal rat pups (N slices). Perfusion with Mg^{2+}-free ACSF induced IEDs in all slices, with lower frequency in FAS (0.10±0.00Hz, n=71) than in N slices (0.16±0.02 Hz, n=93, p=0.002), but longer duration (FAS 490±43ms n=57 vs N 344.2±24ms, n=54, p=0.0032).

Increase of K^+ to 7mM (in Mg^{2+}-free ACSF) increased IED frequency in all slices: although the percent increase was larger in FAS slices (695 ±161%, n=5 vs N 191±59%, n=5; p=0.02), the frequencies reached in these conditions were much lower than those of N slices (0.90±0.2, n=5 vs FAS 0.43±0.11, n=5, p=0.02). The muscarinic agonist oxotremorine (1μM) increased IED frequency significantly more in n=9 FAS vs n=17 N slices (p=0.03) and reduced IED duration in all. Oxotremorine's effects were reversed by the M1 antagonist pirenzepine (1μM) in N slices (n=14) but not in FAS (n=9). The $GABA_A$ antagonist bicuculline (BMI, 20μM) decreased IED frequency in all slices but increased their duration only in N slices (by 346±36%, n=25, p=0.012). In these conditions, the ACh analog Carbamylcholine chloride (CCh, 25μM, in BMI) increased IED frequency in n=17 N and n=25 FAS slices, the effect being more pronounced in the former (N vs FAS p=0.008); the muscarinic antagonist atropine (1μM) fully reversed CCh's effect. In conclusion, ethanol exposure *in utero* changes the dynamics and modulation of hippocampal epileptiform discharges from young rats.

DESIPRAMINE ANXIOLYTIC-LIKE EFFECTS INVOLVE β2 ADRENERGIC RECEPTORS AND TRANSCRIPTION FACTOR FosB IN ADULT ZEBRAFISH (*DANIO RERIO*).

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Previous studies indicate that the transcription factor FosB is induced by chronic antidepressant treatment and that it plays an essential role in rodent models of depression and antidepressant action. Zebrafish (*Danio rerio*) is a promising model to study anxiety-like phenotypes, but, the molecular changes in zebrafish brain have not been well established. The present study aims to examine the effects of acute and chronic administration of desipramine (DMI), a tricyclic antidepressive drug, on adult zebrafish telencephalic FosB and β2 adrenergic receptor expression pattern, using immunohistochemistry and Western blotting. Furthermore, the anxiety-like behaviours were evaluated by a novel tank test (NTT), a utilized behavioural test for quantifying anxiety-like behavioural responses. Our results showed that acute or chronic DMI administration exerted a clear anxiolytic-like effect in parallel to reducing the telencephalic β2 adrenergic receptor expression. Interestingly, chronic or acute DMI exposure induced differential effects on specific isoforms of FosB gene product expression (full length and specific spliced isoforms). Our findings show that DMI is capable of providing anxiolytic-like effects associated with decreases of β2 adrenergic receptor expression in adult zebrafish. In addition, we provide the first evidence for the expression of transcription factor FosB in zebrafish brain, influenced by the tricyclic antidepressive drug DMI. Taken all together, zebrafish is a non-human model-organism constituting a useful tool in the research of anxiety, depression and antidepressants.

EFFECTS OF CHRONIC UNPREDICTABLE STRESS AND DESIPRAMINE EXPOSURE ON BRAIN CB1 RECEPTORS AND BEHAVIOURAL ZEBRAFISH ENDOPHENOTYPES

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Studies indicate that chronic unpredictable stress (CUS) induces depressive-like behavioural phenotypes, while antidepressant treatment, such as, desipramine (DMI), reverses this effect. In addition it has been suggested that CUS modifies endocannabinoid receptors and signaling in rodent brain. To question whether such effects are present in a non-mammalian model organism such as, zebrafish, we applied a 21-day-CUS and DMI exposure protocol. Next, we determined the cannabinoid receptor type-1 (CB1R) expression in adult zebrafish telencephalon including areas homologous to mammalian amygdala, hippocampus and striatum/septum, by using Western blot methodology. Additionally, we measured the hedonic state, and anxiety-like behaviours, using Conditioned Place Preference (CPP) and Novel Tank (NTT) tests, respectively. Our results revealed that, CUS-exposed animals can be descriminated to two different phenotypes in relation to the hedonic state, CUS-sensitive and CUS-resilient. Interestingly, CUS-sensitive (anhedonic-like) group exhibited decreased expression of telencephalic CB1R. In contrast, CUS-resilient and CUS-sensitive groups exhibited a similar pattern in the anxiety-like parameters. CUS increased the erratic movements and psychomotor behaviour (anxiogenic-like effect). DMI treatment seems to reverse most of the CUS effects and produce an anxiolytic-like behaviour in the absence of CUS (decrease of diving response and reduction of erratic movements). Our data suggest that CUS reduced CB1R expression specifically in the zebrafish sub-population associated with anhedonic-like phenotype. Furthermore, the increased psychomotor behaviour may reflect the agitation aspect of depressive-like phenotypes. Taken all together, zebrafish CUS model constitutes a useful tool in the research of mood disorders, especially regarding the agitated depression.

MOLECULAR VIBRATIONS: AN IMPORTANT FEATURE OF ODOUR RECOGNITION BY THE OLFACTORY RECEPTORS

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The mainstream theory of odour perception by olfactory receptors is based on the lock-and-key model. According to this, size, shape and functional groups of odorant compounds determine activation of olfactory receptors. Once an appropriate odorant molecule binds to an olfactory receptor, the receptor is activated and triggers a neural signal (1). However, this theory cannot explain why some molecules with completely different structure have identical smell and moreover why some molecules with almost identical shape have different smell. Alternatively, the vibrational theory posits that differences in key vibrational frequencies of odorant compounds contribute to odour perception (2). It has been demonstrated behaviourally that *Drosophila melanogaster* can distinguish between deuterated and regular odorants despite the fact that the shapes of such compounds are identical, but differ in the vibrational spectra. In this study, we used electrophysiological and Ca-imaging methods to test the response of a group of *Drosophila melanogaster* olfactory sensory neurons to normal and deuterated odours. We provide evidence that odour molecules that have the same shape but different molecular vibration spectra produce different neuronal activation maps in a concentration dependent manner and that the ability to distinguish between isotopologues is a property of many olfactory receptors. We also examine the role of the odour impurities as well as the perireceptor events in the differential activation of the olfactory receptors. Our findings confirm and explain prior behavioral observations and indicate that molecular vibrations contribute to the olfactory receptors activation.

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ADULT HIPPOCAMPAL NEUROGENESIS ALTERATIONS IN THE DSS MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE (IBD)

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The gut-brain axis is a bidirectional communication system that integrates neural, immune and hormonal signals between the brain and the gut. Alterations in this axis have been shown to be implicated in the

pathophysiology of several brain disorders, including depression and anxiety. Although accumulating evidence support a clear association between gut inflammation and cognitive behavior, the molecular mechanisms underlying this connection remain to a large extent unknown. Existing evidence points to the role of adult hippocampal neurogenesis in this process since the constant production of hippocampal neurons is tightly linked to cognitive functions and its disruption is associated with depression and anxiety disorders. Here, we sought to address the question whether intestinal inflammation has an impact on hippocampal neurogenesis and behavior, using a well-characterized mouse model of Inflammatory Bowel Disease (IBD) based on the administration of dextran sodium sulfate (DSS) in the drinking water. Acute administration of DSS (7 days) increased the number of actively proliferating neural stem cells and immature neurons, accompanied by the activation of the resident microglia and astrocytes in the hippocampus. Long-term administration of DSS (28 days), which recapitulates the disease pattern of IBD in humans, also increased the number of neuronal progenitors but surprisingly, did not affect the number of mature newborn neurons. Conclusively, our findings indicate that acute intestinal inflammation stimulates adult neurogenesis but does not increase the total number of newborn neurons. This paradoxical effect is possibly caused by a defect in the neuronal maturation/survival process. The exact molecular mechanisms underlying the DSS-caused alterations in neuronal maturation is under investigation.

OLFACTORY LEARNING REQUIRES NEUROFIBROMIN FUNCTION WITHIN A SUBSET OF GABAERGIC NEURONS IN DROSOPHILA

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Neurofibromatosis type 1 (NF1) is a complex multi-system disorder, occurring in approximately 1 in every 3000 births and inherited in an autosomal dominant manner. NF1 is caused by loss-of-function mutations in the Nf1 gene, which encodesneurofibromin, a protein associated with regulation of multiple processes, including Ras inactivation (RasGTPase-activating protein) and adenylyl cyclase activation. Neurofibromin is a large cytoplasmic protein expressed in almost all tissues, but is more abundant in the brain, the spinal cord and the peripheral nervous system. Nearly half of NF1 patients present with specific cognitive impairments, including deficits in executive functions, attention, language, visual perception, and learning. However, the molecular and cellular circuitry whose perturbation underlies these cognitive dysfunctions remains poorly understood and difficult to study in patients, requiring the use of experimental animal models for their study. Loss of the highly conserved Drosophila *dNf1* ortholog results in organismal size reduction and deficits in associative learning and memory, thus resembling human NF1 symptoms. We have previously shown that pan-neuronal expression of a dNf1 transgene, is sufficient to reverse the associative learning deficit of dNf1 null mutants. The same study has also revealed the functional interaction between dNf1 and the Receptor Tyrosine Kinase Anaplastic Lymphoma Kinase (Alk). dAlk was shown to act as a negative regulator of olfactory associative learning in the fly and pan-neuronal abrogation of its activity restored to normal the reduced size of dNf1 null mutants and ameliorated their learning deficits.Therefore, the main objectives of this work is to define a) the neuronal circuit where dNf1 is normally required for associative learning and b) which of neurofibromin's functions and implicated signaling cascades are important for learning in these specific neurons. Our results a) describe for the first time a novel GABAergic neuronal circuit outside the MBs, the higher brain region essential for learning and memory in Drosophila, where dNf1 expression is necessary and sufficient for normal olfactory associative learning and b) suggest an Alk- and Ras1- pathway-dependent learning deficiency associated with NF1 in these specific neurons.

GPCR COMPLEXES IN THE RAT CLAUSTRUM: KAPPA OPIOID (KOR), MU OPIOID (MOR) AND SOMATOSTATIN 2 (SSTR2) RECEPTOR

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The claustrum is an enigmatic brain region, believed to play a role in human consciousness. The κ-opioid (KOR) and the somatostatin 2 (SSTR2) receptor are strongly expressed in this area. They belong to G-protein coupled receptors (GPCRs), which can form homo- and heteroreceptor complexes that allosterically modulate each other's signaling. Both have been shown to interact with the μ-opioid receptor (MOR). In situ proximity ligation assay (PLA) in rat brain slices was therefore used to examine receptor dimers in the claustrum. Although the presence of KOR-MOR appeared less impactful, KOR-KOR and SSTR2-MOR were considerably dense, while KOR-SSTR2 heterodimerization was demonstrated for the first time. These findings could shed light on claustrum's role in health and disease.

EXPOSURE TO A MILDLY AVERSIVE EARLY LIFE EXPERIENCE LEADS TO PREFRONTAL CORTEX HYPOMYELINATION AND BEHAVIORAL DEFICITS IN THE RAT

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Aversive early life experiences in humans have been shown to result in deficits in the function of the prefrontal cortex (PFC). Social adversity in neonatal life has been linked with cognitive dysfunctions that correlate with white matter alterations. Nevertheless, the impact of early adversity on the myelination of the prefrontal cortex is poorly understood. In order to study this relation, we utilized an experimental model of maternal neglect in rats, in which the mother is present but intermittently unavailable to the pups. This early experience involves exposure of rat pups during postnatal days (PND) 10 to 13 to a T-maze in which they search for their mother but upon finding her they are prohibited contact with her, thus being denied the expected reward (DER). We determined in adult males A. the performance in a PFC-dependent task i.e. reversal learning in a T-maze and B. the levels of myelin basic protein (MBP) and oligondendroglia (O2 positive) cells in the PFC corpus callosum. We found that the DER early life experience results in behavioral inflexibility indicative of impaired PFC function, accompanied by decreased levels of MBP without any effect on the number of oligodendroglial cells. These findings indicate that an adverse early life experience can modify prefrontal cortex myelination in adulthood and thus interfere with normal cognitive function.

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TARGETING NUCLEAR RECEPTOR NR5A2/LRH-1 IN NERVOUS SYSTEM MALIGNANCIES

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Glioblastoma and neuroblastoma are nervous system malignancies, characterized by rapid progression and poor survival rate [1, 2]. These clinical observations underscore the need for novel insights in the mechanisms of malignant transformation and progression. To this end, recent data from our lab have shown that nuclear receptor NR5A2/LRH-1 (Nuclear Receptor Subfamily 5 group A Member 2 or Liver Receptor Homologue 1) exerts a strong anti-proliferative and anti-gliogenic effect on neural stem/precursor cells (NSCs) [3, 4]. Considering that both tumors may

originate from NSCs [1, 5], NR5A2 is a candidate gene with a potential role in suppressing nervous system related malignances. In agreement, meta-analysis of clinical data from TCGA and Oncomine databases, suggest that NR5A2 is down-regulated in Glioblastoma tumors as compared to healthy tissue. Here, we experimentally investigated this hypothesis, by testing whether NR5A2 and its pharmacological agonists (DLPC and DUPC) are able to inhibit glioblastoma and neuroblastoma cancer cell growth. Our studies showed that NR5A2 is sufficient to strongly suppress proliferation of both human and mouse neuroblastoma (SH-SY5Y, Neuro2A) and glioblastoma cells (U87-MG, GL261) without affecting apoptosis. The anti-proliferative effects of NR5A2 are mediated by the induction of negative regulators of cell cycle, $p27^{kip1}$ and $p21^{cip1}$, as well as Prox1,a tumor suppressor protein in neuroblastoma cells [6]. We next examined the ability of DLPC and DUPC to mimic the anti-proliferative action of NR5A2. Indeed, these agonists were able to inhibit proliferation in human glioblastoma cell line U87-MG through the induction of the previously mentioned tumor suppressors. Most importantly, treatment with DLPC reduced tumor growth in xenografts model of NOD-SCID mice. These data reveal a tumor suppressor role of NR5A2 in Nervous System and render this nuclear receptor an important pharmacological target for the treatment of nervous tissue related tumors.

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THE RECEPTOR TYROSINE KINASE ALK IS EXPRESSED IN THE A/B DENDRITES OF ADULT DROSOPHILA MUSHROOM BODIES AND INHIBITS LONG-TERM MEMORY FORMATION

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How Receptor Tyrosine Kinases participate to the formation and regulation of memory remains poorly documented. Using Drosophila as model system, we present evidence here that beyond its established role in learning, the highly conserved fly RTK dAlk is a novel *inhibitor of protein synthesis-dependent long-term memory formation*. We have determined that while learning inhibition requires the presence of dAlk exclusively in neurons located outside of the MBs, LTM inhibition is strictly contingent on MB-expressed dAlk, which is specifically enriched at the postsynaptic active zones in dendrites of the MBs ✔/🚲 intrinsic neurons. We show that attenuation of dAlk expression in adult MBs facilitates LTM formation, resulting in significant improvement of memory performance, whereas overexpression of the receptor precipitates severe LTM impairment. Learning, early memories, or anesthesia-resistant memory, in contrast, are not affected. Finally, we demonstrate that although its activating ligand Jeb is dispensable to inhibit LTM, dAlk itself is required at the earliest stages of the conditioning procedure, when its dendritic levels rapidly increase upon the first association of the two conditioning stimuli, a process which is specifically controlled by the 3'UTR sequence contained in its mRNA. We propose hence that dAlk tempers excessive memory formation by preventing or eliminating, at the earliest stages of conditioning, the formation of irrelevant and energy costly long-lasting memories, therefore acting as a novel memory filter.

CROCIN FROM *CROCUS SATIVUS L.* EXERTS SIGNIFICANT CYTOTOXICITY ON A MEDULLOBLASTOMA HUMAN CELL LINE

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Medulloblastoma is a highly invasive tumour, disseminating throughout the central nervous system early in its course. Despite the high 5-year-survival rate, a significant number of patients demonstrate serious sequelae and high mortality rates, unrelated to the initial malignancy itself but rather to the aggressive treatment. A strong rationale exists for the use of *Crocus sativus L* (saffron) and its bioactive constituents (crocin, crocetin, safranal) as pharmaceutical agents, as they exert significant health-promoting properties. Crocins, unlike other carotenoids, are highly water-soluble compounds, with relatively low toxicity. They have attracted wide attention as promising anti-cancer agents, due to their antioxidant, anti-inflammatory, and immunomodulatory effects, interference with transduction pathways implicated in tumorigenesis, angiogenesis, and metastasis (disruption of mitotic spindle assembly, inhibition of DNA topoisomerases, cell-cycle arrest, apoptosis or cell differentiation) and sensitization of cancer cells to radiotherapy and chemotherapy. The current research aimed to study the potential cytotoxic effect of crocins on TE671 medulloblastoma cell line, which may be useful in the optimization of existing and development of new therapeutic strategies. Crocins were extracted from stigmas of saffron in ultrasonic bath, using petroleum-ether, diethylether and methanol 70%v/v as solvents and the final extract was lyophilized. Identification of crocins according to HPLC analysis was determined comparing the UV-vis spectra and the retention time (t_R) of the peaks with literature data. For the biological assays crocin was diluted to water. TE671 cells were incubated with a range of concentrations of crocins (16, 8, 4, 2, 1, 0.5 and 0.25 mg/ml) for 24, 48, 72 and 96 hours. Analysis of cell viability after incubation with crocins was performed with Alamar Blue viability assay. HPLC analysis indicated that the most abundant crocins in our extract were trans-crocin-4 and trans-crocin-3. Crocins exerted significant cytotoxicity in a dose and time-dependent manner ($p<0.005$ for exposed cells to any concentration at 48, 72 and 96 hours *versus* cells not exposed); as their concentration and time of exposure increased, the reduction of resazurin to resofurin decreased, indicating reduction in cell viability. IC50 values for each time point were calculated ~3.73, 1.72, 0.87 and 0.75 mg/ml at 24, 48, 72 and 96 hours, respectively. The results of our study could afford the basis of research regarding the use of natural carotenoids as anticancer agents and the shift to targeted therapy with higher efficacy and limited toxicity.

Acknowledgements
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GEMININ IS ESSENTIAL DURING EARLY CORTICOGENESIS FOR THE MAINTENANCE OF PROLIFERATING NEURAL PROGENITORS

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Before the onset of neurogenesis, the neural tube is composed of neuroepithelial cells organized in a single layer, the neuroepithelium. Neuroepithelial cells undergo symmetric divisions in order to expand the progenitor pool and during the early neurogenesis, at around the tenth embryonic day, they switch to asymmetric divisions in order to give rise to partially or fully differentiated cell populations. The production of the different cell types that compose the embryonic cerebral cortex demands the coordination of cell fate determination and the regulation between self-renewal and differentiation decisions. Defects in the production of the cell populations can be associated with abnormalities in cortical development leading to syndromes, such as microcephaly. An important regulator of cell fate decisions during embryogenesis is the molecule Geminin[1]. Previous studies from our lab indicated Geminin's role during corticogenesis. Specifically deletion of Geminin from radial glial cells of the CNS (E12) leads to a significant increase of the neural progenitor populations accompanied by a reduction of the deep layer neurons and an increased production of upper layer neurons[2]. In order to study neuroepithelial cells, we used $Gem^{Fl/KO}$;Foxg1Cre transgenic mice knocking out conditionally Geminin from neuroepithelial cells of telencephalon during E8.5. The mice are developing severe microcephaly. In order to understand the mechanism leading to microcephaly, we incorporated thymine analogue in order to investigate the cell cycle profile of neural progenitor cells and performed immunofluorescent staining in E10.5 embryos scoring the neural progenitors and the newly formed neurons. We observed a significant decrease in the number of proliferative neural progenitor cells and a reduced number of neural progenitors capable of exiting the cell cycle and differentiating into neurons implying defects in neurogenesis. In addition, we scored caspase 3 expression and observed increased apoptosis upon loss of Geminin. As a consequence of the above, in the absence of Geminin, a deficient cortical development is observed resulting in the phenotype of microcephaly. Our results aim to shed light on the role of Geminin during the early stages of neuroepithelium establishment in mouse cortical development and to highlight possible mechanisms that lead to microcephaly in humans.

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BLOOD-BORNE LIRAGLUTIDE TRANSPORT INTO THE HYPOTHALAMUS: A TANYCYTIC ROUTE?

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Liraglutide is a glucagon-like peptide-1 (GLP-1) analog that has been shown to mediate several aspects of energy balance regulation through the arcuate nucleus of the hypothalamus (ARH). In order to reach their neuronal targets, circulating peripheral hormones like GLP-1 have to first pass the blood brain barrier (BBB) either at the level of BBB vessels or of circumventricular organs (CVO) such as the median eminence laying just adjacent to the ARH. The median eminence, which forms the floor of the 3rd ventricle, contains highly specialized ependymoglial cells, called tanycytes. These tanycytes play an active role in shuttling circulating metabolic signals, such as leptin, into the cerebrospinal fluid, which may then freely diffuse into the hypothalamus. It has been shown previously that Liraglutide reaches different CVO in a GLP-1 receptor (GLP-1R) dependent manner. However, the cellular mechanism underlying its transport and the nature of the neuronal populations on which it exerts its action, remain to be identified. Here, we show that tanycytes of the median eminence are the first cells of the hypothalamus to perceive Liraglutide as early as 30 seconds after it is intravenously (IV) injected. Furthermore, Liraglutide induces CREB phosphorylation in median eminence explants from mice, after 30 seconds of the IV injection, supporting the involvement of the GLP1-R pathway in the uptake. When the fluorescent Liraglutide reaches the arcuate nucleus, it binds to POMC neurons 60 seconds after its IV injection. To validate this data, *In vivo* (tanycytic primary culture) and *in vitro* studies are currently in progress to decipher the molecular mechanism underlying transcytosis of Liraglutide in tanycytes.

THE ROLE OF SODIUM AND POTASSIUM CHANNELS IN AXONAL CONDUCTION VELOCITY SPEEDING DURING POSTNATAL MATURATION IN DROSOPHILA

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The remodeling of the central nervous system during the period of the postnatal maturation can be decisive for the establishment of either healthy sensory-motor and cognitive abilities or the manifestation of neurological or psychiatric disorders in the adult life. Therefore, it is highly important to elucidate the cellular and molecular mechanisms of the structural (i.e. dendritic architecture) and functional (i.e. passive and/or active membrane properties) changes occurring at the level of central neurons during postnatal remodeling. In vertebrates, central neurons increase dramatically their conduction velocity during post-natal period and, interestingly, this change seems to depend negligibly on myelination or axonal size. In our study, the Giant Fiber (GF) interneuron of the neural circuit Giant Fiber System (GFS), mediating the escape reflex in Drosophila, is subject to functional changes due to postnatal maturation observed during the first day (24h post-eclosion, PE) of adult fly life. Specifically, the conduction velocity in the GF axon is 79% higher (conduction velocity speeding) in mature flies (1 day PE) compared to newborns (1 hour PE). We predicted that this difference is more likely attributable to changes in the number or modulation of ionic channels than in structural changes, given that the GF is a non-myelinated nerve cell and its axon assumes its final diameter (7-8μm) during pupal life. Our data demonstrate that knock down of genes encoding voltage-gated sodium (*para-RNAi*) or potassium (*shaker-RNAi*) channels, specifically in the GF, decreases the GF axonal conduction velocity in mature flies by 62% and 41%, and in young flies by 33% and 7.7% correspondingly. On one hand this suggests that both channels contribute to action potential generation and propagation in the GF axon. On the other hand, lower conduction velocity due to silencing of the aforementioned genes resulted in a decrease of postnatal conduction velocity speeding from 79% in the controls to 47% in *para-RNAi* flies and 37% in *shaker-RNAi* flies. Hence, it is likely that the GF of newly-eclosed flies possess a lower population of functional sodium and potassium channels and that the recruitment of more of these channels is necessary for appropriate conduction velocity speeding during postnatal maturation.

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DIFFERENTIAL ELECTROPHYSIOLOGICAL PHENOTYPES OF HUMAN TAU ISOFORMS IN AN IDENTIFIED NEURAL CIRCUIT IN DROSOPHILA

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Tauopathies are a heterogeneous group of neurodegenerative diseases that include Alzheimer's disease (AD) and the frontotemporal dementia with parkinsonism linked to chromosome-17 (FTDP-17). These diseases are characterized by aggregation of the microtubule associated protein tau into neurofibrillary tangles. In humans, elevated wild-type tau in the CNS leads to AD, while mutations affecting microtubule binding are causal of FTDP-17. However, the mechanisms underlying tau-mediated neuronal dysfunction are not clear. In general, it is believed that dysfunction or loss of synapses might be the main cause for the progressive loss of learning and memory abilities, which are the pathological hallmarks of dementias. To gain more insights into the pathobiology of tauopathies, given the complexity of human brain, we targeted the expression of human wild-type (0N4R, 0N3R) and mutant ($0N4R^{R406W}$) tau isoforms in the neurons of a simple and well characterized neural circuit, mediating the escape reflex in Drosophila, the Giant Fiber System (GFS). The examined part of the circuit consists of a series of three neurons: two interneurons (GF and PSI) and one motorneuron (MN5) innervating two flight muscles (DLM5-6). Our analysis shows that overexpression of 0N4R increases the synaptic delay in the central cholinergic synapse between PSI and MN5, while overexpression of 0N3R decreases the fidelity of responsiveness of the same synapse during high frequency stimulation. On the other hand, the mutant tau isoform $0N4R^{R406W}$, which is associated with Frontotemporal Dementia, has no effect. Thus, presynaptic function is mildly and differentially compromised in a particular cholinergic synapse, suggesting tau involvement in neurotransmitter release or calcium mediated exocytosis mechanisms. The divergent phenotypes indicate that the effect of the tau isoforms is not a generalized consequence of exogenous protein expression, but likely the result of different isoform specific physiological actions leading to distinct synaptic dysfunctions. Furthermore, the aforementioned electrophysiological phenotypes manifest in middle-aged (30-day old) and aged (45-day old), but not in young (10-day old) individuals, which is in agreement to age-dependent onset and progression of degenerative dementias in humans.

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DEVELOPMENT OF AN INTRATHECAL GENE THERAPY APPROACH IN MODELS OF CMT1X INHERITED NEUROPATHY

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Inherited demyelinating peripheral neuropathies are progressive incurable diseases without effective treatment. To develop a translatable gene therapy approach targeting myelinating Schwann cells, we delivered a lentiviral vector using a single lumbar intrathecal injection and a myelin-specific promoter. The human gene of interest, *GJB1*, which is mutated in X-linked Charcot-Marie-Tooth Disease (CMT1X), was delivered into adult *Gjb1*-null mice, a genetically authentic model of CMT1X that develops a demyelinating peripheral neuropathy. We obtained widespread, stable, and cell-specific expression of connexin32 (Cx32) in up to 50% of Schwann cells in multiple lumbar spinal roots and peripheral nerves. Behavioral and electrophysiological analysis revealed significantly improved motor performance, quadriceps muscle contractility, and sciatic nerve conduction velocities. Furthermore, treated mice exhibited reduced numbers of demyelinated and remyelinated fibers and fewer inflammatory cells in lumbar motor roots, as well as in the femoral motor and sciatic nerves. Based on these findings we further tested this approach in mutant mice carrying three different CMT1X mutations, T55I, R75W and N175D, expressed on a Cx32KO background. All three Cx32 mutants were localized in the perinuclear compartment of myelinating Schwann cells *in vivo* consistent with retention in the ER (T55I) or Golgi (R75W, N175D) with loss of physiological expression in non-compact myelin areas. Following intrathecal gene delivery of the human *GJB1* gene we could detect the virally delivered WT Cx32 correctly localized in the non-compact myelin areas at high rates only in T55I/Cx32KO mutant mice, but almost never in the other two mutants, suggesting dominant effects of the R75W and N175D mutants but not of the T55I mutant on co-expressed WT Cx32. *GJB1* treated T55I/Cx32 KO mice showed improved motor performance, along with lower ratios of abnormally myelinated fibers and reduced numbers of inflammatory cells in all tissues examined compared to mock-treated animals. In contrast, N175D mutant mice showed only partial and R75W mutant mice showed no phenotype improvement after treatment. This study demonstrates that a single intrathecal lentiviral gene delivery can lead to Schwann cell-specific expression in spinal roots extending to multiple peripheral nerves. Although this approach seems to be promising for the treatment of demyelinating peripheral neuropathies we also show that certain CMT1X mutants may interfere with gene addition therapy for CMT1X, requiring an alternative design of the gene therapy.

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TARGETING NF1 TRANSCRIPTS WITH SPECIFIC SHRNAS REVEALS ISOFORM-SPECIFIC FUNCTIONS

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Autosomal dominant or sporadic (prevalence 1:3,000) mutations in one copy of the *NF1* gene cause Neurofibromatosis 1 (NF-1), a progressive disease with diverse symptoms stemming from abnormalities in cell proliferation and differentiation, including the formation of benign tumors and unfortunately malignant neoplasms of the CNS and PNS. The latter, despite radical treatments, have an unfavorable prognosis of life expectancy. Thus, it is important to understand the function of the *NF1* protein product, the tumor suppressor neurofibromin. Our most recent data have placed neurofibromin as a protein that regulates chromosome segregation and assures production of euploid daughter cells. More specifically, we have provided a mechanistic model for the regulated nuclear import (due to a functional NLS in the C-terminus) of this tubulin-binding protein into the premitotic nucleus in order to localize on the spindle throughout mitosis. Moreover, we discovered that (RNAi-imposed) loss of this tumor suppressor leads to errors in chromosome congression, a prelude to aneuploidy. The novelty of our data also impacted the way we approach the question of the function of this RasGAP. The first concept, as mentioned, names the dosage of NLS+-neurofibromin as critical for chromosome congression, while the second concerns the existence of multiple transcripts/isoforms that may have the ability or not to enter the nucleus and to be potent or weak RasGAPs. Indeed, previous studies by us and others revealed that human *NF1* transcripts may include or skip the NLS-bearing exon51, that the *NF1*+NLS transcripts are the most abundant in all tissues while the ΔNLS are expressed at very low levels in selected tissues and only those that present pathology in NF-1. Similarly transcripts may include or skip exon 31, resulting in the type II or I isoforms, respectively, with known differential RasGAP activities. These mRNA editing events may occur in the same cell and thus produce as many as four neurofibromins: GRDI/II x NLS/ΔNLS. In our effort to explore the fundamental questions of whether this multi-domain protein exists as different isoforms serving different functions and purposes, none of which is yet understood, we employed the use of shRNAs, delivered by tet-on lentiviruses, that specifically target prospective NF1 mRNAs. Providing proof of concept, targeting of either GRDI or GRDII transcripts resulted in differential increases of Ras activation by EGF, as assessed by Ras-affinity

precipitation assays. Moreover, targeting of NLS sequences resulted practically in depletion of neurofibromin expression, while the majority of metaphasic spindles exhibiting congression abnormalities. Collectively our data suggest that this approach will eventually allow us to prove the existence of multiple neurofibromin as well as define their individual properties.

DEVELOPMENTAL CHANGES IN EARLY POSTNATAL INHIBITORY CIRCUITS OF THE PREFRONTAL CORTEX

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The prefrontal cortex (PFC) controls higher cognitive abilities (working memory, decision making, inhibitory control, focused attention), many of which are affected in neurodevelopmental disorders, such as schizophrenia, autism spectrum disorders and epilepsy. The PFC is characterised by delayed development and matures during adolescence, when cognitive abilities reach adulthood levels. This delay is different compared to other primary sensory areas (such as the somatosensory barrel cortex (BC)) and allows for significant perturbations by environmental factors. Some adaptations in PFC development can lead to emergence of neurodevelopmental disorders. GABAergic interneurons contribute to fundamental aspects of physiological mature brain function and maturation. Therefore, it is important to understand the neuronal mechanisms underlying PFC development, and particularly the properties of inhibitory PFC circuits. Our aim is to investigate the early postnatal development of PFC interneurons, in comparison with BC, and study how their maturation affects the functionality of developing cortical circuits. We performed morphological and cellular experiments in the PFC and BC at postnatal day (P)10 and P20. Our experiments revealed differential changes in PFC and BC cell density. Moreover, during development of BC the total cell density is increased, but during PFC development, the cell density isdecreased. Overall, we observe a decrease in cell density in PFC vs BC at P20 and P45.In particular, we found decreased cell density of $Lhx6^+$ interneurons in contrast to early development of BC. The reduced number of $Lhx6^+$ cell density is dependent on decreased PVA^+ cell density and not SST^+ interneurons. We also performed whole-cell patch clamp recordings from pyramidal neurons and interneurons of the PFC and BC, at P10 and P20. PFC pyramidal neurons at P10 exhibited action potentials with reduced amplitude and rate of rise of action potential compared to P20, indicative of incomplete maturation of sodium channels. Also, no fast-spiking (PVA) interneurons were recorded in PFC at P10. In addition, GABAergic synaptic function also changes from P10 to P20 in a different way between PFC and BC. Specifically, field excitatory postsynaptic potential (fEPSP) responses in the BC do not change upon application of diazepam, a GABA-A receptor agonist. However, in the PFC, diazepam increases fEPSPs at P10, indicating a depolarizing effect of GABA-A receptor activation. At P20, diazepam does not alter the fEPSPs in either structure. These results show that significant maturational events are on-going between P10 and P20 in the PFC, both in the excitatory and inhibitory neurons.

GEMININ DELETION FROM EARLY NEURAL PROGENITORS RESULTS IN REPLICATION-STRESS RELATED MICROCEPHALY

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Mutations in the genes that form the pre-replication complex (Pre-RC) are responsible for a severe type of primordial dwarfism with microcephaly, known as Meyer-Gorlin Syndrome (MGS). The increased replication stress that was detected in cells isolated from MGS patients is the main cause of the clinical features that are observed indicating that specific tissues are susceptible to defected DNA replication. Intriguingly, there is a group of MGS patients that carry mutations in the gene of Geminin, a known inhibitor of DNA replication[1]. Previous studies have shown that in vitro inactivation of Geminin causes re-replication, a source of replication stress and subsequent DNA damage-induced apoptosis[2]. Moreover, our lab has shown that Geminin regulates self-renewal and differentiation decisions of neural progenitor cells during mid-corticogenesis, by regulating the S phase duration[3]. To further investigate the role of Geminin in the regulation of DNA replication and brain development we have addressed a Foxg1Cre transgenic mouse model to delete Geminin from early telencephalon. We demonstrate that upon deletion of Geminin a dramatic decrease in the size of the developing cortex and incomplete formation of facial characteristics are observed, characteristics similar to human microcephaly. Neuroepithelial cells reveal increased DNA damage and cell cycle defects, resulting in gradual loss of stem cells pool and incomplete neurogenesis. In the remaining population of neural progenitors, we identified increased signs of replication stress. Our current research focuses on deciphering the differential necessity for Geminin during organismal development that will suggest a possible mechanism leading to developmental defects in mice and humans.

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PROX1 AFFECTS NEURONAL MATURATION DURING CENTRAL NERVOUS SYSTEMDEVELOPMENT

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Prox1 is a master regulator of embryonic and adult neurogenesis viainduction ofcell cycle exit and acquisition of early neuronal identity in neural stem/progenitor cells[1-5].Paradoxically, in the majority of newly born neurons, Prox1 expression is heavily down-regulated during the transition from the state of immature to terminal differentiatedneurons, 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 activity.Gain-of-function analysis showedthat 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 lengthin both cortical and spinal cord neurons.To delineate 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, MAPK and neuronal activity-dependent signaling pathways. By real time RT-qPCR analysis, we confirmed the effect of Prox1 on these genes.Moreover, in primary mouse neuronal cells we confirmed theProx1-mediated reduction in *Camk2b* gene expression, while ChIP assays revealed an interaction between Prox1 transcription factor and the promoter of *Camk2b* gene.Camk2b is aCa(2+)/calmodulin-dependent protein kinase playing an important role in synaptic activity/ plasticity and neurite outgrowth[6-8]. Collectively, these observations suggest that Prox1may act as a potent suppressor of neuronal maturationthrough the Ca^{+2} signaling pathway during the early stages of

neurogenesis. In the later stages, Prox1 is down-regulated to allow axon elongation and acquisition of terminal neuronal identity.

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GEMC1/LYNKEAS IS THE MASTER REGULATOR OF RADIAL GLIAL CELLS FATE INITIATION TO THE EPENDYMAL LINEAGE

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In the brain, multiciliated ependymal cells line the walls of the lateral ventricles extending multiple motile cilia that acquire a coordinated beating to propel the cerebrospinal fluid (CSF). Ependymal precursors, radial glia, become specified to this cell lineage during embryogenesis, whereas differentiation takes place the first two postnatal weeks. Ependymal cilia dysfunction abolishes CSF circulation leading to neurological disorders, such as hydrocephalus. Investigation of key-molecules which drive the pathway of multiciliogenesis will shed light to the molecular mechanisms involved in these disorders. Previous studies from our laboratory revealed that GemC1/Lynkeas participates in the fate commitment of radial glial cells towards the ependymal cell lineage[1]. To address the mechanisms regulating the generation of ependymal cells, we established an in vitro primary culture of radial glial cells (RGCs). We initially studied the expression of McIdas, one of the upstream regulators of multiciliogenesis, in differentiating radial glial cells[2]. McIdas was expressed in high levels during the first steps of differentiation of RGCs when centriole amplification takes place. To further investigate the role of GemC1/Lynkeas in the derivation of ependymal cells, we generated transgenic mice lacking the expression of GemC1/Lynkeas. Loss of GemC1/Lynkeas eliminates the expression of multiciliated cells markers in vivo during embryogenesis, like McIdas and Foxj1. Their expression is undetectable in differentiating radial glial cells, suggesting that commitment to the lineage is blocked. Furthermore, primary cultures derived from GemC1/Lynkeas deficient mice are enriched in cells with astroglial characteristics. Summarizing our research provides evidence on the pivotal role of GemC1/Lynkeas in the fate commitment of RGCs towards the ependymal lineage.

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EFFECTS OF GABAA AND GABAB MEDIATED INHIBITION ON CORTICAL NETWORK ACTIVITY ACROSS THE LIFESPAN

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Neuronal populations have the ability to self-organize into functional networks that generate spontaneous synchronized network activity. During "quiescent states" (slow wave sleep, anesthesia), these spontaneous fluctuations are called "slow oscillations" and are characterized by recurring transitions between a depolarized "Up-state" and a hyperpolarized "Down-state" (~1Hz). This spontaneous network activity (SNA) is a feature of both developing and mature cortical networks and its initiation, maintenance and termination is regulated both by recurrent excitatatory and inhibitory connections, and by neuromodulation. However, both the SNA and the inhibitory circuitry undergo profound changes with development and aging, and the specific effects of GABAergic inhibition on Up states at each stage have not been studied. Hence, the aim of this study was to investigate the distinct roles of fast (GABAa-mediated) and slow (GABAb-mediated) inhibition in spontaneous Upstate activity throughout the mouse lifespan.
Our study was conducted in acute brain slices of the barrel cortex and SNA was assessed by local field potential (LFP) recordings. Spontaneous Upstates were recorded in 3 age-groups: (a) pups (16-20do), (b) adolescents (42-48do), (c) adults (3-9mo). Increasing concentrations of Gabazine (50nM, 100nM, 200nM) and CGP55845 (50nM, 100nM, 250nM, 1000nM) were used for the blockade of GABAa and GABAb receptors, respectively; and several parameters of Upstate activity were quantified. The experiments examining **fast inhibition** revealed that the effect of gabazine was both age and dose-specific: Low doses of the drug (50-100nM) led to an *increase* in Upstate *occurrence*, but only in adolescent and adult mice. This age-specific increase brought the occurrence of the two older age groups up to the higher level of the pups, thereby eliminating the developmental differences. In addition, low gabazine levels also caused an *increase* in Upstate *duration* but only in pups, thereby reinforcing the existing developmental differences. In contrast, the high dose of gabazine (200nM) caused a significant *decrease* in Upstate activity (both in occurrence and duration), and triggered the appearance of SWD activity at all ages. The experiments examining **slow inhibition** showed that blockade of GABAb receptors caused a uniform *increase* in Upstate *duration* in all age groups; but had an age-dependent "biphasic" effect on Upstate *occurrence*: it caused a *decrease* in young animals and an *increase* in adults.
These results indicate that inhibition has a highly complex and age-dependent effect in Upstate mechanisms, and that GABAergic developmental differences should be considered when studying the ontogeny of this phenomenon.

POTENTIAL USERS OF VISUAL PROSTHESIS: THE IMPORTANCE OF FOCUS GROUP DATA IN ENROLLMENT AND RETENTION OF IDEAL CANDIDATES

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Clinical trials for visual prosthetics pose many challenges, including identification of the ideal patient, pre-and post-operative testing [1, 2, 3], and more ethical considerations than pharmacological clinical trials. Visual prosthetic and, generally, neuro-prosthetic trials are limited by small number of participants as well as extended testing processes and high risk. With three prosthetic devices approved for clinical trials [4, 5], the investigation of patient enrollment and retention is highly germane. Little is known about patient attitudes toward implantation, testing, along with hopes, fears, perceived risks and benefits about involvement. Moreover, what is known mostly concerns cortical visual prostheses [6, 7]. Data directly from affected individuals could maximize trial benefits as early as the enrollment phase. Here, we propose a focus group to investigate blind individuals' perspectives in Greece about implanted visual restoration devices, concentrating on patient willingness and motivations for participation, preferred prosthesis device, concerns, levels of involvement, and type of informed consent, using both qualitative and quantitative data. As compared to previous studies, higher levels of willingness to participate due to lack of support/care networks are expected, along with an emphasis of the importance of patient-researcher communication in the selection and pre-operation phase, and of the involvement in choices about preferred functions and meaningful tests. The proposed project will contribute both to theoretical understanding of perspectives of visually impaired individuals, and to refining the selection strategies of ideal candidates for a neuro-prosthetic device.

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SYNTHETIC MICRONEUROTROPHIN BNN27 AMELIORATES AB PATHOLOGY AND PROMOTES ADULT NEUROGENESIS IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by progressive neuronal loss and cognitive decline. The major pathological hallmark of AD is the accumulation of the β-amyloid (Aβ) peptide within the brain. There is now strong evidence that the reduction of neurotrophin Nerve Growth Factor (NGF) is also involved in cell death and reduced neurorestoration. 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 $p75^{NTR}$ (*microneurotrophin*), promoting neuronal survival[1,2]. We examined the ability of BNN27 to ameliorate AD-related neuropathology, reducing Aβ toxicity as well as promoting the proliferation and/or survival of neuronal precursors *in vitro* and *in vivo*. BNN27 pellets were sub-dermally applied to 5xFAD mice, which harbor five familial AD mutations, prior to the development of any Aβ pathology (1.5 months of age). The pellets allowed a slow release of the compound over 6 weeks. BNN27 treatment significantly decreased the formation of Aβ plaques within the dentate gyrus of the hippocampus. Additionally, BNN27 effectively promoted adult hippocampal neurogenesis, significantly increasing the number of doublecortin (DCX) positive neurons, a marker of neuronal precursor cells and immature neurons, within the dentate gyrus of the hippocampus. BNN27 partially reduced the accumulation of oligomeric Aβ1-42 in the hippocampus of 5xFAD mice. It is of note that oligomeric Aβ1-42 reduces the proliferation of hippocampal neural stem cells. Furthermore, the integrity of myelin, axons and cholinergic neurons was also investigated. No significant changes of myelin and axonal integrity were observed in the hippocampus after treatment of 5xFAD mice with BNN27. However, BNN27 reduced cholinergic atrophy in basal forebrain of 5xFAD mice, significantly increasing the mean soma size of Choline Acetyltransferase (ChAT) positive 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. This hypothesis is currently under investigation.

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TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 (TRPA1) CHANNELS ON THE SCIATIC NERVE CONTRIBUTE TO THE DEVELOPMENT OF NEUROPATHIC PAIN

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Neuropathic pain is a persisting condition arising from damage in the somatosensory system. The prevalence of neuropathic pain is high, but the treatment options are limited. Nerve entrapments are a common cause for neuropathic pain (spinal canal stenosis, carpal tunnel syndrome), however the cellular and molecular mechanisms involved in the pathogenesis are not well studied. Here we postulate that mechanical pressure on the nerves during the entrapment might activate mechanosensitive ion channels on the axonal membranes which could cause Ca^{2+} to accumulate in the axoplasma and lead to sensitization and degeneration of axons. TRPA1, are Ca^{2+}-permeable ion channels and are considered as multimodal sensory receptors sensitive to a wide range of stimuli. These include environmental irritant chemicals like mustard oil and allicin, as well as endogenous products of ROS mediated oxidation and lipid metabolites. In addition TRPA1 are also sensitive to mechanical pressure and cold temperatures. They are expressed in subpopulations of primary afferent neurons involved in nociception. Using electrophysiological recordings of compound action potentials in isolated rat sciatic nerves and TRPA1 agonists and antagonists, we provide evidence of their functional expression on peripheral sensory axons. We then used the TRPA1 agonist allylisothiocyanate (AITC) on the sciatic nerve *in vivo*, and found that a single application of AITC can cause the development of mechanical hypersensitivity several days later. This indicates that activation of TRPA1 may underlie the development of neuropathic pain. In additional experiments, using fluorescence and isolated sciatic nerves, we explored the involvement of axonal mitochondria and metabolic activity in the AITC induced neuropathy. Our results provide a strong indication that chronic mechanical and chemical stimuli may involve endogenous mechanisms (i.e. mechanosensitive and chemosensitive ion channels) for the initiation and establishment of neuropathy and nociceptive hypersensitivity.

EVIDENCE OF SEX DIFFERENCES IN THE MICE PLASMA AFTER *I.P.* ADMINISTRATION OF TRANS-CROCIN-4 BASED ON A NOVEL UNTARGETED UPLC-HRMS-BASED METABOLOMICS APPROACH

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An important carotenoid constituent of saffron stigmas is trans-crocin 4 (TC4) a bis-ester of crocetin with gentiobiose [1]. TC4 has shown remarkable activity against Alzheimer's Disease due to its antioxidant and antiamyloidogenic properties [2]. Metabolomics is an emerging scientific field that enhances biomarker discovery and reveals underlying biochemical mechanisms aiming towards the early subclinical diagnosis of diseases. So far, scarce data demonstrate the changes induced to mice plasma metabolome after TC4 administration and none of them following *i.p* administration. Thus, an untargeted UPLC-High Resolution Mass Spectrometry (HRMS) metabolomics approach has been employed to determine the alteration to the metabolic fingerprint after *i.p* administration of TC4 in male and female mice. The protocol of this study included *i.p* administration of TC4 in 58 male and female mice (including control animals), plasma collection of the plasma samples in predefined time points and further analysis by UPLC-HRMS (Thermo Orbitrap Discovery XL). The results were statistically evaluated by multivariate analysis (MVA) in order to discover the variables contributing to the discrimination between a) treated and untreated groups, b) male and female and c) time points of sacrifice. MVA was performed through the open-source package of R-

language "mixOmics" including Principal Component Analysis PCA and (sparse) Partial Least Squares–Discriminant Analysis (s)PLS-DA. The variables contributing to each clustering were identified using comparisons to online databases (e.g., Metlin) along with software manipulations e.g. adduct and fragment identification, covariance searching etc. Due to the high variability imposed by various factors e.g., sex, dose and time points, the multilevel PLS–DA e.g. splitting variation to each individual component, has proven to be the only effective approach. Employing this methodology, the time sequence of metabolome changes due to the administration of TC4 has been made apparent. Furthermore, it is evident that there is a sex-related effect on the metabolome, denoting that the administration in both genders is indispensable in order to acquire safe conclusions as well as reliable metabolome pictures.

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SELECTIVE κ-OPIOID RECEPTOR LIGANDS EXERT A NEUROPROTECTIVE ROLE THROUGH INDUCTION OF AUTOPHAGY

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It is already evident that κ- and δ-opioid receptors exert a neuroprotective role in animal models attenuating histologic brain injury and promoting the recovery of global and focal cerebral ischemia with an unknown yet mechanism. Autophagy is a lysosomal degradation pathway, which eliminates misfolded proteins and dysfunctional organelles, essential for cellular survival and homeostasis. Recent observations have shown that methamphetamine induces autophagy in endothelial cells through the κ-opioid receptor(1) (κ-OR) however it is unknown whether, selective κ-opioid agonists mediate these effects and which is the signalling pathway that controls the pro-survival and pro-cell death effects of opioid induced autophagy.The primary goal of this study was to investigate whether selective κ-OR agonists induce autophagy in Neuro-2A cells stably expressing this receptor and define the molecular mechanism leading to these effects. Our data indicate that administration of κ-selective agonists increase the levels of the pre-autophagosomal and autophagosome proteins Beclin 1 and LC3-II respectively in a dose and time dependent manner. These effects are reversed upon exposure of neuroblastoma cells with the opioid receptor antagonist naloxone, suggesting that it is aκ-OR-mediated effect. Pre-treatment of cells with pertussis toxin and an inhibitor of ERK1,2 kinase activation blocked the κ-OR-induced Beclin 1up-regulation and LC3-II formation, suggesting that Gi/Go proteins and ERK1,2 signaling regulate κ-OR induced autophagy. Additional studies have shown that treatment of Neuro-2A cells with rotenone (an inhibitor of mitochondria complexI) increased ROS production and apoptosis while administration with the κ-OR specific agonistU-50488H reduces these effects. Collectively, the present results demonstrate a novel regulatory mechanism through which activation of κ-OR with specific ligands modulates cell death and autophagy promoting cellular survival.

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HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN A1 INDUCES ALTERNATIVE POLYADENYLATION OF ALPHA-SYNUCLEIN mRNA

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Converging evidence supports the concept that alpha synuclein (SNCA) pathology is integral to the pathological process in Parkinson's disease (PD) and a number of other diseases now categorized under the term alpha-synucleinopathies. Unraveling the physiological mechanisms that control α-synuclein expression at the mRNA level may provide critical insight into ways of interfering with protein accumulation and or localization. SNCA mRNA is alternatively polyadenylated to produce a transcript with approximately 2500 nt long 3'UTR. This transcript has been selectively linked to pathological processes in PD relative to the shorter transcript with 370-550 nt 3'UTRs. Using the long SNCA 3'UTR RNA as bait, we pulled-down from murine brain extracts HNRNPA1 as an interacting protein. Using overexpression and silencing studies, we found that HNRNPA1 significantly induced alternative polyadenylation towards the longer isoform in both HEK293 and SKNSH cells. In addition, HNRNPA1 reduced SNCA mRNA levels in SKNSH cells. Expression analysis of SNCA plasmid constructs bearing either the long or the short 3'UTRs revealed that the mRNA with the long 3'UTR was less stable and produced significantly less protein. Taken together, these findings identify for first time a mechanism controlling SNCA polyadenylation.

REPRESSION OF ALPHA-SYNUCLEIN EXPRESSION BY AU-RICH ELEMENT RNA-BINDING PROTEIN 1

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Genetic and biochemical studies have established a central role for α-synuclein (SNCA) accumulation in the pathogenesis of Parkinson's disease. Delineating and subsequently interfering with physiological mechanisms that control α-synuclein expression is one approach to limit disease progression. To this end, the highly conserved long and AT-rich 3'-UTR of SNCA was investigated for its role on mRNA translation. Using biotinylated SNCA 3'UTR RNA as bait, we pulled-down AUF1 from murine brain extracts. AUF1 is an RNA binding protein (RBP) that has been linked, among other nucleus-associated processes, to mRNA instability. Detailed examination of AUF1 binding affinity for the different segments of SNCA mRNA, revealed that it interacts strongly with the distal part of SNCA 3'UTR but not with the 5'UTR or CDS. Using overexpression and silencing studies, we showed that AUF1 lowers SNCA mRNA levels in SKNSH cells and protein expression in HEK293 as well as SKNSH cells. We, further, showed that this effect was mediated by the 3'UTR and that AUF1 preferentially regulated the SNCA isoform with the long 3'UTR. Finally, we found that AUF1 expression is significantly decreased during brain development. Taken together, these findings reveal a novel mechanism by which SNCA expression is regulated at the pre-translational level.

MIRK/DYRK1B KINASE INDUCES CELL CYCLE EXIT AND DIFFERENTIATION OF NEURAL PROGENITORS IN EARLY EMBRYONIC CHICK SPINAL CORD

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Regulation of cell cycle progression/exit of neuronal precursors is essential for proper generation of the nervous system. DYRKs constitute an evolutionarily conserved family of protein kinases with key roles in the control of cell proliferation and differentiation. Until to date only Dyrk1A has been reported to be expressed and involved in CNS development. Mirk/Dyrk1B kinase has been studied as a cell cycle regulator in skeletal muscle differentiation. We have first demonstrated that Dyrk1B is also expressed in the brain and in primary cortical neurons in culture, while it promotes cell cycle exit and neuronal differentiation in Neuro 2a cells. Here we cloned the chick Dyrk1B ortholog and showed that it is expressed by cycling neuronal progenitors, as well as by differentiated motor neurons in the embryonic chick spinal cord. Dyrk1B protein expression is decreased during embryonic CNS development. Furthermore, we used a *gain-of-function* approach to investigate the role of Dyrk1B *in vivo* in the chick spinal cord. Expression of Dyrk1B/GFP or control GFP protein was achieved by unilateral *in ovo* electroporation in the neural tube of E2 chick embryos, which were analyzed at E4. Analysis of $Dyrk1B^{+}/GFP^{+}$ cells showed reduced BrdU incorporation by 2.3-fold and decreased expression of mitotic marker PH3, of transcription factor Prox1 and of pluripotent marker Sox2, by 10, 4.9 and 1.5-fold respectively, as compared with GFP^{+} cells in control embryos. Moreover $Dyrk1B^{+}/GFP^{+}$ cells showed a neuronal fate phenotype, as indicated by increased expression of dorsal neurogenesis markers Pax3 and Pax7 by 2.3 and 1.9-fold respectively and differentiation markers Nkx6.1, Islet-1, Doublecortin and βIII-tubulin by 1.9, 2.1, 1.4 and 1.2-fold respectively. In conclusion, we identified Mirk/Dyrk1B as a novel dual inducer of cell cycle exit and neuronal differentiation *in vivo*. Phosphoproteomics and transcriptome analysis for the elucidation of signaling pathways affected by Dyrk1B are in progress.

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EVALUATION OF ANXIETY BEHAVIOR AND THE LEVELS OF COTININE IN PLASMA IN ADULT MICE AFTER WHOLE BODY EXPOSURE TO SMOKE

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The present study sought to investigate the effects of smoking (whole body exposure to smoke) on a) anxiety behavior and b) levels of cotinine in plasma blood in adult male mice (3-4 months old). Balb/c mice were randomly divided into 2 groups (12mice/group). The first group was exposed to clear air (Control group) and the other group was exposed to smoke of conventional cigarettes (Experimental group). The exposure of the mice to air/conventional cigarette smoke took place in an innovative smoking device, in which the animals were kept in a special chamber (3 animals/session). The control group was exposed to clear air for 30 minutes in the device's chamber.The experimental group was exposed to smoke of 1.5 conventional cigarettes containing 0.8 mg of nicotine/cigarette for 30 minutes too (whole body exposure).The behavioral analysis was assessed by using the open-field test. During an individual 10 min task, we measured the time mice spent in the periphery of the open field apparatus (thigmotactism time). Cotinine is the primary metabolite of nicotine, the major alkaloid of tobacco, the active ingredient in cigarettes and the biomarker of exposure to cigarette smoke. The open field test was conducted 90min after the exposure and some preliminary data showed that the mice that exposed to cigarette seem to be more anxious than the control mice. Furthermore, LC MS/MS was used for the determination of cotinine in blood plasma at specific times after the exposure.It was found that the peak concentration of cotinine (C_{max} of 13.30 ± 0.01 ng/mL) was at 115min after the smoke exposure. Experiments are in progress as we try to investigate further the behavioral changes after the exposure in different amount of cigarettes.

IMPAIRED AUTOPHAGY AND LYSOSOMAL ACTIVITY IN A HUMAN iPSC-BASED MODEL OF FAMILIAL PARKINSON'S DISEASE

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Parkinson's disease (PD) remains an incurable neurodegenerative disorder with variable clinical characteristics, age of onset and course of progression [1]. The hallmark of PD, whether sporadic or familial, is the deposition of protein aggregates, which are composed mainly of alpha-synuclein (αSyn) [2]. αSyn is a pre-synaptic protein with N-terminal binding to acidic lipids that can sense and generate changes in membrane curvature, suggesting its participation in presynaptic events, including endocytosis and exocytosis [3]. Its involvement in pre-synaptic organization has been postulated in mice [4], but there are no reports showing how mutant αSyn affects synaptic organization in a human setting. αSyn is the major gene linked to sporadic Parkinson's disease (PD) [5], while the G209A (p.A53T) αSyn mutation causes a familial form of PD characterized by early onset and a generally severe phenotype, including non-motor manifestations [6]. Using cell reprogramming technologies, we have 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 protein aggregation, compromised neuritic outgrowth and contorted axons with swollen varicosities containing αSyn and tau, as well as disrupted synaptic connectivity [7]. To further explore the morphological characteristics of p.A53T neurons we now use electron microscopy (EM), which indicates microtubule disorganization and reveals a striking accumulation of autophagic vacuoles within mutant neurons. In agreement, we show impaired autophagic activity and lysosomal protein degradation by immunofluorescence and biochemical analysis. A better understanding of these processes will help us elucidate αSyn-associated pathology and develop new therapeutic approaches.

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ACTIVATION OF THE δ-OPIOID RECEPTOR PHOSPHORYLATES THE NEURONAL PROTEIN SPINOPHILIN IMPLICATED IN RECEPTOR ENDOCYTOSIS

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Spinophilin is a neuronal multifunctional scaffold protein that regulates cytoskeletal functions and modulates G protein-coupled receptor (GPCR)

signaling by altering receptor'sphosphorylation, internalization and desensitization [1]. We have previously demonstrated that spinophilin, interacts with the δ-opioid receptor (δ-OR) and selective members of G and RGS4 (Regulator of G protein Signaling 4) proteins to form a multiprotein complex interfering in opioid receptor signaling [2]. Other studies have shown that phosphorylation of spinophilin modulates GPCR endocytosis and trafficking [3]and thus wonderedwhether δ-OR activation by specific ligands, phosphorylates spinophilin and interferes in receptor internalization and desensitization. Flow cytometry studies in Neuro-2A cells indicated that spinophilin expression reduces the levels of internalized δ-opioid receptors, stabilizing the receptor in the cell membrane. Moreover,DSLET-activation of δ-OR in HEK293 cells leads to tyrosine-phosphorylation of spinophilin, an effect that is abolished by the presence of PP1, a c-Src kinase inhibitor suggesting thatit is mediated by c-Src. Parallel co-immunoprecipitation studies indicated that c-Src kinase interacts with spinophilin and is activated by DSLET administration. Site-directed mutagenesis on specific tyrosine residues of spinophilin defined the critical sitesimplicated in spinophilin phosphorylation. In Neuro-2A cells expressing mutant spinophilin carrying the Y398F or the Y483Fmutation DSLET-induced internalization of the δ-OR isaltered.Collectively, our results demonstrate that spinophilin is regulated by δ-OR activation and plays a critical role in receptor endocytosis revealing new pathways on the role of spinophilin in opioid receptor desensitization of neuronal cells.

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CRH PROMOTES THE NEUROGENIC ACTIVITY OF ADULT NEURAL STEM CELLS VIA BMP4 SUPPRESSION

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Local cues in the adult neurogenic niches dynamically regulate neural stem cells homeostasis, whereas their identity and associated molecular mechanisms remain poorly understood. Here, we show that Corticotropin-releasing hormone (CRH), the major mediator of mammalian stress response and key neuromodulator in the adult brain is necessary forhippocampal neural stem cells (hiNSCs) activity under physiological conditions. In particular, we demonstrate functionality of the CRH/CRH receptors (CRHRs) system in mouse hiNSCs and conserved expression in humans. Most importantly, we show that genetic deficiency of CRH impairs hippocampal neurogenesis, affects spatial memory and significantly compromises the responsiveness of hiNSCs to environmental stimuli. Additionally, we provide evidence that local disruption of the CRH/CRHRs system *in vivo* reduces neurogenesis, while exposure of adult hiNSCs to CRH promotes neurogenic activity via BMP4 suppression. Our findings suggest a novel physiological role of CRH on adult neurogenesis, independently of its stress-related systemic function.

MILKING THE POSTNATAL BRAIN NEURAL STEM CELL NICHE: A METHOD FOR ISOLATING ENDOGENOUS NEURAL STEM AND PROGENITOR CELLS FROM THE CEREBROSPINAL FLUID

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Cell replacement therapy is an attractive treatment option for CNS disorders that involve cell type-specific defects, such as hypomyelinating disorders, Parkinson's disease and amyotrophic lateral sclerosis. Foetal neural stem cells or patient-specific iPSCs are promising sources but necessitate aggressive immunosuppression and raise concerns regarding tumorigenesis, respectively. We are developing a method that enables the release of postnatal brain neural stem and progenitor cells (pbNSCs) from the subependymal zone (SEZ) neurogenic niche into the cerebrospinal fluid, facilitating their isolation via liquid biopsies. This process we have named "milking of the SEZ" and involves the intracerebroventricular injection of a 'release cocktail' containing neuraminidase and β1-integrin blocking antibody. We have assessed cell yields at various time-points ranging from 3 to 30 days post-release and immunohistochemical analysis has confirmed that collected cells include Sox2+ and Nestin+ neural stem/progenitor cells, Dcx+ neuroblasts, PDGFaR+/Olig2+ oligodendrocyte progenitor cells and GFAP+ astrocytes. Furthermore, their cell-type profile matches that of the SEZ niche and even accurately reflects changes in the source area, for example after the co-injection of FGF-2 that results in increased numbers of Sox2+ cells. Our histological analyses revealed that the wall of the lateral ventricles at the site of the SEZ is more specifically targeted by the release procedure and that milking does not result in impaired function of the affected niche. Finally, the collected cells exhibit features compatible with those of dissected and acutely dissociated SEZs, for example increased proliferation when plated on laminin and when exposed to culture media conditioned with choroid plexus-derived factors. Overall, our results suggest that this is a promising new source of pbNSCs, while preliminary data from the histological analysis of human infant brains indicate that this method can be transferred to the clinic.

microRNA-124 OVEREXPRESSION LEADS TO DIRECT REPROGRAMMING OF GLIAL CELLS TOWARDS THE NEURONAL LINEAGE IN A MOUSE MODEL OF MECHANICAL BRAIN INJURY

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The central nervous system (CNS) has limited capacity for self-repair following neurodegeneration, thus neuronal cell replacement has been the ultimate goal of several experimental approaches aiming at CNS recovery. Direct reprogramming of endogenous non-neuronal CNS cell populations to neurons through forced expression of neurogenic factors is a promising method for *in vivo* neuronal replacement. Astrocytes have the capacity to trans-differentiate into various subtypes of neurons *in vitro*, and to some extent *in vivo*, through forced expression of transcription factors such as Mash1, Neurogenin-2, Pax6 and NeuroD1. MicroRNAs (miRNAs) are small, non-coding RNAs that act as post-transcriptional regulators of gene expression and have been recently shown to participate in direct reprogramming mechanisms. Each miRNA has the ability to modify the expression of multiple genes, regulating a variety of developmental processes, including neuronal differentiation. Unpublished work of our group indicates that miR-124, a brain-enriched miRNA leads to *in vitro* reprogramming of astrocytes towards MAP2+ and βIII-tubulin+ neurons, mainly of glutamatergic, and to a lesser extent of GABAergic phenotype, through up-regulation of pathways induced by proneural factors Mash1 and Neurogenin-2. miR-124 is thus rendered into an attractive candidate for studying astrocytic reprogramming *in vivo* as well. Here we have used a lentiviral vector for overexpressing mature miR-124 together with GFP into the cortex parenchyma of young FVB mice, four days after the infliction of a mechanical cortical trauma. Six days after the stereotaxic injection, the virus has been uptaken primarily by glial cells, and in particular by GFAP+ astroglia (~60%), mature oligodendrocytes (~19%), and microglial cells (~13%) while only 8% of the transduced cells were NeuN+ neurons. Already 3 weeks after the viral injection, overexpression of miR-124 leads to an elevated percentage of NeuN+ cells amongst the transduced parenchymal cells (~35%). Furthermore, parallel treatment with valproic acid, a histone deacetylase inhibitor and α-Tocopherol along with miR-124 infusion, significantly enhances glial reprogramming towards the neuronal phenotype, with ~70% of GFP+ cells exhibiting NeuN+ phenotype. Currently we are studying the effect of combined forced expression of miR-124 along with another neurogenic miRNA, miR-125b, in the above *in vivo* setup, as *in vitro* data indicate that their combined action leads to the reprogramming of astrocytes into more differentiated neurons, predominantly of glutamatergic specificity.

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LAYER- AND FREQUENCY-DEPENDENT DIVERSIFICATION IN SHORT-TERM SYNAPTIC PLASTICITY ALONG THE SEPTOTEMPORAL AXIS OF THE RAT HIPPOCAMPUS

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The hippocampus is a brain structure that displays functional diversity along its long (septotemporal or dorsoventral) axis. It has been recently revealed that the functional segregation is accompanied by important specializations that occur at various levels of local neural network along the hippocampus long axis. The intrinsic dorsoventral diversification may be crucially involved in tuning information processing along the hippocampus long axis. One of the most important mechanisms that control information processing is synaptic plasticity. Indeed, short-term synaptic plasticity is importantly involved in information processing and therefore may be involved in diversifying synaptic properties along the hippocampus.In the hippocampus, the CA1 field presents the peculiarity that represents the last stage in the unidirectional flow of information, originating in the entorhinal cortex, along the dentate gyrus-CA3–CA1 loop and simultaneously receives a direct input from the entorhinal cortex. Thus, the CA1 receives its main excitatory input from the CA3 field through Schaffer collaterals (Sc) that make synaptic connections at the stratum radiatum and it is also activated in the stratum lacunosum-moleculare by the direct entorhinal input, the so-called temporoammonic path (TA). In this study we aimed to examine short-term synaptic plasticity in CA1 and compare responses between Sc and TA inputs using extracellular recordings of excitatory postsynaptic potentials (fEPSPs) from slices prepared from the dorsal (DH) and the ventral hippocampus (VH). Short-term synaptic plasticity was studied following repeated stimulation of either Sc or TA. Specifically, stimulation consisted of a ten-pulse stimulus train at frequencies from 0.1 to 100 Hz. We found different frequency-dependent patterns of responses between DH and VH and between layers. In general, DH synapses in both Sc and TA facilitated for stimulus frequencies 1-50 Hz, while they depressed at higher frequencies (75-100 Hz). However, the facilitation in TA was higher than in Sc (for 3-20 Hz) and peaked at a lower frequency (10 Hz vs 20 Hz). In addition, depression in TA of DH was weaker compared with Sc. More striking differences were observed in VH. In particular, Sc synapses in VH consistently depressed over the entire range of frequencies while TA synapses displayed significant facilitation for 1-30 Hz. Both Sc and TA synapses showed robust differences between DH and VH. Given the importance of short-term synaptic plasticity in the processing of neural information, these results suggest that the integration of converging information in the CA1 circuitry through Sc and TA significantly differs between DH and VH.

BIOGENIC AMINES AFFECT COURTSHIP BEHAVIOUR OF DROSOPHILA MALES

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In Drosophila, courtship of a male towards a recently mated female is unsuccessful. The mated female smells bad (repulsive olfactory cues) and behaves erratically towards the male. The male becomes frustrated and reduces subsequent courtship behaviour even towards virgin females. This experience-dependent behavior modification is called courtship conditioning and has been one of the major paradigms used to study learning and memory in *Drosophila*. The courtship behaviour is not metabolically very costly, but males exhibit a good strategy to obtain the maximum benefit of reproductive investment. Adult males are typically repulsive to other males hence futile homosexual behavior can be inherently avoided in wild-type Drosophila males. However, ectopic expression or mislocalization of a member of the adenosine triphosphate (ATP)-binding cassette (ABC) family of transmembrane transporter proteins, the tryptophan/guanine transmembrane transporter gene, *white* (*w*), induces male-male courtship. Tryptophan is the direct precursor of 5-hydroxytryptamine (5-HT) or serotonin, and GTP is a precursor of 4 hydroxybiopterin, an essential cofactor required for tyrosine hydrolase to produce serotonin and the catecholamine dopamine. Thus point mutation of *white* gene, results in flies (strain w^{1118}) with white eyes and reduced levels of serotonin and dopamine. We investigated whether loss of white protein affects male courtship and courtship conditioning. We found that naïve w^{1118} flies court normally virgin females but when they face first other males their behaviour changes afterwards by exhibiting reduced courtship towards virgin females. Interestingly, no distinct behavioural interactions –such as courtship behaviour or aggression- were observed during the pre-exposure period, suggesting that courtship frustration may be caused by male olfactory repulsive cues. In contrast, males of the parental Canton-S strain court normally towards virgin females independently of pre-exposure to other males. Thus our study provides a link between biogenic amines and frustration threshold and establishes a new courtship conditioning assay based on male-to-male interactions.

THE IMPORTANCE OF HYPOTHALAMIC NNOS-DERIVED NO SIGNALINGFOR OLFACTORY BEHAVIOR AND COGNITION

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The gonadotrophin-releasing hormone (GnRH) neurons are the key players in a complex neural network that is controlling sexual maturation, puberty onset and adult fertility in mammals. Interestingly, these GnRH neurons are not born inside the brain. They find their origin in the olfactory placode and migrate into the hypothalamic preoptic region during further embryogenesis. Defects in the migration of these GnRH neurons or the capacity to secrete their neurohormone result in a delay in puberty onset and fertility problems, and are related to genetic disorders like congenital hypogonadotropic hypogonadism (CHH) and Kallmann syndrome (KS). Nitric oxide synthase (nNOS or NOS1)-containing neurons have been suggested to interact with GnRH neurons and regulate their activity and neurosecretory capacity. Recently, mutations in the NOS1 human gene have been discovered in patients with CHH and KS, and it appears that several of them are associated with cognitive impairments/mental retardation and anosmia. Because of these interesting findings, we decided to further elucidate the putative new role of hypothalamic NO signaling in behavioral processes. In accordance, our results provide evidence for a novel role of hypothalamic nNOS-derived NO signaling in cognitive and memory processes, as well as in olfactory behavior. We hope that our results can contribute to a better understanding of the different roles of hypothalamic NO signaling and pathophysiology of related disorders.

LEARNING TO RECOGNIZE OBJECTS IN A LOW-VISION SIMULATION

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Vision after restoration is not nearly as efficient as typical vision. Adult visual plasticity, that relies on the brain's capacity to functionally reorganize itself on an experience-dependent basis, forms the underlying scaffolding to implement devices that restore sight to the blind. Artificial vision studies have attempted to identify the significant variables that affect the outcome of visual restoration. Here, utilizing a visual perceptual learning paradigm, we propose a simulation experiment to assess the beneficial impact of training on visual rehabilitation protocols that could potentially promote adaptation to restored visual function. These training procedures would also allow us to establish a common framework so as to compare and evaluate findings between visual restoration approaches. While recent evidence has provided valuable insight into the letter recognition abilities of humans when presented with simulated phosphenes [1,2], the benefits of long-term training remain only partially explored. Based on recent findings in letter recognition [3], we aim to examine whether general object recognition can also be enhanced by training. We, therefore, introduce a four-alternative-forced-choice (4AFC) paradigm that requiresnormal sighted participants to identify a previously cued image out of a predefined set (cf. [4]). All objects will be depicted with the use of phosphenes (cf. [1,2]) with varying levels of phosphene density in a virtual-reality gaze contingent paradigm. Trial-by-trial and block-based feedback will be provided in order to promote learning. This paradigm would allow us to examine the potential of artificial vision-induced plasticity in a simulation model. Expected results would have strong implications for the design of pre-surgical testing along with rehabilitation training for implanted patients.

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REGION SPECIFIC EFFECTS OF IMMATURE STATUS EPILEPTICUS ON THE ADULT HIPPOCAMPAL CA3 – MEDIAL ENTORHINAL CORTEX CIRCUITRY *IN VITRO*

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The network formed by communicating neurons between the hippocampus proper and the medial entorhinal cortex (mEC) plays a crucial role in cognitive processes but also in the generation, propagation and amplification of synchronous discharges, and seizures. We have earlier demonstrated that a sustained generalized seizure in immature rats is associated with lasting neurobiological changes persisting to adulthood. In this work, we investigated communication modalities between the hippocampal CA3 and mEC deep layers, by addressing the effects of total severance of their functional connections on the adult Interictal Epileptiform Discharge (IED) frequency and concurrent HFOs after immature Status Epilepticus (SE).In this setting, we have also aimed at correlating IED frequency changes with those of Ripples (R) and Fast Ripples (FR) of the High-Frequency Oscillations (HFOs), if any. Temporal hippocampal-mEC slices were obtained from adult (>P60) Sprague-Dawley rats, >40 days after aSE Pentylenetetrazole-induced seizure (SE-slices) or from their Normal littermates (N-slices).Field potentials were recorded simultaneously from the hippocampal CA3 and mEC deep layers of hippocampal-mEC slices during continuous application of 50 uM 4-Aminopyridine. Paired recordings from the pyramidal CA3 and mEC deep layers of combined (N or SE) slices demonstrated that IED frequency was significantly lower in mEC compared to CA3(n=16N, 9SE, $p<0.0001$); isolation of the 2 areas did not alter IED frequency in CA3 or in mEC, and thus did not modify their relationship.CA3 IED frequency was similar in all slices (N, SE) however, mEC IED frequency was higher in SE vs N slices (n=16N, 9SE, $p=0.014$). CA3 HFOs had 100 times higher power than those recorded from mEC. FR/R ratio was lower in CA3 vs mEC when they were functionally connected (n=14N, $p=0.032$ n=8SE, $p=0.0087$), a difference that disappeared when they were isolated. The FR/R ratio increased in CA3 in the absence of mEC; both in N($p=0.0007$) and SE ($p=0.005$) slices. Interestingly, the direction of the R and FR power changes post-SE was a mirror image between CA3 and mEC and between N, SE slices, that is power increases in mEC corresponded to power decreases in CA3 post-SE. Immature SE has a profound impact on the communication modalities of CA3-mEC circuitry in the long term. The post-SE change sappear to be region specific and may enable mEC circuitry to serve as an independent epileptogenic area post-SE. Overall, the sequelae of early-life convulsions may conceivably depend on the affected area and on the stimuli that the adult brain faces.

INVESTIGATING THE EXPRESSION PATTERN OF GEMC1/LYNKEAS, P73 AND FOXJ1 IN THE DIFFERENTIATION OF RADIAL GLIAL CELLS TOWARDS THE EPENDYMAL LINEAGE *IN VITRO*

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The subventricular zone (SVZ) of the lateral ventricles is one of the main neurogenic niches in the adult brain in mammals. The niche contains proliferating neural stem cells (NSCs) that are separated from the lateral ventricles through a layer of ependymal cells. Ependymal cells have multiple cilia and their proper function is important for the appropriate flow of the cerebrospinal fluid, which contains secreted molecules and morphogens that

control the self-renewal and differentiation decisions of adult NSCs. Their progenitors, radial glial cells (RGCs), are committed to the ependymal lineage during the 14th -16th day of fetal development, while their differentiation starts after birth and is completed in the following two weeks. However, the molecular cascade that controls the differentiation of radial glial cells into ependymal cells remains to be further elucidated. Aiming to study the molecular pathway which controls the commitment of RGCs and the generation of ependymal cells, we sought to establish an in vitro model to follow the process of their differentiation. For this purpose, we isolated RGCs from the subventricular zone of newborn mice and cultured them upon proliferating and differentiating conditions. We investigated the expression of molecules that act at the initial stages of ependymal cells generation, such as GemC1/Lynkeas, p73 and Foxj1, in different time points. To investigate the expression of GemC1/Lynkeas we have used mice in which the gene of b-galactosidase has been inserted between the exons 2 and 3 of GemC1/Lynkeas allele. The detection of its expression was carried out through staining using the substrate x-gal. Our results showed that GemC1/Lynkeas expression is observed since the first stages of differentiation. Also, through immunofluorescence experiments we tracked the expression pattern of p73 and Foxj1. We saw that p73 and Foxj1 are expressed in a low percentage of cells in proliferating conditions. A higher percentage of cells express them in the early stages of differentiation, further suggesting that they are important for the initial steps of the differentiation process. In accordance with this, their expression is reduced during the completion of this process. In conclusion, we established and characterized with the expression of important factors an in vitro system that allows ependymal cell differentiation. This system could be further utilized to characterize the molecular mechanisms that underlie the differentiation process of radial glial cells into ependymal cells.

MORPHOLOGICAL, MOLECULAR AND GENETIC ASPECTS OF THE GnRH MIGRATORY PROCESS

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The control of reproduction is mediated by a hypothalamic network that regulates the periodic secretion of gonadotropin-releasing hormone (GnRH). GnRH neurons originate in the nose and enter the brain along vomeronasal and terminal axons during embryonic development. Here we provide new insights into the development of the GnRH migratory process during the first trimester of human development by imaging intact whole embryos and fetuses at a cellular resolution. This allows for the first time, a true representation and appreciation of cells in their native, *in vivo* context. This approach has highlighted previously unknown features, whilst also revealing that not only is the GnRH population in humans significantly higher than previously thought, but that GnRH cells target several extra-hypothalamic brain regions in addition to the hypothalamus. Their presence in these areas raises the possibility that GnRH has non-reproductive roles, creating new avenues for research on GnRH functions in cognitive, behavioral and physiological processes. Currently it is known that alterations in the development of this system or in the secretion of GnRH are associated with congenital hypogonadotropic hypogonadism (CHH) and Kallmann syndrome (KS) in humans: characterized by a failure of sexual competence. This work also provides evidence that Anti-Müllerian hormone (AMH), a hormone involved in male urogenital development is mutated in CHH and KS patient cohorts and suggests that defective AMH signaling may contribute to these pathologies through regulation of the migration of GnRH cells.

UNRAVELING THE MECHANISM OF ALPHA-SYNUCLEIN SEEDING IN OLIGODENDROCYTES

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Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder characterized by the presence of distinctive glial cytoplasmic inclusions (GCIs) within oligodendrocytes. The major constituent of GCIs is the neuronal protein alpha-synuclein (aSyn) and the oligodendroglia-specific protein TPPP/p25a. Relocation of TPPP/p25α from the cell processes to the soma, occurring prior to aSyn accumulation, is closely linked to the oligodendroglial degeneration present in MSA. Since mature oligodendrocytes do not normally express aSyn, it is proposed that the protein is entering oligodendrocytes following its release by neurons. Up to date the precise aSyn species responsible for the formation of GCIs and the proteolytic machineries responsible for their clearance remain enigmatic. In the current study, we have used rat oligodendroglial cells, either controls (OLN93-WT) or stably overexpressing human aSyn (OLN93-AS), in order to study the uptake, subcellular localization and potential seeding capability of exogenously added human recombinant aSyn (haSyn) species (monomers, oligomers, fibrils), in the presence or absence of TPPP/p25α. Our results show that haSyn fibrils are readily uptaken by both OLN93-WT and OLN93-AS cells and induce aggregation of endogenous rat aSyn, in a seeding mechanism. Pre-formed aSyn fibrils also induce collapse of the cytoskeleton. Moreover, concurrent aSyn and TPPP/p25αoverexpression accelerates the seeding of endogenous rat oligodendroglial aSyn and augments the formation of aberrant aSyn species. Further study will enable us to fully elucidate the role of TPPP/p25α in these processesand to identify the proteolytic pathways responsible for aSyn clearance, which may eventually represent potential therapeutic targets for MSA and related synucleinopathies.

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UNRAVELING THE ROLE OF CANONICAL TGFβ-SUPERFAMILY SIGNALING IN A PRECLINICAL ALPHA-SYNUCLEINOPATHY MOUSE MODEL

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Parkinson's disease (PD) is a dilapidating neurodegenerative disorder, characterized by the progressive loss of the dopaminergic neurons that reside in the substantia nigra pars compacta and project in the striatum. The pathological hallmark of the disease is the presence of intracellular proteinacious aggregates in the degenerating neurons, major component of

which is alpha-synuclein (ASYN), an abundant presynaptic brain protein genetically and biochemically linked to PD. Although the cause of the disease is poorly understood, several processes have been suggested to contribute to PD pathogenesis, including oxidative stress, mitochondrial dysfunction, abnormal protein handling and neuroinflammation. Clinical as well as *in vivo* and *in vitro* studies have classified TGFβ-superfamily signaling system amongst the molecular culprits regulating PD pathophysiology. However, to date its precise role remains elusive. To address this, we have utilized a mouse alpha-synucleinopathy model, recapitulating major histopathological features of PD, including the loss of the nigral DAergic neurons and their DAergic nigrostriatal projections. Alpha-synucleinopathy was established via unilateral injections of increasing titers of recombinant adeno-associated viruses (rAAV) expressing the human PD-linked mutant A53T ASYN (or the control protein GFP) in the substantia nigra of adult male C57/Bl6 mice. Immunohistochemical, biochemical and behavioral analyses of the described model demonstrate a dose-dependent DAergic pathology, related to the increasing protein load of ASYN. Ongoing analysis of the distinct degrees of pathology unravels aspects of the inter-relationship between ASYN-mediated neurotoxicity and the TGFβ-superfamily signaling activation. Data stemming from the immunohistochemical and biochemical profile of the rAAV-ASYN-injected mice, will give insights for the construction of the spatiotemporal map of the canonical TGFβ-superfamily signaling activation in a PD-related context. Collectively, our study will pave the way for an integrated validation of the role of the TGFβ-superfamily signaling in PD and related synucleinopathies.

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POSTNATAL TREATMENT WITH GnRH ANTAGONIST RESCUESTHESEVERE NEUROENDOCRINE PHENOTYPE OF A NEW PCOS MOUSE MODEL

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Polycystic ovary syndrome (PCOS) is the most common form of female infertility, affecting up to 10% of women worldwide. It is typically associated with excessive ovarian androgen secretion and chronic oligo-anovulation. Most PCOS women also exhibit increased luteinizing hormone (LH) levels, suggestive of rapid gonadotropin-releasing hormone (GnRH) release. Moreover, women with PCOS exhibit 2-3x higher levels of circulating Anti-Müllerian Hormone (AMH). While the exact origin of PCOS is unknown, data from clinical and animal studies suggest that it may originate in utero and that environmental factors, such as hormonal imbalances during fetal life, could be important for etiological factors of PCOS. Our team established a new PCOS mouse model and showed that fetal exposure to excess AMHimpacts the hypothalamic-pituitary-gonadal axis and induces the acquisition of PCOS-like traits in theoffspring. Prenatal AMH-treatedfemale offspring recapitulated the major PCOS cardinal neuroendocrine reproductive features, namely hyperandrogenism, elevation in LH pulse frequency and oligo-anovulation, and a persistent rise in the GnRH neuronal firing activity in adulthood. Because, our electrophysiological results uncovered a persistent hyperactivation of GnRH neurons in PCOS-like offspring postnatally, we reasoned that normalizing GnRH secretion in adulthood could ameliorate the neuroendocrine dysfunctions observed in these mice. Using a pharmacological approach, wedemonstrate that tempering GnRH signaling pathway rescues the neuroendocrine phenotype of PCOS-like animals, restoringtheir normal hormonal levels (LH and testosterone), estrus cyclicity and ovarian morphology. Our findings support the hypothesis that AMH-dependent deregulation of GnRH release could be involved in the aetiology of PCOS and highlight a critical role for GnRH in the neuroendocrine dysfunctions of this disease.

ENCODING SCHEMES FOR VISUAL PROSTHESIS SYSTEMS: A REVIEW

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The purpose of this review is to examine the methods and encoding schemes to transfer visual information from camera sensors to the brain in visual prosthesis systems. Due to fundamental limitations of brain-machine interfaces, the spatial and temporal resolution of current and near-future visual prostheses is low. While all visual prostheses perform a transformation of video signals to electrical stimulation patterns, a variety of preprocessing strategies may improve the legibility and utility of images delivered to the brain. Given the availability of ever-increasingly powerful computational engines available to the prosthesis designer, processing-intensive approaches previously precluded because of their computational demands are becoming realistic for today's systems. Such processing strategies could facilitate the prosthesis user's navigation, situational awareness, and identification of objects and people of interest in a visual scene. Here, computational imaging methods are reviewed with regard to potential applications in visual prosthesis systems. Despite often thorough characterization of the computational implementation of these methods, there is limited data regarding their performance as prosthesis preprocessing strategies: we know how to create these filters, but do they actually help the patient? To address this question, rigorous psychophysical testing is required. Therefore, we propose two groups of experiments to assess the applicability and efficiency of different prosthesis encoding schemes. The first group will evaluate algorithms designed to aid in object segregation and detection through object identification tasks using static images. A second group of experiments will assess navigational aids through mobility tasks using an obstacle course. These experiments will be the first systematic investigation of different image encoding schemes in the field of visual prosthetics.

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GENERATION OF A HIGH-RESOLUTION PROTEIN MAP OF THE MOUSE BRAIN

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The determination of the spatial distribution of proteins in the nervous system, at a scale of resolutions ranging from regional, to cellular and subcellular sites, can provide valuable and decisive insights into the molecular organization of the brain and a knowledge base to study the functional aspects of both healthy and diseased brain. Here, we have generated a high-resolution protein map of the mouse brain for the first 157 protein targets using an antibody-based, bioimaging strategy. For that, we have utilised the unique collection of antibodies generated within the Human Protein Atlas (HPA) consortium(covering more than 85% of the human proteome) while the close homology of many human and mouse protein orthologs has made this mouse brain attempt possible. The objective is to generate a whole mouse brain protein distribution map based on Immunohistochemical data on series of coronal sections having a 400 μm section interval and spanning from olfactory bulb to medulla oblongata. Using this sampling approach, we have generated protein distribution maps with 90-95% of all known brain regions represented in at least one brain section. These comprehensive and complete mouse brain distribution maps

will eventually contribute to the identification of the molecular signature of cells and specialized cellular compartments and outline the anatomical boundaries of brain regions and cells.

BNN-20, A SYNTHETIC MICRONEUROTROPHIN, INDUCES REGION-SPECIFIC, ADULT DOPAMINERGIC NEUROGENESIS IN THE SUBSTANTIA NIGRA OF THE "WEAVER" MOUSE, A MOUSE MODEL OF PARKINSON'S DISEASE.

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Neurotrophic factors, are among the most promising therapeutic candidates against Parkinson's Disease (PD), but their clinical use is limited due to their inability to cross the Blood Brain Barrier (BBB). Our previous results, using a preclinical model of PD, the "weaver" mouse, suggest that the micromolecular neurotrophin BNN-20 exhibits extraordinary neuroprotective effect on the weaver SNpc dopaminergic neurons, through the TrkB-PI3K-Akt-NFkB pathway, acting as a mimetic of BDNF, while penetrating the BBB [1]. The goal of this study was a) to confirm the existence of adult dopaminergic neurogenesis in the SNpc, which remains controversial [2,3], and b) to evaluate the possible effect of long-term BNN-20 administration (P14-P60) on the rate of neurogenesis. Adult dopaminergic neurogenesis was evaluated by BrdU/tyrosine-hydroxylase(TH) immunofluorescence double-labeling on wild-type and weaver SNpc cryo-slices, following BNN-20 administration (P14-P60) and daily 5-bromo-2'-deoxyuridine(BrdU) injection(P40-P60 or P20-P40). Adult hippocampal neurogenesis was also assessed by quantification of BrdU+ neurons in the SGZ of the mice. The neurogenic capacity of the midbrain was further confirmed, through cell isolation from wild-type and weaver midbrain and cultivation of adult neural stem cells (NSCs) into neurospheres*in vitro*. The rate of neuronal differentiation of midbrain NSCs was assessed using appropriate differentiation markers (Sox2, BIII-tubulin). Results suggest that wild-type SNpc (P60) contains a few BrdU+/TH+ neurons, significantly increased in the weaver SNpc, probably as a compensatory mechanism. Long-term BNN-20 administration led to a further, vast increase of the double-labeled dopaminergic neurons in the weaver SNpc, suggesting neurogenic properties. These are consistent with *in vitro* observations, where few primary neurospheres could be grown from the wild-type midbrain, while significantly more were grown from the "weaver" midbrain. Long-term BNN-20 administration enhanced the differentiation into neurons without affecting the proliferation of the NSCs. Preliminary in vitro results from SVZ-derived NSC cell cultures, confirm the latter, suggesting that BNN-20 promotes the differentiation of the NSCs towards neural and glial phenotypes. Adult hippocampal neurogenesis was increased in weaver SGZ compared to wild-type (P60). However, no effect was observed after BNN-20 administration in the SGZ, suggesting a lesion-specific neurogenic activity. In conclusion, BNN-20 exerts dramatic neurogenic properties, increasing the number of newly-born dopaminergic neurons in the SNpc in a region-specific manner, probably by enhancing neuronal differentiation. Hence, it represents a compelling therapeutic candidate against PD.

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REGIONAL DISTRIBUTION AND CELLULAR LOCALIZATIONOF GLYCOCORTICOID RECEPTOR GRAISOFORMS IN THE ADULT ZEBRAFISH BRAIN (*DANIO RERIO*)

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Glycocorticoid receptor GRα is a member of the nuclear receptor family, through which glycocorticoids mediate their cellular actions. Specifically, sequence alignments of human, monkey, rat, and mouse GRs have shown eight GRα isoforms (GRα-A, GRα-B, GRα-C1, GRα-C2, GRα-C3, GRα-D1, GRα-D2, and GRα-D3). The present study questioned the expression pattern of GRα in the adult zebrafish (*Danio rerio*) brain, by means of western blot, immunohistochemistry and double immunofluorescence. Our results identified five GRα isoforms (95kDa, 67kDa, 50kDa, 45kDa and 25kDa)in telencephalon, mesencephalon and hindbrain of zebrafish brain. These isoforms were widely distributed throughout the antero-posterior brain axis, e.g.dorsal (Dm, Dl και Dc) and ventral (Vv, Vl,Vd) telencephalon, preoptic and hypothalamic areas (PPa,PPp, LH, Hv), thalamus, midbrain (PM, RT ,NIII, NLV, PL,MLF ,LLF ,GC), tectum, cerebellum (Cce, Val, Vam) and brain stem (GC ,IMRF , IRF ,LC , LLF , MON , SRF, IR). Interestingly, GRα were localized in dorsal telencephalon in glial cells expressing GFAP protein, and in the ventral telencephalon in neurons expressing TH. Within hypothalamus, GRα were colocalized with GFAP, Chat, TH and v-Glut. In the thalamus GRα was detected on cells expressing TH and in the hindbrain on neurons expressing v-Glut, Chat and glial cells expressing GFAP. The results of this study clearly support the evolutionary conserved expression of glucocorticoid receptors in zebrafish brain and suggest the neuronal and glial localization of GRα in key brain areas, influencing catecholaminergic, cholinergic and glutamatergic transmission.

THE MGLUR5-ARC PATHWAY IN MEDIAL PREFRONTAL CORTEX IN STRESS RESILIENT NEONATALLY HANDLED ADULT RATS

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Neonatal handling is an experimental animal model of early life experiences known to affect the hypothalamic-pituitary-adrenal (HPA) axis. Neonatally handled rats exhibit attenuated HPA axis response to stressors and are characterized as resilient to stress. The group I metabotropic glutamate receptor 5 (mGluR5) is a G-protein coupled receptor. mGluR5 activate Gq/G_{11} phospholipase C-mediated signaling and is important for modulating several forms of synaptic plasticity and excitatory synaptic transmission. mGluR5 has been implicated in various psychiatric disorders, such as anxiety and depression and it has been identified as a novel therapeutic target. The activation of mGluR5 increases the translation of the immediate-early gene Arc (activity-regulated cytoskeletal-associated protein). Arc is an activity neuronal marker localized post-synaptically and involved in various forms of neuronal plasticity, such as AMPA receptor endocytosis and LTD. Additionally, Arc is involved in actin polymerization and LTP consolidation. This study aimed at identifying the role of the mGluR5-Arc pathway in medial prefrontal cortex in the stress resilience of neonatally handled adult rats. Rats were subjected to maternal separation for 15 min from postnatal day 1 to 21. Open-field behavioral responses and mGluR5 and Arc mRNA levelsin the prefrontal cortex of adult rat male brain using in situ hybridization were evaluated. In the open field task, we observed higher movement duration, total distance travelled, time spent and number of entries in the center in neonatally handled compared to non-handled rats. Furthermore, we found that mGluR5 and behaviorally induced Arc mRNA levels were higher in neonatally handled rats compared to non-handled rats in prelimbic and anterior cingulate cortex. Our results demonstrate that the neonatally handled animals exhibit lower fear and anxiety levels, and possibly, a better adaptation to the new environment. The activation of Arc translation observed in the mPFC of neonatally handled rats may be correlated to the upregulation of mGluR5 mRNA levels. Our data suggest that a brief and repeated maternal separation during neonatal life by altering

the mGluR5-Arc pathway leads to increased neuronal activation of mPFC. Since mPCF exerts a suppressive effect on the stress response, the mGluR5-Arc pathwaymay be implicated in the stress resilience of neonatally handled rats.

CHRONIC ANTIPSYCHOTIC-INDUCED METABOLIC SYNDROME IN THE RAT: EFFECTS OF ENVIRONMENTAL MODULATORS OF THE BRITENING PROCESS AND THERMOGENESIS: A PILOT STUDY

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The metabolic syndrome (MetS) associated with use of antipsychotic medication represents a significant health hazard. It is therefore critical to understand the mechanism through which antipsychotics influence energy expenditure, inducing MetS. This mechanism must involve changes in thermogenesis via brown adipose tissue (BAT). The first aim of this pilot study was to examine chronic antipsychotic effects on thermogenesis as reflected by the distribution and activity of BAT, using microPET/CT scanning. The second aim was to examine whether antipsychotics-induced MetS may be curbed through environmental manipulations stimulating the "browning" process which transforms white adipocyte cells into cells with BAT-like characteristics. 24 female Sprague-Dawley rats were subjected to one of two drug conditions Olanzapine [DRUGTx: 1. Olanzapine (DRUGTx-OL) vs 2. Vehicle (DRUGTx-VEH), n=12]. OL dose was chosen according to Paabøl Andersen and Pouzet model for schizophrenia (1). Animals from each drug condition were subjected to one of 4, 25-day behavioural treatments [BEHTx: a. control (BEHTx-CON), b. cold exposure (BEHTx-CE), c. Environmental Enrichment (BEHTx-EE), d. Chronic Unpredictable Stress (BEHTx-CUS); n=3]. Measures taken were Cumulative Weight Change (CWC), Standardized Uptake Value (SUV) and total BAT volume (TBATV). The biomarkers irisine and corticosterone were also monitored. Descriptive statistics were used on these pilot results (final N=19). Group means across the micro PET/CT sessions were calculated. CWC in the OL groups was increased compared to VEH, and decreased after BEHT [$F(2,36)=3.23$, $p<.05$]. An increase in SUV mean was noted after DRUGTx, with a reduction after BEHTx. An increase in TBATV means was observed in all groups after DRUGTx, with a reduction after BEHTx. However the VEH-EE group demonstrated increased TBATV after BEHTx. Irisine (μg/ml) maximum concentration was detected in groups DRUGTx-OL x BEHTx-CON and DRUGTx-VEH x BEHTx-EE. Corticosterone (ng/ml) concentration tended to be lower in all DRUGTx-OL groups compared to DRUGTx-VEH ones. Chronic OL treatment followed by BEHTx seemed to modify BAT activity (SUV mean, BAT volume) in the predicted direction and also influense irisine and corticosterone levels. Therefore these pilot results confirm the appropriateness of the methods used for further investigation of our main hypotheses.

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THE ROLE OF LONG NON-CODING RNAS IN MAMMALIAN BRAIN DEVELOPMENT

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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. 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 our efforts on lncRNAs, which are transcribed from genomic loci in close proximity with protein coding genes, encoding for transcription factors (TFs) with critical roles in brain development. We hypothesized that these lncRNAs may be implicated in the regulation of neighboring TF genes. To this end, we characterized the changes in the expression profile of the most interesting from the identified lncRNAs-TF pairs during development of mouse brain (telencephalon). In this study, we further investigated the functional role of two lncRNAs, e.g. TCONS_00034309 and Ariel (AK142161), in the differentiation of neural stem cells by *in vitro* and *in vivo* overexpression and knock-down studies. Our data suggest critical roles for these lncRNAs in neuronal differentiation and astrogliogenesis during brain development. To conclude, 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.

MICROGLIAL ACTIVATION IN THE ABSENCE OF CLASSICAL NEURONAL APOPTOSIS IN THE SUBSTANTIA NIGRA OF THE HUMAN NEONATE AFTER PERINATAL HYPOXIC/ ISCHEMIC INJURY

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The recently emerging theory of the "fetal basis of adult disease" supports that early environmental stressors, in utero or perinatally, such as hypoxia/ischemia, by interacting with genetic vulnerability, may establish sensitivity to certain diseases later in life. Epidemiological studies have shown that perinatal hypoxia/ischemia is a major risk factor for the later development of learning, language and memory impairments, developmental forms of parkinsonism, attention deficit hyperactivity disorder and schizophrenia. Our previous studies on human autopsy brain material from neonates with neuropathological lesions of prolonged perinatal hypoxic/ischemic injury (PHI) have shown a dramatic reduction in the expression of tyrosine hydroxylase (first and limiting enzyme of dopamine synthesis) in the neurons of substantia nigra (SN), with parallel reduction of their cellular size. The question raised was whether these observations indicate an early stage of SN degeneration or a delay in its development. Since experimental studies have shown increased number of apoptotic neurons in the SN of rat and sheep after PHI (Oo et al., *Neurosci*, 1995,69:893-901; Castillo-Melendez et al., *Pediatr Res*, 2004,55:864-71), we immunohistochemically investigated the expression of cleaved caspase-3 (a widely used marker for detecting the early stages of apoptosis) and Iba-1 (a marker for microglia) in relation to the severity/duration of PHI, as estimated by neuropathological criteria. Our material included human brain tissues from 22 autopsied neonates with a corrected (prenatal plus postnatal) age ranging from 34 to 46,5 weeks obtained at postmortem examination after written parental consent. Our preliminary results showed absence of early signs of classical apoptosis in SN neurons after PHI. Only few positively caspase-3-positive glial cells were occasionally observed in the SN

of some neonates with neuropathological changes consistent with acute/severe PHI. In Iba-1 stained sections, a morphological variety of microglial phenotypes was revealed, indicating a different degree of microglial activation after PHI. The most intense microglial activation was seen in the neonates with acute/severe neuropathological PHI, while in cases with signs of prolonged or older PHI, intense activation was observed only in the presence of an underlying infection. These findings suggest that microglial proliferation in the absence of neuronal apoptosis appears to occur in the human SN after PHI. Similar findings have been reported for SN neurons in Parkinsonism (Graeber et al., *Parkins & Relat Disord,* 1999, 5:187-92).

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A NOVEL REGULATORY ROLE OF RGS4 IN NEURITE OUTGROWTH AND CELL PROLIFERATION MEDIATED BY STAT5B TRANSCRIPTIONAL RESPONSES UPON δ-OPIOID RECEPTOR ACTIVATION

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Regulator of G protein signaling 4 (RGS4), a member of the B/R4 family of RGS proteins, is a key regulator of opioid receptor signaling (ORs) that confers selectivity for G protein coupling,inhibits OR-mediated ERK1,2 phosphorylation and accelerates receptor internalization[(1,2)]. RGS4 is multitask protein that is expressed in the developing neurons and is highly implicated in neurodegenerative diseases however, the molecular mechanisms of how RGS4 could be involved in neurotropic pathways remains unclear.Based on our previous findings that δ-opioid receptor (δ-OR) forms a multiprotein signaling complex, consisting of Gi/Go proteins and the Signal Transducer and Activator of Transcription 5B (STAT5B) that leads to neurite outgrowth upon δ-OR activation [(3,4)] and having demonstrated that RGS4 directly interacts with STAT5B,we wondered whether RGS4-STAT5B interplay could regulate neuronal responses mediated by δ-OR activation.Our data demonstrate that RGS4 is a negative regulator of STAT5B activation that interferes in STAT5B phosphorylation, dimerization and transcriptional activation mediated uponδ-OR and/or erythropoietin receptor activation. Additional studies in adult brain extracts from RGS4$^{-/-}$ mice, lacking a functional RGS4, revealed increased levels of p-STAT5B. Moreover, RGS4 expression attenuated neurite outgrowth and neuronal differentiation of neuroblastoma cells upon δ-OR activation with specific analogs; whereas Neuro2A cells expressing RGS4 exhibited a significant reduction in cell number and proliferation rate.Primary neuronal cultures of RGS4$^{-/-}$ mice, lacking a functional RGS4, exhibited differential neuronal sprouting compared to wild types,by displaying increased axonal length and number of branches, upon δ-OR activation. On the other hand, isolated neural stem cells from the subventricular zone of adult RGS4$^{-/-}$ mice brain exhibited elevated proliferative properties as assayed by phopho-Histone3 immunostaining, with a concomitant increase of the mRNA levels of the STAT5B anti-apoptotic target genes, *Bcl-2* and *Bcl-xl*. Collectively, these results demonstrate for the first time a non-canonical function of RGS4 in STAT5B-mediated transcriptional responses providing also insights into therole of RGS4 involvement in neuronal development and synaptic signaling upon opioid administration.

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C1Q ABLATION EXACERBATES AMYLOID DEPOSITION: A STUDY IN A TRANSGENIC MOUSE MODEL OF ATTR V30M AMYLOID NEUROPATHY

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ATTR neuropathy is a lethal autosomal dominant sensorimotor and autonomic neuropathy due to deposition of amyloid fibrils composed of aberrant transthyretin (TTR) protein. A substitution of valine for methionine at position 30 of the protein is the commonest amyloidogenic mutation [1]. ATTRMet30 neuropathy exhibits a great degree of variability among different populations. Genetic and epigenetic factors have been implicated and although we have previously demonstrated a correlation of complement C1q polymorphisms with age of onset among the Cypriot population, the exact mechanisms remain undetermined [2]. The complement cascade, as a whole, has long been investigated for its association with inflammation and macromolecule aggregate clean-up. Using a double transgenic mouse model of ATTR V30M amyloid neuropathy in which C1q is ablated will help to shed a light into the possible modifier role for C1q in amyloidogenesis and the progression of the disease. A transgenic mouse model of ATTR Met30 was cross bred with a C1q knockout strain in order to produce a complement deficient ATTR Met30 strain. Thioflavin S, immunocytochemistry and immunoblotting were utilized to assess the amount of amyloid load and the expression of a number of molecular markers of apoptosis, oxidative stress, endoplasmic reticulum stress and phagocytosis were also investigated. Amyloid deposition was found to be increased by 60% in the absence of C1q. Significant up regulation was also recorded in apoptotic and cellular stress markers reflecting extracellular toxicity of pre-fibrillar and fibrillar TTR. Our data further indicate that in the absence of C1q there is marked reduction of macrophages in association with amyloid deposits and thus less effective phagocytosis of TTR as shown in Figure 1.

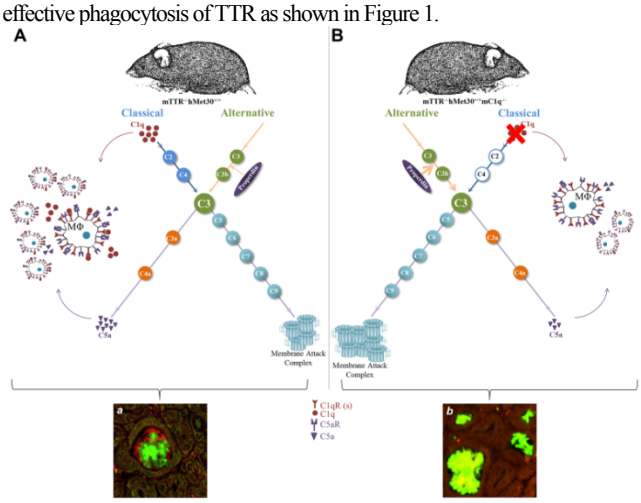

Figure 1. Classical and alternative pathway activation lead to the hydrolysis of C3 initiating the formation of the membrane attack complex (MAC) and production of the chemoattractant C5a. C1q and C5a recruit and activate phagocytic cells such as macrophages through receptors located on their surfaces (A). In the absence of C1q, properdin increases along the entire alternative pathway and the terminal MAC complex. Concurrently, the presence of CD68 positive phagocytes was decreased along with the

expression of C5a anaphylatoxin and its receptor CD88 (B). As a result, amyloid deposition increases following C1q ablation (b) versus the original model (a).

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PERIPHERAL TUMOR NECROSIS FACTOR-ALPHA (TNF-A) MODULATES AMYLOID PATHOLOGY BY REGULATING BLOOD-DERIVED IMMUNE CELLS AND GLIAL RESPONSE IN THE BRAIN OF AD/TNF TRANSGENIC MICE

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Increasing evidence has suggested that systemic inflammation along with local brain inflammation can play a significant role in Alzheimer's disease (AD) pathogenesis. Identifying key molecules that regulate the crosstalk between the immune and the central nervous system can provide potential therapeutic targets. TNF-α is a pro-inflammatory cytokine implicated in the pathogenesis of systemic inflammatory and neurodegenerative diseases such as rheumatoid arthritis (RA) and AD. Recent studies have reported that anti-TNF-α therapy or RA itself can modulate AD pathology, although the underlying mechanism is unclear. To investigate the role of peripheral TNF-α as a mediator of RA in the pathogenesis of AD, we generated double transgenic 5XFAD/Tg197 AD/TNF mice that develop amyloid deposits and inflammatory arthritis induced by human TNF-α (huTNF-α) expression. We found that 5XFAD/Tg197 mice display decreased amyloid deposition, compromised neuronal integrity and robust brain inflammation characterized by extensive gliosis and elevated blood-derived immune cell populations, including phagocytic macrophages and microglia. To evaluate the contribution of peripheral huTNF-α in the observed brain phenotype, we treated 5XFAD/Tg197 mice systemically with infliximab, an anti-huTNF-α antibody that does not penetrate the blood-brain-barrier and prevents arthritis. Peripheral inhibition of huTNF-α increases amyloid deposition, restores neuronal damage and suppresses gliosis and recruitment of blood-derived immune cells, without affecting brain huTNF-α levels. Our data report for the first time a distinctive role for peripheral TNF-α in the modulation of the amyloid phenotype in mice by regulating blood-derived and local brain inflammatory cell populations involved in beta-amyloid clearance.

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β-ADRENERGIC RECEPTORS LOWER THE THRESHOLD FOR LTP INDUCTION AND STABILIZATION IN THE VENTRAL BUT NOT THE DORSAL HIPPOCAMPUS

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The hippocampus is a functionally heterogeneous structure with the dorsal hippocampus (DH) implicated in memory and spatial navigation, while the ventral hippocampus (VH) mediates anxiety-related behaviours and generally emotional signal processing with, however, poorly understood mechanisms[1] . Hippocampus is densely innervated with noradrenergic fibers, that mainly originate in the locus coeruleus, which release noradrenaline during emotional arousal, modulating synaptic plasticity and memory consolidation via activation of β-ARs[2]. We demonstrate here that long-term potentiation (LTP) induction at CA1 synapses of rat hippocampal slices by minimal theta-burst stimulation, which is composed of two identical bursts of four pulses at 100 Hz with a 200 Hz inter-burst interval (2-burst)[3], that mimics a physiological firing pattern of hippocampal neurons, is remarkably more sensitive to β-AR activation in VH compared to DH. Thus, in the presence of β-ARs agonist isoproterenol, the previously subthreshold 2-burst stimulation, reliably induced NMDA receptor-dependent LTP in the VH, without facilitating LTP in the DH. Mobilization of endogenous noradrenaline was assessed by the presence of β-ARs antagonist propranolol during LTP induction by five theta-bursts or three ten-burst trains (3TBT). It was proved that noradrenaline contributes to LTP induction under these conditions in the VH but not in the DH. Isoproterenol also increased 3TBT-induced LTP in both hippocampal segments, but enhanced voltage-gated calcium channel-dependent LTP only in the VH. Importantly, isoproterenol enhanced postsynaptic excitability during theta-burst stimulation in the VH only. These results suggest that β-AR activation probably acts as a switch that promotes synaptic plasticity in the VH in emotionally arousing conditions.

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DIFFERENT CONTRIBUTION OF DOPAMINE D1/D5 RECEPTORS TO LTP IN THE DORSAL AND VENTRAL RAT HIPPOCAMPUS

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The hippocampus is a medial temporal lobe structure that is involved in episodic memory and learning. Hippocampus is divided in the dorsal pole (DH) which is important for memory and spatial navigation and the ventral pole(VH) that is involved in emotional behaviour such as arousal and anxiety[1]. Dopamine is a neurotransmitter, involved in signaling salient events and facilitates synaptic plasticity in the hippocampus, originating from the ventral tegmental area and the substantia nigra pars compacta. Its action is required for multiple forms of hippocampal synaptic plasticity in pyramidal neurons and is mediated by binding to the D1-like family of receptors which include D1 and D5 receptors[2]. In this study we used slices taken from the dorsal and ventral pole of the hippocampus and we induced long term potentiation (LTP) using theta burst stimulation, specifically thirty bursts of four pulses at 100 Hz with a 200 Hz inter-burst interval. The aforementioned protocol induces a robust LTP in slices from both poles[3]. We found that the selective activation of D1/D5 dopamine receptors by SKF38393 during LTP induction, strongly enhances the magnitude of the resulting NMDA receptor-dependent LTP, only in dorsal slices and not in ventral. Also the contribution of endogenous receptor activation to LTP was found similar in the two hippocampal segments, after the use of D1-like family receptor antagonist SCH23390. These results reveal a higher capacity of the DH compared to the VH for D1/D5 receptor-dependent synaptic plasticity, which may suggest

that the dopamine-dependent neural signaling of strong stimuli involves the DH but not the VH circuitry.

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A GRADIENT OF FREQUENCY-DEPENDENT SYNAPTIC PROPERTIES ALONG THE LONGITUDINAL HIPPOCAMPAL AXIS

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The hippocampus is a functionally heterogeneous brain structure along its longitudinal axis and this functional specialization may be attributed on the different organization of the intrinsic neuronal network[1]. Short-term synaptic plasticity (STP) is the ability of synapses to undergo lasting changes in their effectiveness[2]. STP is fundamental in hippocampus for information processing and may be significant in diversifying the properties of local neuronal network along its long axis. Therefore, we aimed to examine the properties of the CA1 synapses along the entire dorsoventral axis of the rat hippocampus using field excitatory postsynaptic potentials (fEPSPs) from transverse rat hippocampal slices and a frequency stimulation paradigm. Specifically we applied a ten-pulse stimulus train at frequencies ranging from 0.1 to 100 Hz to the Schaffer collaterals and we found a gradually diversified pattern of frequency-dependent synaptic effects along the dorsoventral hippocampus axis. The first conditioned response was facilitated along the whole hippocampus for stimulus frequencies 10-40 Hz. However, averaged responses generally ranged from maximum synaptic facilitation in the dorsal segments of the hippocampus to maximum synaptic depression in the ventral segments of the hippocampus. In particular, dorsal synapses facilitated for stimulus frequency up to 50 Hz while they depressed at higher frequencies (75-100 Hz). Facilitation at dorsal synapses was maximal at stimulus frequency of 20 Hz. On the contrary, ventral synapses showed depression regardless of the stimulus frequency, only displaying a transient facilitation at the beginning of 10-50 Hz stimulation. Importantly, the synapses in the medial hippocampus displayed a transitory behavior. Finally, as a whole the hippocampal synapses maximally facilitated at 20 Hz and increasingly depressed at 50-100 Hz. In conclusion we observed that the short-term synaptic dynamics change gradually along the hippocampal long axis, conveying distinct properties of information processing to successive segments of the structure. These findings are in line with the functional segregation along the dorsoventral axis of the hippocampus.

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GAZE AND HEAD CONTINGENCY IN ARTIFICIAL VISION

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Despite appearing automatic, perceiving the visual world is a highly complex process that depends on intact visual and oculomotor function. Visual scanning is necessary to efficiently integrate individual glances into more coherent perceptions [1]. To-date, however, most prosthetic devices do not deliver stimulation based on full gaze position (head direction plus eye position within the head), and thus provide suboptimal visual information to the user, while also requiring substantial training to hold the eyes fixed forward while scanning with the head [2]. Here, we report attempts of current visual prosthetic devices to overcome the hurdle of gaze contingency and address the effects of head versus eye movements on processing visual information in a simulated prosthetic vision paradigm [1]. Previous studies of visual performance with normal, sighted subjects using a simulation of artificial vision used a fully gaze-contingent mode [3, 4]. We now propose to perform a new study that includes a similar head-contingent mode so as to examine the potential improvements of adding full gaze contingency to existing visual prosthesis designs in order to optimize prosthetic utility for everyday activities. At the same time, such a study could answer questions regarding the level of training in holding the eyes still that is required for optimal head-only use. Answering these questions could help overcome the limitations of current prosthetic devices and would hold the premise to contribute to the post-implantation rehabilitation strategies that could assist patients make use of artificial visual signals that they provide.

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PERCEPTION, COGNITION, AND ACTION IN AGING: EXPLOITING THE BENEFITS OF MULTISENSORY INTEGRATION

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Aging is accompanied by degradations in sensory, temporal, and cognitive processing due to alterations in the functioning of the central nervous system and other physiological systems. Attempts to ameliorate the negative effects of normal aging have mainly focused on research to prevent falls, enhance cognitive processing, or provide assistive companions. To-date, however, no effort has been made to exploit the beneficial impact of multisensory integration so as to promote successful aging. The aging brain tends to rely increasingly on multisensory processing and integration by using sensory information from several modalities to improve gait performance, postural stability, and sensorimotor coordination [1]. The higher multisensory gains for older adults, along with evidence supporting a shared neural substrate for multisensory processing, executive functions, and motor control, suggest an important interplay between perception, cognition, and action [1-2]. We will examine this interplay by reviewing the current research on the sensory, cognitive, and temporal processing degradations of normal aging, as well as mobility-related impairments and their interactions with the aforementioned processes.Specifically, we will focus on how both gait and cognitive deficits are associated with several anatomical and functional changes such as: reduction in frontal white matter,decrease of functional connectivity in large-

scale networks, and altered oscillatory activity[1-3]. We will also coverhow cognitive, sensory, and temporal processing is associated to age-related gait impairments [1-3] and how gait can potentially serve as a predictor for cognitive decline (e.g., Alzheimer's Disease). We will conclude with evidence showing that altered multisensory processing during aging can be linked to both gait and cognitive impairments and propose that the introduction of multisensory training and intervention can lead to the amelioration of age-related degradations and the promotion of successful aging.

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ANTENNAL SENSORY NEURON CALCIUM RESPONSES TO OLFACTORY STIMULI REMAIN ROBUST THROUGHOUT LIFE IN DROSOPHILA

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The sense of olfaction is progressively compromised (hyposmia) during aging and age-dependent degenerative diseases such as Alzheimer's and Parkinson's disease and loss of olfaction ability, (anosmia) is a predictor of death. In particular, the number of olfactory receptor neurons decreases during aging but to what extend receptor survivors undergo age-dependent functional changes has not been documented in humans and mammals. In this study, we employed the genetic model organism Drosophila melanogaster to investigate changes in olfactory receptor neuron responses during aging. Drosophila manifests healthy aging, thus excluding any co-morbidities that follow aging in higher organisms. We hypothesized that olfactory neuron responses decay gradually with age. We divided a population of aging flies – meaning animals that have surpassed a 60-day lifetime – into three groups, depending on their motor performance in response to mechanical stimulation, thus utilizing a hybrid chronological-phenotypical classification of aging that allows defining the physiological rather than the chronological age of individual flies. Young fit animals, <20 days old, served as the control group. Animals were expressing the photofluorescent molecule GCamp6 on all their sensory neurons, including the olfactory receptor neurons in the antenna. Their activity and condition were evaluated by means of in vivo real-time calcium imaging during stimulation with a single and a series of two (with interval of 1 second between them) and four (with interval of 2 seconds between them) olfactory stimuli (2.5% hexanol in IPM). We found that calcium response amplitude and calcium influx and efflux duration do not change during aging. Only animals that were permanently impaired exhibit smaller response amplitude but still calcium handling by the receptor neurons was unaffected. Finally, strong calcium deregulation, in the form of spontaneous calcium waves and spikes, was evident in sensory neurons of flies that had entered the terminal stage of life. Indeed for dying flies, calcium influx increases considerably in receptor neurons and eventually the cells loose their ability to respond to the odor. In conclusion in contrast to our hypothesis, calcium responses of olfactory receptor neurons do not progressively decay with age, but remain robust throughout health span and impairment span, and collapse only few hours prior to death.

ACUTE AND REPEATED EXPOSURE WITH THE NITRIC OXIDE (NO) DONOR SODIUM NITROPRUSSIDE (SNP) DIFFERENTIALLY MODULATE RESPONSES IN A RAT MODEL OF ANXIETY

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The nitric oxide (NO) donor sodium nitroprusside (SNP) actually is under investigation for the treatment of schizophrenia. That anxiety disorders are noted to occur commonly in schizophrenia patients is known. Contradictory results were reported however, concerning the effects of SNP in animal models of anxiety disorders. The present study investigated the effects of acute and repeated administration of SNP on anxiety-like behaviour in rats assessed in the light/dark test. The effects of SNP on motility in a locomotor activity chamber were also investigated in rats. Acute administration of 1 mg/kg SNP 30 but not 60 min before testing induced anxiolytic-like behaviour which cannot be attributed to changes in locomotor activity. Conversely, a single injection of 3 mg/kg SNP at 30 min before testing depressed rats' general activity, while at 60 min this dose did not influence performance of animals either in the light/dark or in the motor activity test. Repeated application of SNP (1 and 3 mg/kg, for 5 consecutive days) did not alter rodents' performance in the above described behavioural paradigms. The present results suggest that the effects exerted by SNP in the light/dark test in rats are dose, time and treatment schedule-dependent. The current findings propose also a narrow therapeutic window for SNP in this animal model of anxiety.

EFFECTS OF THE ACTIVE CONSTITUENTS OF *CROCUS SATIVUS* L. CROCINS AND THEIR COMBINATION WITH MEMANTINE ON RECOGNITION MEMORY IN RATS

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Crocus sativus L., (saffron) is a plant cultivated in various parts of the world. Saffron and its active components affect a number of neural processes (anxiety, depression, schizophrenia e.t.c.). Crocins are among the active constituents of *Crocus Sativus* L. and their implication in cognition is well documented. In a first dose-response study, the effects of crocins on different stages of recognition memory (encoding, storage and retrieval) were assessed in rats. Further, the potential role of crocins as adjunctive agents for the treatment of memory disorders was investigated. For this second study, the effects exerted by a combination of sub-thresold doses of crocins and memantine, a compound approved for the treatment of Alzheimer's disease, on recognition memory were examined. For these experiments, the novel object recognition task (NORT), a procedure assessing recognition memory in rodents was used. Administration of crocins (15 and 30 but not 5 mg/kg, i.p.) reversed delay-dependent deficits in the NORT in the normal rat, suggesting that these saffron derivatives affected acquisition, storage and retrieval of information. In addition, the combination of sub-thresold doses of crocins (5 mg/kg) and memantine (3 mg/kg, i.p.) counteracted delay-dependent deficits in the same task in rats. These findings suggest that crocins may modulate different aspects of recognition memory and support a functional interaction between crocins and memantine on recognition memory. The latter support a potential role for crocins as adjunctive agents for the treatment of memory disorders.

ADOLESCENT CANNABINOID EXPOSURE IN FEMALE RATS: TRANSGENERATIONAL EFFECTS ON Δ^9-TETRAHYDROCANNABINOL BRAIN STIMULATION REWARD AND LOCOMOTION IN ADULT MALE OFFSPRING

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Marijuana is currently the most commonly abused illicit drug. According to recent studies, cannabinoid use occurring prior to pregnancy can impact brain plasticity and behavior in future generations. The purpose of the present study was to determine whether female adolescent exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC) induces transgenerational effects on Δ^9-THC brain stimulation reward and locomotion in adult male offspring. Female Sprague-Dawley rats received Δ^9-THC (0.1 or 1 mg/kg, ip) or vehicle (controls) during postnatal days 28-50. As adults, females were mated with drug-naïve males. We then assessed potential alterations of Δ^9-THC's (0, 0.1, 0.5 and 1mg/kg, ip) reward-facilitating effects, using the curve-shift variant of the intracranial self-stimulation (ICSS) procedure, and changes in its effects on locomotion using the open field test (OFT), in their adult male F1 offspring. The reward-facilitating effect of the 0.1mg dose of Δ^9-THC was abolished in the F1 offspring of females that were exposed to Δ^9-THC, whereas the reward-attenuating effect of the 1mg dose of Δ^9-THC remained unaltered. Furthermore, 0.1mg of Δ^9-THC that slightly increased locomotion in control animals induced hyperlocomotion in the F1 offspring of females that were exposed to 0.1 and 1mg of Δ^9-THC, and 0.5 and 1mg/kg of Δ^9-THC that slightly decreased locomotion in controls induced hyperlocomotion in the F1 offspring of females that were exposed to 1mg/kg of Δ^9-THC.The present results reveal that maternal Δ^9-THC exposure during adolescence can diminish the reward-facilitating effects of Δ^9-THC and induce marked locomotor-stimulating effects of Δ^9-THC in adult male offspring. These transgenerational effects occur in the absence of any in utero exposure. It is speculated that Δ^9-THC-exposure during female adolescence may affect neural mechanisms that are shaping reward- and psychomotor-related behavioral responses in a subsequent generation, as indicated by the shifts in the reward-facilitating and the locomotor-stimulating effects of Δ^9-THC.

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GBA AND A-SYNUCLEIN: IS IT A TOXIC RELATIONSHIP?

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Recent evidence indicates an association between mutations in the GBA gene that encodes glucocerebrosidase (GCase), the lysosomal enzyme deficient in Gaucher disease, and Parkinson's disease (PD). α-synuclein (AS), the key player in PD pathogenesis, and GBA have been demonstrated to synergistically cause lysosomal dysfunction. In the current study, we sought to further explore thisrelationship byexamining endogenous GBA downregulation with AAVs expressing microRNAs in the presence or absence of human wildtypeα-synuclein (AS). Experiments were performed in wt C57B6 mice in which AAVs expressing microRNA control, microRNA GBA or rescue microRNA +/- AS (6 treatment groups) were injected unilaterally in the striatum and dopaminergic system integrity was subsequently assessed 8 weeks following injections. The transgenes were efficiently delivered to the striatum and were also detected in the cortex and substantia nigra. GCase levels were reduced by approximately 60% with *in vitro* downregulation and 40% in striatal dopaminergic neurons. Interestingly, the combination of endogenous GBA downregulation and AS overexpression induces dopaminergic cell loss in the substantia nigra and decreased dopamine levels in the striatum while either treatment alone (GBA downregulation or AS overexpression), do not.AS secretion levels are currently being assessed with *in vivo*microdialysis. The present results support a synergistic toxic relationship between GCase (loss of function) and AS. Furthermore, GCase downregulation via microRNA AAV delivery in combination with increased human wildtype AS burden may represent a novel PD model of GBA heterozygosity.

ESCALATING LOW-DOSE Δ^9-THC ADMINISTRATION DURING ADOLESCENCE IMPAIRS PSYCHOMOTOR AND COGNITIVE-RELATED FUNCTIONS AND INDUCES NEUROBIOLOGICAL ALTERATIONS IN SPECIFIC BRAIN REGIONS IN ADULT MALE RATS

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Preclinical studies suggest that delta-9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive component of cannabis, during adolescence can trigger long-term behavioral and neurobiological alterations in adulthood. The evaluation of behavioral, neurochemical and neurobiological profile of adult rats following escalating low-dose adolescent Δ^9-THC exposure. Between post-natal day (PND) 35 and 45 adolescent male rats received escalating low-dose Δ^9-THC treatment twice daily (0.3 mg/kg PND 35–37; 1 mg/kg PND 38–41; 3 mg/kg PND 42–45) or vehicle. On PND 75: a) open-field motor activity was recorded b) sensorimotor gating was assessed with the use of the Pre-Pulse Inhibition (PPI) test c) neurochemical variables were measured in the prefrontal cortex (PFC) and the hippocampus (HIPP) and d) specific molecular variables were evaluated in both PFC and HIPP of adult rats. Δ^9-THC-treated rats showed increased reactivity to novelty versus vehicle. Δ^9-THC-treated rats didn't show PPI deficits compared with vehicle. In PFC, dopamine turnover ratio was decreased in Δ^9-THC-treated rats, versus vehicle, while the opposite effect was found in HIPP. Higher CB1 receptor protein levels were found in HIPP following Δ^9-THC treatment versus vehicle. Region-specific effects were also observed in dopamine transporter (DAT) levels, in HIPP and PFC following Δ^9-THC treatment versus vehicle. Present findings show that low-dose adolescent Δ^9-THC administration induced psychomotor stimulation, neurobiological impairment without psychosis-like profile, as deduced by PPI, and region-specific effects on the dopaminergic activity in HIPP and PFC. Based on these findings and our previous results low-dose Δ^9-THC during adolescence induces a profile mostly related to psychomotor and cognitive impairment in parallel with regionally specific neurobiological alterations.

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MICRO-RNA-934 IS A NOVEL REGULATOR OF EARLY HUMAN NEUROGENESIS THROUGH TARGETING THE WNT RECEPTOR FRIZZLED-5

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To identify neurodevelopmentally relevant networks, we performed whole transcriptome RNA-sequencing of coding and non-coding RNAs at distinct stages of directed neuronal differentiation of human embryonic stem (HUES) cells. Systematic analysis of RNA-Seq data on miRNA expression revealed that mir-934 is specifically expressed during neural induction, a stage characterized by neural progenitor expansion and early neuron generation. Interestingly, this miRNA is only found in certain primates, including humans, indicating that mir-934 is a novel miRNA that may be

involved in human neurogenesis. We confirmed the expression of mi934 during neural induction by qRT-PCR, concomitantly with a reduction of the mRNA of its *in silico* predicted target, Fzd5 receptor, which is a member of the Wnt signaling pathway. To test the interaction between mir-934 and Fzd5 we cloned the respective miRNA response element from the 3' UTR of Fzd5 into the 3' UTR of a dual luciferase reporter construct, pmirGLO. We found that co-transfection with mir934 and the Fzd5 reporter construct suppressed luciferase activity in HEK293T cells, thus verifying binding of mir934 on Fzd5. Moreover, functional studies showed that overexpression of mir934 had an effect on the process of neurogenesis *in vitro* by significantly reducing the number of PAX6+ progenitors while increasing the number of differentiated DCX+ neuronal cells. Inhibition of mir934 had the opposite outcome. Taken together, our data suggest that mir934 regulates human neurogenesis *in vitro*, by tuning down the levels of Fzd5 expression to prompt neural precursors to progress towards neuronal differentiation.

HUMAN T CELLS EXPRESS FUNCTIONAL KAINATE CHANNELS: A MODEL CELL TO STUDY NATIVE NON-NMDA RESPONSES IN NEUROLOGICAL DISORDER?

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Glutamatergic neurotransmission possesses a central role on both physiology and pathophysiology of the mammalian CNS. In the last decade, however, glutamate and its receptors have emerged as crucial regulators of the human immune system. More specifically, extracellular glutamate has been found to affect human T-cell stimulated calcium responses, antibody or mitogen induced proliferation, cytokine secretion and migration, all process that are known to be strongly dependent on the activity of the voltage-gated potassium channel Kv1.3. In our lab we have previously shown that extracellular glutamate exerts a complex modulation on the activity of the Kv1.3 channel in human T-cells. Additionally we showed that some, but not all, of the effects of glutamate could be accounted for by the activation of cognate group I and II metabotropic receptors. Herein by the use of the patch-clamp technique we present data showing for the first time the presence of functional kainate (KA) channels by recording KA-evoked single channel events and whole cell currents from freshly isolated T cells from healthy individuals. Additionally, we show that some of the effects of glutamate on the main potassium conductance of T-cells namely; the Kv1.3, are mediated through the activation of endogenous KA receptors. The above finding, that KA affects the activity of the Kv1.3 channel, when taken together with data from other labs that show: a) during the immune-synapse formation T-cell activation requires the release of glutamate by the antigen-presenting cell and b) that the Kv1.3 channel, which is the main regulator of T-cell activation, is located within the immune synapse, suggest that the non-NMDA channels may contribute to the glutamatergic regulation of the immune response. Moreover, our finding that freshly isolated human T-cells express functional non-NMDA channels with similar characteristics with the ones recorded from neurons may provide an *ex-vivo* model for the study of the activity of these channels in neurological and psychiatric disorders (epilepsy, mood disorders, schizophrenia, chronic pain) where glutamatergic transmission is thought to be involved.

BRUTON'S TYROSINE KINASE (BTK) FUNCTIONS PROMOTES HABITUATION TO FOOT SHOCKWITHIN THE MUSHROOM BODIES OF*DROSOPHILA MELANOGASTER*

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To survive animals cope with the constantly changing environment by adapting to novel stimuli. Habituation is a major mechanism to decrease responsiveness to repetitive or prolonged, non-reinforced stimuli because it filters the massive amount of information acquired from the environment. By habituating to less important signals, an animal can selectively focus its attention to more important stimuli. Habituation deficits have been related to schizophrenia, learning disabilities and migraines. However, the molecular basis underlying habituation is not fully understood. Our goal is to combine the powerful tool of Drosophila genetics with behavioral analysis in order to identify genes implicating in habituation. Assessing this question, we used the Minos transposon technology. In particular, we used MiMIC insertions (Minos Mediated Integration Cassette) to screen for habituation mutants. Among the genes being identified in this screen, we focused on a group of insertions within the gene encoding the Drosophila homologue of Bruton's Tyrosine Kinase (BTK), a protein necessary for B-cell maturation in humans. The Drosophila Btk (Btk29A) is implicated in adult survival and male genital formation. Flies bearing MiMIC insertions inside the Btk locus do not habituate to foot shocks under conditions that elicit strong habituation in control flies. We verified this result, using different mutants as well as pharmacological inhibition and RNA interference approaches strongly suggesting that Btk is necessary for normal habituation to electric foot shocks. To identify specific neuronal subsets requiring functional BTK to promote habituation to foot shock, we used RNA interference and a series of GAL4 drivers to attenuate it in different neuronal subsets. We found that BTK is required within the mushroom bodies (including α, β and γ lobes) for normal habituation. On the other hand, overexpression of both BTK isoforms did not result in premature habituation. Because deficient habituation has been lined to schizophrenia, we tested two of very common antipsychotic drugs, Clozapine and Risperidone in *btk* mutant flies. In both cases, drugs restored normal habituation in the mutants. It appears therefore, that common molecular mechanisms may link defective habituation in flies and schizophrenia in humans.

GENE THERAPY APPROACH USING A LENTIVIRAL VECTOR FOR TREATING CHARCOT-MARIE-TOOTH TYPE 4C

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Charcot-Marie-Tooth Type 4C (CMT4C) is the most frequent form among recessively inherited demyelinating neuropathies and results from mutations in the *SH3TC2/KIAA1985* gene. *SH3TC2* mutations cause loss of function of the SH3TC2 protein suggesting that gene replacement therapy may be useful for treating CMT4C. *Sh3tc2*-/- mice develop an early onset progressive peripheral neuropathy with hypo- and demyelination along with decreased motor and sensory nerve conduction velocities, offering a relevant model for testing treatments for CMT4C. Our aim is to rescue the phenotypic and pathological alterations of *Sh3tc2*-/- mouse model by gene replacement strategy. In order to do this, we generated a novel lentiviral vector, LV-Mpz-*SH3TC2.myc*, to drive expression of the human *SH3TC2* cDNA under the control of the myelin protein zero (*Mpz*/P0) promoter specifically in myelinating Schwann cells. A myc tag was added to facilitate expression analysis. A control vector (mock) was also produced in which the *SH3TC2* cDNA was replaced by the EGFP reporter gene. We first confirmed expression of hSH3TC2 in Hela cells byimmunofluorescence analysis showing strong expression of SH3TC2 specifically at the plasma membrane with additional localization in a dotted pattern intracellularly. For *in vivo* gene delivery we used both intraneural and intrathecal injections of the LV-Mpz-*SH3TC2.myc* vector in 2-week to 4-month old *Sh3tc2*-/- mice. Expression of virally delivered h*SH3TC2* was assessed 4 and 8 weeks after injection. Immunofluorescence analysis showed hSH3TC2 immunoreactivity in perinuclear Schwann cell cytoplasm in sciatic nerve teased fibers of *Sh3tc2*-/- mice following both intraneural and intrathecal delivery, while lumbar intrathecal gene delivery resulted additionally in expression of hSH3TC2 in the lumbar roots. For treatment trials we have injected intrathecaly litters of *Sh3tc2*-/- at the age of P20 with either full or mock vector. Behavioral analysis showed improved results in fully treated *Sh3tc2*-/- in rotarod and foot grip tests. Moreover, motor nerve conduction velocities were significantly increased in fully treated *Sh3tc2*-/- compared to mock injected mice. Morphological analysis confirmed significant improvement in g-ratios, myelin thickness, and numbers of demyelinated fibers in lumbar roots and sciatic nerves of fully treated compared to mock-treated *Sh3tc2*-/- mice. Thus, we have developed a novel lentiviral vector for Schwann cell targeted gene delivery to treat CMT4C and for testing possible therapeutic effects in the mouse model of the disease.

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BEHAVIORAL DEFICITS AND DISRUPTED CYTOARCHITECTURE IN CEND1 NULL MICE CORRELATE WITH PERSISTENT PROGENITOR CELL PROLIFERATION AND INCREASED APOPTOSIS

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During nervous system development, neural progenitors arise in proliferative zones, then exit the cell cycle and differentiate to neurons as they migrate away from these zones to reach their final destinations. Cend1 (for cell cycle exit and neuronal differentiation 1) is a neuronal-lineage specific modulator involved in the coordination of cell cycle exit and differentiation of neuronal precursors. We have previously shown that Cend1$^{-/-}$ mice show alterations in cerebellar layering arising from increased proliferation of granule cell precursors, delayed radial granule cell migration and impaired Purkinje cell differentiation. These structural abnormalities resulted in functional impairment, which was manifested by ataxic gait and deficits in motor coordination. To further characterize the impact of Cend1 genetic ablation, here we performed a battery of behavioral tests, including spatial learning and memory trials in the Morris Water Maze, associative learning in the fear conditioning test, exploratory behavior and anxiety in the elevated plus maze and novel object recognition. We observed significant deficits in all but the latter test, suggesting structural alterations in brain regions such as the cortex, amygdala and hippocampus. In agreement, immunohistochemistry revealed reduced numbers of GABAergic interneurons in the adult cortex and the amygdala, particularly in the basolateral amygdaloid nucleus. The paucity in GABAergic neurons in adult Cend1$^{-/-}$ mice correlated with increased proliferation and apoptosis as well as reduced migration of neuronal progenitors at the embryonic ganglionic eminence from which these cells originate. Further, we noted increased astrogliosis and aberrant neurogenesis in the adult dentate gyrus of the hippocampus, which may be associated with the spatial learning and memory deficits noted. Our data highlight the necessity for Cend1 expression in the establishment of a structurally and functionally normal phenotype.

DYNAMIC ENGAGEMENT OF DISTINCT NEURONAL CIRCUITS IN STIMULUS EVALUATION AND HABITUATION

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Animals continuously evaluate and respond to thousands of different stimuli. Behavior is formed in response to stimuli in the environment and modification of the initial response to a stimulus is essential for survival and thus, well conserved among the species. Habituation is the decrease in response to repeated inconsequencial stimuli. This form of non-associative learning enables the animal to ignore insignificant stimuli and focus on more salient ones. Impaired habituation indicates improper evaluation of stimuli and is linked to various disorders such as schizophrenia, ADHD, migraines and autism spectrum disorders. Since the molecular mechanisms that govern it are largely unknown, we developed a new paradigm of odor avoidance habituation in Drosophila to begin elucidating them at the circuit and molecular level. This novel assay permitted dissection of the perceived value of the stimulus in two phases. During the initial phase, brief exposure to the odor does not change its aversive value and the response of the flies remains unaltered. However, in the second phase, extended continuous exposure to the odorant leads to a significant response decrement, the habituated response. Using genetic tools to functionally silence or activate specific neuronal subsets, our aim is to identify the neuronal circuits underlying these two phases of stimulus evaluation. We report on our circuit analyses, focused on neuronal subsets involved in odor processing along with the mushroom bodies, the centre of olfactory learning and memory in Drosophila. Furthermore, we investigate the role of the adenylyl cyclase Rutabaga in habituation, a protein with a prominent role in mushroom body-dependent associative learning.

STRESS GRANULES AND AUTOPHAGY INTERPLAY IN STRESS-DRIVEN TAU PATHOLOGY

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Imbalance of neuronal proteostasis associated with misfolding and aggregation of Tau protein is a common neurodegenerative feature in Alzheimer's disease (AD) and other Tauopathies. Consistent with suggestions that lifetime stress maybe an important precipitating factor of AD, we previously reported that environmental stress and high glucocorticoid (GC) levels evoke accumulation of aggregated Tau; however, the underlying molecular mechanisms remain unclear. We now demonstrate that chronic stress and GC trigger an mTOR-dependent inhibition of autophagic process, the cardinal clearance pathway for aggregated proteins, leading to accumulation of Tau aggregates and cell death in mice and cells stably expressing P301L-Tau. Considering the interplay of autophagy with Stress granules (SGs) dynamics, we also show that environmental stress/GC stimulate the induction of SGs, recently shown to promote Tau misfolding, aggregation and neurotoxicity. Notably, pharmacological intervention that stimulates autophagic process (Temsirolimus) attenuates the GC-driven elevation of Tau, SGs and cell death. This work provides novel insights about the mechanisms through which neuronal cells convey the detrimental impact of environmental (HPA-related) stress to intracellular "stress" signaling and transcriptome response precipitating Tau-driven brain pathology.

HUMAN LEUCOCYTE ANTIGEN-DRB1* GENOTYPING IN EARLY-ONSET MULTIPLE SCLEROSIS IN A HELLENIC SAMPLE

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Multiple sclerosis (MS) is a demyelinating disorder of the central nervous system triggered by both genetic and environmental factors[1]. It is considered mainly a disease of early adulthood, although approximately 5% of all MS patients have symptom- onset during childhood and adolescence.[1,2] Today, HLA-DRB1*15 alleleis considered as the strongest genetic risk factor for both pediatric and adult MS.[3] The aim of this study is to investigate for the first time the causative or protective effect of the HLA-DRB1* alleles in a Hellenic sample of early-onset MS-patients, in comparison with both adult-onset MS and healthy controls. A group of 195 MS-patients diagnosed according to McDonald criteria and a group of 107 healthy volunteers with no history of autoimmune or inflammatory disease were studied.

Bloodcollection, DNA extraction and HLA-Class II-Genotyping were performed. Frequencies of the DRB1* alleles (low-resolution) were compared across groups using the two-sample chi-square test.Comparisons were made according to the Benjamini-Yekutieli method. 72 early-onset MS-patients (30 males and 42 females, median age: 28 years, median age disease onset: 16 years, median EDSS: 2,6) and 123 adult-onset(46 males, 77 females, median age: 37.5 years, median EDSS: 3) were identified. Regarding the early-onset group, HLA-genotyping has been performed in 42 patients so far and the preliminary results are reported here. The DRB1*15 allele appeared with significantly higher frequency in MS-patients than healthy controls, while theDRB1*16 allele was absentin the pediatric group, indicating a possible protective effect. A tendency towards a higher frequency of HLA-DRB1*03 in patients with early-onset of the disease should be confirmed in a larger sample. Our study confirms the role of HLA-DRB*15:01 as the main risk allelein both early-onset and adult MS-patients. New findings include the putative predisposing role of DRB1*03 allele, which ismainly associated with NMOand the protective role of the DRB1*16 allele in early-onset MS. Confirmation of these results in a larger sample is considered mandatory.

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TEST-RETEST RELIABILITY OF FUNCTIONAL TRANSCRANIAL DOPPLER ULTRASONOGRAPHY FOR MEASURING CEREBRAL LANGUAGE LATERALIZATION USING A WORD-GENERATION TASK

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Functional transcranial Doppler ultrasonography (fTCD) is performed by placing probes on either side of the head and using a 2 MHZ pulsed sound wave to insonate through areas of the temporal bone in order to measure blood flow in the middle cerebral arteries (MCAs). It thereby provides continuous measurement of blood flow changes which can be associated with cortical activation during a cognitive task. FTCD has been validated for the measurement of cerebral language lateralization against both fMRI and the Wada test and has been extensively used in the last couple of decades towards this end. FTCD is completely safe and thus suitable for both research purposes and clinical use, it is easily applicable, cost-effective and it has excellent temporal resolution. However, its test-reliability has not been adequately studied to date. The goal of the present study was to assess the test-retest reliability of fTCD for the measurement of cerebral language lateralization using intervals spanning from 24 hours to 6 months. Nineteen healthy right-handed adult volunteers (9 men) were recruited and asked to perform 20 trials of a word-generation task while the changes in blood flow velocity in their right and left MCAs were being recorded by means of fTCD. The signal was clear in, at least, 10 cycles for 17 participants on both examinations. Results showed good test-retest reliability (rho = 0.0,64, $p < 0.01$). The reliability improved, albeit slightly, when only 13 of the participants, who had at least15 cycles with clear signal, were included in the analysis (rho = 0.69, $p < 0.01$). Overall, fTCD can provide reliable measurement of cerebral language lateralization even when there is a time interval of 6 months between the first and second examination.

INTENTIONAL BINDING IN MULTISENSORY EVENT SEQUENCES: THE ROLE OF INTENTIONALITY, CAUSALITY, AND TEMPORAL PREDICTABILITY

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The temporal illusion of subjectively experiencing a shorter interval duration between a voluntary action and its produced sensory effect is known as intentional binding (i.e., IB) [1]. The research on IB has mainly focused on the use of abstract events, that lack an inherent causal link between actions and their effects, and, thus, they require the use of adaptation strategies. We proposed the adoption of a more naturalistic approach by using multisensory events that are familiar and learned and, thus, they have inherent causal associations.We hypothesized that this established causal link between the event sequences would lead to an increased IB magnitude without the need for adaptation strategies. We conducted five experiments, where we manipulated the action-effect causal relations, while participants performed a simultaneity judgment task [2]. That is, we presented an audiovisual impact action effect, which could be causally related or not, with a preceding voluntary action and an initial cue. Moreover, we tested for the impact of intentionality by the presence or absence of a voluntary action, while the temporal intervals between the action and effect were fixed or random (i.e., manipulation of temporal predictability). Participant data showed no IB induction for audiovisual abstract (Experiment 1) or naturalistic action effects (Experiment 2). However, an enhanced action-effect causal link from the initial cue up to the effect pair (Experiment 3) for temporally predictable intervals, displayed a robust IB effect. In conflict situations, where the event sequences were either mismatched (unrelated initial cue to action and effect; Experiment 4) or had a mismatched response mapping (different type of voluntary action; Experiment 5), no IB effect was obtained. These findings suggest that IB is bound toan integrated sense of causality, which can be obtained only for causally-linked and temporally predictable sequences of multisensory events. Any disruption in this causal sequence, or absence of voluntary action and temporal action-effect predictability, led to the attenuation of the IB effect.

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DOUBLECORTIN-POSITIVE NEURAL PROGENITORS IN NON-NEUROGENIC REGIONS OF THE MOUSE BRAIN FOLLOWING SVZ MECHANICAL AND CHEMICAL INSULT

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It is largely accepted that in the adult mammalian brain neural stem/progenitor cells have a spatially restricted distribution, and neurogenesis takes place only in specific brain areas. Thus, all brain regions beyond the SVZ and dentate gyrus are considered "non-neurogenic". Recently, it is proposed that a low level of neurogenesis occurs also in "non-neurogenic regions", such as the striatum and, to a lesser extent, the cortex. Importantly, brain lesions have succeeded to unmask neurogenic activity in such regions in laboratory rodents. In this study we aim to investigate whether disturbance of the adult mouse SVZ, activates cells of neighboring non-neurogenic regions to acquire a neural progenitor potential. To this end, we have performed a protocol aiming to reduce the SVZ-stem cell population, using a combination of mechanical trauma in the SVZ area followed by subsequent repeated, stereotaxic intraventricular injections of the mito-toxic agent

arabinoside-C (Ara-C). Our preliminary results indicate SVZ mechanical trauma triggers increased proliferation and production of doublecortin$^+$ (DCX$^+$) neural progenitors not only in the SVZ, but at a low rate in adjacent striatal and cortical areas. The infliction of mechanical trauma followed by intraventricular infusions of Ara-C amplifies the number of neural progenitors in the "non-neurogenic regions". Additionally, most of the DCX$^+$ clusters in the striatum co-localize with myelin bundles. Moreover, a large and spatially disorganized population of DCX$^+$ cells is found to migrate from ventral SVZ towards the anterior commissure, following SVZ trauma – AraC administration. Our studies are on-going to further characterize the spatio-temporal distribution and molecular phenotype of neural progenitors arising in non-neurogenic brain regions.

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$GABA_B$ RECEPTORS DIFFERENTLY CONTROL SYNAPTIC TRANSMISSION BETWEEN THE DORSAL AND VENTRAL CA1 RAT HIPPOCAMPAL SYNAPSES

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The hippocampus is an elongated brain structure that displays functional heterogeneity along its long axis (called also dorsoventral or septotemporal axis). Furthermore, recently accumulated experimental evidence has revealed the existence of important diversification in the endogenous neuronal circuitry along the along the longitudinal axis of the hippocampus and in particular between the dorsal (DH) and the ventral hippocampus (VH). Among the most important mechanisms that regulate the function of local neural networks are those that control the release of neurotransmitters from presynaptic terminals. In the hippocampus, presynaptic $GABA_B$ receptor ($GABA_BR$) represents a major mechanism that controls the release of both glutamate and GABA from excitatory and inhibitory neurons respectively. Using field recordings of excitatory postsynaptic potentials (fEPSPs) from transverse slices prepared from the DH and VH we examined the effects of the agonist of $GABA_BRs$ baclofen over a wide range of concentrations, from 0.5 to 100 μM. We found that baclofen produced a significant and concentration-dependent reduction in excitatory synaptic transmission (measured by the slope of fEPSPs) in both DH and VH. Remarkably, this action was significantly higher in VH than in DH for relatively low drug concentrations. Furthermore, baclofen significantly increased paired-pulse facilitation of fEPSP (at an inter-pulse interval of 50 ms) in a concentration-dependent manner. This action was similar in DH and VH. All baclofen actions were completely reversed in the presence of the specific $GABA_BR$ antagonist CGP 52432 (10 μM). We propose that the dorsoventral differences in the actions of $GABA_BRs$ on synaptic transmission, by shaping the balance between excitation and inhibition is importantly involved in tuning the balance between excitation and inhibition in the local circuitry, thereby importantly influencing information processing along the hippocampus long axis.

NR5A2 AFFECTS NEURONAL DIFFERENTIATION OF NSCS IN ADULT HIPPOCAMPUS

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Neurogenesis in the dentate gyrus (DG) of adult hippocampusis actively involvedin brain homeostasis.Identification of novel regulators in adult neurogenesis could significantly contribute to new therapies. Accordingly, we have recently unraveled the regulatory role of NR5A2, a druggable orphan nuclear receptor in embryonic neurogenesis.However, its involvement in adult neurogenesis is an open question. To this end, here we showed that NR5A2 is expressed in the DG of adult hippocampus. Higher expression levels of NR5A2 were observed in DG neurons than adult neural stem cells (aNSCs) or progenitor cells, suggesting a correlation with neuronal differentiation. In agreement, NR5A2 overexpression in *ex vivo* cultured aNSCs, led to a reduction of proliferation and increase of neuronal differentiation. Moreover, conditional deletion of NR5A2 in DGcells*in vivo* caused a decrease in the number of NeuN as well as Calbindin positive neurons, indicating its necessity for the maintenance of neuronal identity. Our data propose a regulatory role of NR5A2 in adult hippocampal neuronal differentiation and identity.

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CANNABINOID RECEPTOR 1 ACTIVATES RAS AT THE LIPID RAFTS

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The Cannabinoid 1 receptor, CB1, is now considered as a major regulatory molecule of the development and function of the CNS. The abundance of CB1 receptors in the cerebral cortex, striatum, cerebellum, hippocampus and hypothalamus, and the discovery of several endogenous ligands have led to research that established the CB1 system as an extensive base for the regulation of CNS physiology, ranging from proper CNS cellularity to complex behaviors such as fear, appetite, and addiction. Understanding the signal transduction pathways that CB1 utilizes for its acute and long term actions is therefore pivotal, and in particular the activation of its major effector ERK in discrete amplification waves. More specifically, we have previously shown that the specific CB1 agonist Methanandamide (R(+)-MA) induced a biphasic ERK1/2 activation at 5 and 15 min, mediated by sequential activation of $G_{q/11}$/PLC/PKCε/Src/Fyn, and subsequent $G_{i/o}$/Src/Fyn/FGFR recruitment, respectively, all requiring integrity of lipid rafts. In our effort to further analyze the proximal CB1R signaling events and their topology, we now show with confocal analysis that rafts are required for proper CB1 presentation at the plasma membrane. Most CB1-fluorescence was seen at the plasma membranes and lesser in the cytoplasm, attributed to cycling of the receptor between plasma membrane and endosomes after autocrine or paracrine stimulation of the CB1 ("constitutive endocytosis"). When cells were incubated with R(+)-MA (9nM), plasma membrane localization became minimal, while the intensity of the intracellular juxta- and peri-nuclear pools of CB1 receptors significantly increased by a 3-fold. Disruption of the lipid rafts with Methyl-β-Cyclodextrin (MCD), or of microtubules, resident proteins in raft microdomains, with nocodazole, or of the closely associated F-actin cytoskeleton with cytochalasin resulted, in all cases, in minimal presentation of the receptor at the plasma membrane and in its increased concentration in intracellular pools. Moreover, we show for the first time that R(+)-MA induced acute Ras activation (<2 min) in a time-dependent manner, as assessed in cortical neurons in culture and in rat hippocampal ex vivo preparations; this activation also required integrity of the lipid rafts and the associated cytoskeleton. These data collectively suggest that CB1 targeting and signalling is regulated by lipid rafts and its functional cytoskeleton partners, and that Ras activation is the earliest event towards ERK activation.

THE FUNCTIONAL ROLE OF CONNEXINS INPERIPHERAL MYELINATED FIBERS IN HEALTH AND DISEASE

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Gap junctions (GJs) are membrane channels foundin most tissues connecting adjacent cells or differentcell compartments as in Schwann cells. They areinvolved in electrical connectivity and metabolichomeostasis allowing the passage of smallmolecules such as ions, second messengers, nucleotides and

peptides. An important functionalrole of peripheral nerve connexins is suggested bytheir involvement in X-linked inherited neuropathy aswell as in acquired neuropathy caused by oxaliplatin.Although GJs play a role in electrical connectivitytheir specific role in the formation of the sciatic nervecompound action potential (CAP) remains unclear.The aim of this study was to investigate the role ofperipheral nerve connexins in the electricalresponses of the mouse sciatic nerve under normaland stress conditions. For this purpose we usedsciatic nerves of three different mouse models, theCx32 knockout (KO), Cx29 KO and the Cx32/Cx29double knockout (dKO) mice. Using our *ex vivo*model for extracellular recordings we exposed sciaticnerves from different genotypes to three different GJblockers: octanol, 18-beta-glycyrrhetinic acid (GRA)and octanoic acid (OA) and recorded the CAP.Amplitude and duration of the CAP were used as anindication for the effects of the different blockers onthe CAP formation. All GJ blockers caused a gradualdecrease of the CAP without any changes in theduration of the CAP in all genotypes, suggestingprogressive disturbance of axonal membraneexcitability in the absence of one or two GJ proteins. Comparison of the three genotypes showed thatCx32 may play a dominant role in the maintenance ofthe CAP formation since nerves from Cx29KO miceproved to be more sensitive to the GJ blockerscompared to the Cx32KO nerves showing a fasterdecline of the CAP amplitude. Moreover the effect ofGJ blockers was similar in Cx32KO and dKO nerves. Finally, the effect of GJ blockers on the dKO nervesimplies the presence of another GJ protein. Nerves from dKO mice were additionally exposed to a combination of oxaliplatin and octanol. We further investigated the morphological properties of nerves exposed to the inhibitors and of dKO nerve fibers showing that nor the exposure to inhibitors or the lack of the known gap junctions cause any morphological abnormalities. Inconclusion, our results confirm the direct functionalinvolvement of Cx32 GJ channels and Cx29hemichannels in the CAP formation and indicate theexistence of at least one more connexin in peripheralnerve.

ALS-ON-CHIP: A NOVEL ORGAN-ON-A-CHIP DEVICE FOR HIGH-THROUGHPUT STUDIES OF MOTOR NEURON DISEASE

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Organ-on-chip devices is an emerging technology that provides advanced *in vitro* 3D tissue models based on cells growninside biomaterial models of the extracellular matrix. Organ-on-chip designs usually utilize organ-specific cells, derived from pluripotent stem cells such as ESC and iPSC. Several organ-on-chip platforms have focused on central nervous system (CNS) studies, providing novel ways to probe physiology and pathology, including motor neuron diseases [1,2]. Despite their elegance, existing organ-on-chip designs are usually complex, provide low sample throughput, and are not always easy to quantify using state-of-the-art high-throughput "-omics". These drawbacks limit their application in several key applications including preclinical drug discovery and personalized medicine. This work describes the first prototype of ALS-on-chip, a novel organ-on-chip platform developed for high-throughput studies of ALS pathology and ALS drug screening. The platform is based on studying appropriate cell players (motor neurons, glia, myocytes) inside miniaturized porous collagen-based scaffolds derived from biologically active biomaterials utilized clinically in induced regeneration [3]. Existing ALS-on-chip designs utilize mouse ES-derived motor neurons [4] co-cultured with differentiated C2C12 myoblasts. The device design provides easy quantification of large numbers of motor neuron samples inside a biologically-relevant 3D matrix by automated fluorescence imaging or high-throughput proteomics. Compared to existing organ-on-chip designs that focus on CNS disorders, our ALS-on-chip device enablesimportantnovel applications such as preclinical drug screening, and systems-level studies on drug mode of action analysis. Here, we describe the key features of the ALS-on-chip device, provide detailed characterization of the device, describe the procedures for generating the 3D cultures and co-cultures of mouse motor neurons in the chip, and provide pilotHCS data for quantifying excitotoxicity effects to mouse ES-derived motor neurons or motor neuron cell line models of ALS. Future work aspires to provide a novel important platform for preclinical ALS drug discovery by utilizing mouse motor neurons derived from the hSOD1G93A mouse model and human iPS-derived motor neurons.

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CHALLENGING THE POINT NEURON DOGMA: FS BASKET CELLS AS 2-STAGE NONLINEAR INTEGRATORS

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Fast Spiking interneurons play a critical role in the proper functioning of numerous brain areas. Yet dendritic integration in these cells and its role in neuronal output remain largely unknown. Recent experiments revealed that dendrites of interneurons in the cerebellum support sublinear summation of EPSPs, often coupled to supralinear calcium accumulations, thus hinting to the importance of dendritic computations. Using biophysical models of hippocampal and L5 medial PFC interneurons, we predict that dendritic integration in FS basket cells is bimodal: supralinear, characterized by local sodium spikes within large-volume dendrites and sublinear, occurring within small-volume dendrites in the absence of local spiking. Synaptic activation of varying sets of these dendrites leads to neuronal firing variability that cannot be explained by the widely adopted point neuron reduction. Instead, a 2-stage artificial neural network, with both sub- and supralinear hidden nodes, captures most of the variance. This is the first systematic computational study that aims to shed light on the dendritic computations of the Fast Spiking interneurons across hippocampal and neocortical areas. We propose that FS basket cells have substantially expanded computational capabilities subserved by their non-linear dendrites and are better described by a 2-layer ANN rather than a linear point neuron.

REAL-TIME STRATEGY GAMING FOR THE ENHANCEMENT OF COGNITIVE FUNCTIONING IN ADHD

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Attention deficit hyperactivity disorder (ADHD) is the most common childhood behavioral disorderand it is characterized by inattention and/or hyperactivity/impulsivity. ADHD has been traditionally treated using medication, behavioral therapy, and/or cognitive-social skills training. The need for less medication during childhood and the large cognitive training programs drop-out rates have lead researchers to venture into the adoption of video gaming for rehabilitation given their pervasiveness in everyday life and induction of high motivational states that promote release of striatal dopamine [1].Current studies have focused on action video gaming, we, however, present the FocusLocussolution that uses Real-Time Strategy (RTS) gaming.The use of RTS gaming is fairly limited, however, the data collected to-date has demonstrated benefits that span across development and cognitive skills[2-4] and it has been supported that RTS training is ideal for tuning multiple cortical networks due to the sustained maintenance and rapid switching across multiple information sources at a high workload for long periods of time over several weeks [4]. Here, wedescribe the development of a tablet- and intervention center-based (via virtual reality) RTS game that targets: delay aversion/timing, inhibitory control, selective and sustained

attention, motor coordination, and working memory. These cognitive skills were selected given their association with the control of specific ADHD symptoms and behaviors and evidence of improvement of these skills through other training techniques. The FocusLocus solution is a one-of-a-kind RTS game that in combination with other treatments will assist for the control of ADHD symptomatology and the individual's efficient integration to daily functioning.

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EFFECT OF CORTICOTROPIN RELEASING FACTOR RECEPTOR ANTAGONISTS ON LONG-TERM POTENTIATION IN THE MALE AND FEMALE PREFRONTAL CORTEX

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The 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. CRF binds to two types of receptors, namely CRFR1 and CRFR2. CRFR1 is the predominant receptor expressed in the PFC. CRFR1 signaling exhibitssignificant differences between females and males. The aim of this study was to investigate the effect of CRFR1 on synaptic plasticity in the 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. Field 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. LTP was induced with a tetanic stimulus in layer II synapses. LTP was induced with similar magnitude in the PFC of both male and female mice. The presence of a-Helical CRF9-41 reduced the degree of potentiation of synapses in male PFC but slightly increasedLTP in females. In the MAM model of schizophrenia, we identified sex differences in LTP deficits in the PFC. Specifically, LTP issignificantly reduced in male MAM-mice compared to control mice but it is not affected in female-MAM mice. We administered antalarmin to control and MAM female mice to test the hypothesis that increased CRF signaling in females protect PFC function in the MAM model. Administration of antalarmin significantly increased the levels of synaptic potentiation in control female mice, while in MAM-female mice it prevented the emergence of synaptic plasticity. Our results, so far, support the sexual dimorphism of CRF receptor function both in normal and pathological states. Future studies are planned to delineate the mechanisms involved in this sexual dimorphism of the CRF system in the mouse PFC.

MODELLING TAUOPATHY-ASSOCIATED LEARNING AND MEMORY DEFICITS IN *DROSOPHILA MELANOGASTER*

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Tauopathies are a group of neurodegenerative disorders characterised by altered levels of phosphorylation or mutations in the neuronal microtubule protein Tau. WT and mutant human Tau-encoding transgenes expressed pan-neuronally in the Drosophila Central Nervous System (CNS) yielded specific and differential toxicity in the embryonic neuroblasts that generate the mushroom body (MB) neurons and adult specific learning deficits and premature lethality suggesting cell type-specific effects of Tau in the CNS. All Tauopathies involve changes in the phosphorylation, but we have linked learning deficits and premature toxicity to occupation of specific sites. Also, aggregation of Tau into filaments and neurofibrillary tangles is one of the defining pathological hallmarks of Alzheimer's disease and other tauopathies. Thus, therapeutic strategies have focused on inhibition of tau phosphorylation or disruption of aggregation. The first aim of this study was to investigate the role of human Tau accumulation on Long Term Memory (LTM). Adult specific expression of a human Tau isoform associated with most Tauopathies in the fly adult CNS results in age-dependent learning and deficits specifically in LTM, but not another form of consolidated memory Anesthesia Resistant Memory. Our second objective was to clarify if these deficits are reversible and if the neurons can regain their function, suggesting that the CNS is not permanently damaged and should respond to pharmaceuticals. Interestingly, upon switch-off of transgene expression, LTM deficits were reversible and surprisingly increased levels of aggregates weredetected. With the use of specific compounds which act as Tau aggregation inhibitors, we aimed to clarify whetheraggregates aretoxic or protective for disease development.

SCHIZOTYPAL TRAITS AND NEUROCOGNITION IN UNAFFECTED RELATIVES OF SCHIZOPHRENIA PATIENTS WITH FAMILIAL OR SPORADIC SCHIZOPHRENIA: THE EFFECTS OF PATERNAL AGE

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Schizophrenia is caused by the interaction of genetic and environmental (e.g. age of father at the patient's birth) factors. According to the segregation of the illness in families, it can be classified into familial and sporadic, according, with familial schizophrenia being highly genetically-determined. A promising approach to investigate the vulnerability to the disorderis via the study of endophenotypic markers in unaffected first-degree relatives of patients. Previous studies examining cognitive and clinical endophenotypes inunaffected relatives of patients with these two subtypes of schizophrenia have yieldeddiscrepant findings. In the present study,we compared unaffected sporadic (simplex, n=65) and familial (multiplex, n=35) relatives of schizophrenia-spectrum patients with control individuals (n=114) on a range of neurocognitive functions and schizotypal traits, controlling for paternal age, which has not been consistently studied so far. We foundthat only the multiplex grouphad higher negative and paranoid schizotypal traits compared with controls (all Ps<.005). We also found that the control group outperformed only the multiplex group in measures of strategy formation and executive working memory (all Ps<0.01) and both groups of relatives in psychomotor speed/set-shifting (all Ps<0.001); both the control and the simplex groups had superior cognitive flexibility compared with the multiplex group (all Ps<0.005). The present findings suggest dissociable neurocognitive profiles between simplex and multiplex relatives, when paternal age is taken into consideration. Overall, multiplex relatives present with a "riskier" personality and cognitive profile possibly due to the combined effects of genetic and environmental factors. Nevertheless, simplex relatives, who lack the high genetic risk, are also impaired in fundamental cognitive processes, thus highlighting the detrimental effects of paternal age on neurocognition.

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A HIGH RESOLUTION EEG HUMAN SLEEP DATASET

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Physiological research and evaluation of tools for automated sleep analysis either require resource-demanding data acquisition or rely on open-access databases. Publicly available polysomnographic data feature poor spatiotemporal resolution, usually less than 10 electroencephalography (EEG) channels and sampling rates below 250 Hz; therefore, are unsuitable for analyses with high resolution requirements. We aim to present a comprehensive dataset suitable for physiological investigations of human sleep and benchmarking of algorithms with high spatiotemporal resolution requirements. Our dataset consists of 62 whole-night polysomnographic recordings of 40 subjects with no reported psychiatric or neurological conditions, obtained at the Neurophysiology Unit, University of Patras between 2007-2016. Acquired biosignals include EEG, electrooculogram, electrocardiogram and maseter electromyogram. A referential montage of 56 EEG channels allows for topographical analyses with fine spatial resolution. Moreover, a subset of 18 recordings is complemented by electrode positioning data captured using a and MRI scans, thus can be useful to EEG source localization. Original sampling rate of 2.5 KHz offers sub-millisecond temporal resolution, thus allows for challenging applications (e.g. EEG high frequency oscillations, heart rate variability analysis). The amplifier's low frequency filter was 0.05 Hz, therefore data are suitable for investigations of broadband electrophysiological dynamics, including slow (<1 Hz) EEG activity. Sleep scoring, artifact rejection and annotation of EEG microstructural events -namely, sleep spindles and K-complexes- have been performed by at least one and usually more experts. This dataset is intended to become available to the sleep research community on the basis of research collaborations.

AN *IN VITRO* AND *IN VIVO* STUDY OF iPSC-DERIVED NEURONS FROM PATIENTS WITH FAMILIAL PARKINSON'S DISEASE

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AIM: Parkinson's disease (PD) is the second most common neurodegenerative disorder. Point mutations in the α-synuclein gene SNCA cause an autosomal dominant form of PD often characterized by early onset. The G209A substitution (substitution of guanine to adenine at position 209) in SNCA results in expression of the pathological α-synuclein protein p.A53T-αSyn. Towards better understanding PD pathology we have developed and characterized a "disease-in-a-dish" model of human induced pluripotent stem cell-derived neurons from PD patients that harbor the p.A53T- αSyn mutation (Kouroupi et al. PNAS 2017 114(18):3679-3688). These neurons develop *in vitro* neuropathological features closely resembling those previously identified in brains of p. A53T-patients. Moreover, mutant neurons show disrupted synaptic connectivity and widespread transcriptional alterations in genes involved in synaptic signaling. The goal here is to study further the p.A53T neurons *in vitro* in a co-culture system with mouse cortical neurons and *in vivo* after transplantation in a hemi-parkinsonian mouse model. **Materials/ Methods:** p.A53T-patient-derived iPSCs and healthy donor-derived iPSCs were differentiated to dopaminergic neurons following dual SMAD inhibition. For the co-culture system, hiPSC-derived neural precursor cells (iPSC-NPs) were cultured with dissociated cortical neurons obtained from E16.5 mouse embryos. Analysis was performed up to 15 days later. For development of a hemi-Parkinsonian *in vivo* model, 6-OHDA was injected unilaterally in the striatum of immumosupressed NOD/SCID mice, using a stereotactic device. Two weeks later, amphetamine-induced rotational scores were used as an estimate of the extent of dopamine depletion. Lesioned animals then received at the same coordinates a transplant of iPSC-NPs, either patient- or healthy donor-derived. Analysis was performed up to 3 months post transplantation to determine survival, differentiation and integration of the transplanted cells in the host brain, using immunohistochemistry, confocal microscopy and image analysis. **Results:** Our data show that when co-cultured with mouse cortical cells, p.A53T-patient-derived cells cause activation of mouse glia without affecting the survival of mouse neurons. Furthermore, *in vivo* analysis shows that the transplanted cells from p.A53T-patient as well as from a healthy donor survive, 3 months after transplantation. Further analysis is in progress.

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