1 **Role of Metabolism in Pathological Aggregation of TDP-43 and its Down-Stream Toxicity**

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) are two fatal neurodegenerative disorders with considerable clinical, pathological, and molecular overlap. Cytoplasmic aggregation of transactive response DNA binding protein of 43 kDa (TDP-43) in neurons and glia, is a consistent feature of a majority of ALS and FTLD cases. At the clinical level, conditions associated with a conventionally risky metabolic profile, such as type 2 diabetes mellitus (T2DM), high body mass index, and dyslipidemia have been consistently associated with delayed onset, slower disease progression, and longer survival in both ALS and FTLD. These observations, combined with emerging evidence suggesting that TDP-43 regulates cellular and whole-body metabolism is the basis of our hypothesis that changes in metabolism might regulate TDP-43 pathology. Our study aims to ascertain how TDP-43 mislocalization, which is considered an early event in the pathological aggregation of TDP-43 is regulated by changes in glucose and lipid metabolism. Furthermore, we aim to investigate if TDP-43 mislocalization and the subsequent loss of nuclear functions dysregulate cellular metabolic cascades to drive TDP-43 pathology. Preliminary experiments performed in NSC-37 mouse motor neuron-like cells reveal alteration of key enzymes involved in utilizing lipids as an energy source. Our ongoing investigations involve functional validation of these findings and modelling of TDP-43 pathology in brain organoids via reprogramming of induced pluripotent stem cells (iPSCs) derived from patients' fibroblasts.

2 **Combination of Dasatinib and Quercetin improves cognitive abilities in aged Wistar rats**

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Introduction. Neurons and other glial cells have the potential to acquire senescent characteristics that could lead to defect in neuronal plasticity and alteration of cognition which negatively impact quality-of-life of elders. Eliminating senescent cells that accumulates with age, using senolytics drugs, has proven to be effective in alleviating symptoms of aged-related diseases.

Hypothesis. Combination of Dasatinib and Quercetin senolytics (D+Q) might prevent cognitive decline observed in aged rats. Objectives. Quantify systemic inflammation level since inflammaging is a key component of unhealthy aging. Assess synaptic plasticity in hippocampal structures that are principally involved in memory processing, spatial processing and navigation. Investigation epigenetic and senescence hallmarks to shed light on relevant molecular pathway affected by D+Q treatment.

Methods. Young (3-month-old) and naturally aged male Wistar rats (18-/22-month-old) were treated with D+Q for eight weeks and tested in the active allothetic place avoidance task. Arterious blood was collected to assess cytokines level. Fresh hippocampal slices were stained with Dil to analyze dendritic spine morphology. Epigenetic and senescence markers were quantified from fixed hippocampal slices or lysates.

Results. We confirmed the cognitive decline of aged rats compare to younger animals. We observed in aged but not young rats treated with D+Q a reduction in systemic inflammation and an alleviation of aged-related learning deficits and memory impairments associated with changes in synaptic plasticity and epigenetic but not senescence markers. Furthermore, D+Q treatment retains long lasting effects up to six weeks after treatment.

Conclusion. Our study brings new insights on the effects of D+Q senolytics in alleviating age-associated cognitive dysfunctions.

3 **Central amygdala-ventral tegmental area-cortical circuits for social and food rewards.**

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Social interaction is a complex behavior essential for the survival of many species, and its impairment is a hallmark feature of major neuropsychiatric disorders. Social dysfunctions are often explained by a deficit in motivation and reward processing specific to social stimuli. However, the functional dissociation between the neural circuits processing social and non-social rewards remains unknown. Here, we compared two cortical circuits connecting the central amygdala (CeA) and ventral tegmental area (VTA), in order to ascertain if they are the underlying motivation for social interaction and for food. Using opsins targeted at behaviorally activated neurons, we tagged CeA cells implicated in social and food reward. Optogenetic manipulations revealed that these circuits only partially overlap. Through chemogenetic manipulations of specific projections, we identified a crucial role of the CeA-VTA pathway and the dopaminergic VTA-anterior cingulate (ACC) and VTA-orbitofrontal cortex (OFC) pathways in social motivation, but not in terms of motivation for a food reward. In addition, we found that the ACC-CeA and OFC-CeA inputs are involved in both social and non-social motivation. Together, these findings establish the CeA-cortical pathways as a node for regulating social motivation, providing new insights into the regulation of social reward.

4 **Camel/Chl1a regulates development of the brain ventricular system**

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The hereditary forms of hydrocephalus cause an expansion of the brain ventricular system (BVS). Development of the brain ventricular system (BVS) is not fully understood. The developmental genes expressed in the BVS components [ependyma and circumventricular organs (CVO)] regulate cell adhesion during BVS development, which is critical for brain morphogenesis and function. camel/chl1a, the distantly related member of the zebrafish L1-CAM family, is a novel gene involved in this process. Its zygotic transcripts express in the axial structures associated with BVS, including the midbrain roof plate, several CVOs (SCO, OVLT, median eminence, paraventricular organ, flexural organ), and inter-rhombomeric boundaries suggesting a role of Camel in neural tube development. Several isoforms of Camel generated by differential splicing of exons encoding the sixth fibronectin type III domain enhance cell adhesion differentially. The antisense oligomer morpholino-mediated loss-of-function (LOF) of Camel affects cell adhesion and causes hydrocephalus. The vento-lateral surfaces of SCO secrete specific proteins which assembly produces the two thin filaments that then fuse forming the Reissner fiber (RF),which regulates the flow of cerebrospinal fluid. Upon morpholino-mediated Camel LOF the RF diminishes and fails to form properly. The Camel mRNA–mediated gain-of-function causes the excessive

secretion and deposition of SCO proteins resulting in RF misdirection. These results demonstrate a contribution of Camel/Chl1a into development of SCO and RF. They support an idea that the CHL1 plays an important role during brain development as one of the BVS regulators.

5 **Voltage-gated potassium channels regulate inner ear development**

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Voltage-gated potassium channels selectively regulate transport of K+ along electrochemical gradient in plasma membrane. They are involved in a variety of biological processes including mechanotransduction, protein transport, neuronal impulse, etc. The zebrafish Kcnb1 encodes the electrically-active subunit of the slow-inactivating delayed rectifier (IK) Kv2.1, a member of the Kv2 subfamily of Kv channels expressed in the mammalian, Xenopus' and zebrafish's inner ear. Moreover, it is known that of all bodily fluids, a fluid of the inner ear – endolymph, has the highest concentration of K+. Kcnb1 is expressed in cells lining the cavity of developing inner ear of zebrafish (Shen et al., Development, 2016). Hence, Kv2.1 could be important for ear development, where it may be required for proper hearing and spatial orientation. Using the loss-of-function (LOF) kcnb1 mutant, a role of this gene was studied during zebrafish development and a link between deficiency of Kcnb1 and abnormal development and functioning of the inner ear has been shown. The ear of developing zebrafish kcnb1-/- and its hearing stones - otoliths are reduced. The orientation of kinocilia of mutants' mechanosensory cells is affected. The Kcnb1 LOF causes defects in hearing and balance, which affect animal survival. This demonstrates the novel developmental role of Kcnb1 during development and function of inner ear.

6 **Neural correlates of social behavioral states in Cntnap2-knockout mice**

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Specific mutations of the Cntnap2 gene are associated with behavioral deficits linked to ASD, such as lack of social interactions, social anxiety, and impaired neuronal excitation/inhibition (E/I) balance. Though previous studies have shown mechanistic impairments in specific brain regions, it is unclear how the Cntnap2-/- mutation affects inter-areal dynamics during emotionally arousing interactions with conspecifics. We used a multi-electrode array implant to record local field potentials

from the multiple areas of the murine brain during social interactions with either group-housed or isolated stimuli. These experiments highlight the brain activity that defines behavioral state components and their modifications associated with ASD-linked impaired social functioning.

We found that Cntnap2-/- (KO) mice interacted similarly between isolated and group-housed stimuli, while C57 (WT) mice preferred to interact with the isolated stimuli. Further, the change in Theta (4-12Hz) power of all recorded brain regions during the encounter with stimuli doesn't differ in WT and KO. However, the change in Gamma (30-80 Hz) during the encounter with stimuli was significantly higher for KO compared to WT.

Moreover, correlating the change in Gamma power as the mouse initiates an interaction bout with the stimuli indicated that interactions with group-housed stimuli involved AcbC and AcbSh in WT and KO, respectively. Though IL gamma power in WT inversely correlated to duration of interaction with isolated stimuli, the gamma power in IL of KO mice did not correlate.

We found that the overall arousal of the mice doesn't change; however, in KO mice, the local activity in brain regions is high compared to WT. Further, the interaction bouts to isolated stimuli involve different brain regions compared to group-housed stimuli. Altogether, our study revealed a previously unreported deficit in the interaction of Cntnap2-/- mice with social stimuli with affective valence

7 **Siah-1-interacting protein regulates mutated huntingtin protein aggregation in Huntington's disease models**

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Huntington's disease (HD) is a neurodegenerative disease caused by the expansion of CAG triplets (encoding glutamine, Q) in the huntingtin gene (HTT). In the striatum of YAC128 mice, the model for HD, we observed an increased level of SIP (also called CacyBP) dimers compared to control mice (Czeredys et al., 2013). These results may suggest that the physiological function of SIP is disturbed in this model. It is known that SIP participates in the ubiquitin-mediated degradation of β-catenin as a

component of the ubiquitination complex. Therefore we aimed to verify the role of wild-type SIP and its mutants in the dimerization domain on ubiquitination of mutant HTT. Using in silico methods such as Rosetta and Molecular Dynamics we predicted mutations that should stabilize the SIP dimerization domain (K21W and T30R_S33E) and then introduced them into SIP. As a cellular model of HD, we used the HEK293 line, which was transfected both with plasmids encoding mutant HTT with 72Q-RFP or wild-type HTT with 25Q-RFP (as a control) and with plasmids containing wild-type SIP or its dimerization mutants. With the application of fluorescent microscopy in cells overexpressing both SIP and mutant HTT we found the decreased number and size of aggregates as compared to control. Using immunoprecipitation and western blot analysis we detected that SIP decreases the protein level of mutant HTT and increases its ubiquitination. We also found that SIP dimerization mutants are less active in the inhibition of mutant HTT aggregation and mutant HTT is less ubiquitinated in their presence. Our results indicate that SIP is responsible for the inhibition of mutant HTT aggregation by facilitating its ubiquitination. The dysregulation of SIP dimers could be potential pathology mechanisms of HD.

8 **The role of N-aryl piperamides on the regulation of MMP-9 via NF-kB translocation inhibition during neuroinflammation induced by LPS**

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Matrix metalloproteinase-9 (MMP-9) plays a pivotal role in the turnover of extracellular matrix (ECM) and various inflammatory responses to various stimuli. The lpopolysaccharide (LPS) stimulation induces the transcription of the MMP-9 gene in the astrocytic glial cells. It is reported that the NF-kappaB binding site of the MMP-9 gene contributes to its expression in the LPS-signaling pathway. In addition, the degradation of the IkB-α subunit and the presence of myeloid differentiation protein (MyD88) and tumor necrosis factor receptor-associated kinase 6 (TRAF6) are required for LPS-activated MMP-9 expression. NF-kappaB contributes to LPS-induced MMP-9 gene expression in a mouse macrophage cell line. To better understand the connection between NF-kB and MMP-9 associated with neuronal inflammation, we planned series of experiments using NF-kB inhibitor entitled D4 ((2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N-(4-(hydroxymethyl)phenyl)penta-2,4-dienamide). The real-time PCR results indicated that D5 significantly down-regulates the MMP-9 gene around 4 and 13 folds in primary astrocyte cell culture stimulated by LPS at 3 and 6 h time points, respectively. The cell immunostaining (immunocytochemistry) using the NF-kB p65 antibody demonstrated that the NF-kB nuclear translocation rate was decreased significantly after 1h treatment by D5. Results indicated that the pattern of redundancy NF-kB translocation rate correlated to the ratio of down-regulation of the MMP-9 gene. However, further investigation is necessary to confirm our result.

9 **Selective cortical 5-HT1A receptor biased agonists, NLX-101 and NLX-204, has rapid-acting antidepressant effects in the rat chronic mild stress model of depression and TRD.**

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Ketamine has a rapid antidepressant effects in depressed patients but it also elicits considerable side-effects. There is therefore interest in identifying safer rapid-acting antidepressants (RAADs), based on ketamine's mechanism of action. Rodent studies have pointed to a prominent role of cortical serotonin 5-HT1A receptors in mediating ketamine's RAAD activity. Recently, novel 'biased agonists', NLX-101 and NLX-204, have been identified, which show preferential activity at cortical 5-HT1A receptors. The present study evaluated antidepressant-like activity of these compounds and ketamine in a robust rat model of depression: the chronic mild stress model (CMS).

The CMS procedure was conducted on both Wistar and Wistar-Kyoto (WKY) rat strains. Ketamine was administered at 10 mg/kg, NLX-101 and NLX-204 were each tested at doses of 0.08 and 0.16 mg/kg. Anhedonia was assessed by CMS-induced decrease in sucrose solution consumption. Elevated Plus Maze (EPM) and Novel Object Recognition (NOR) tests were used to assess anxiety-like behavior and cognitive impairment, respectively.

In Wistar rats, both doses of NLX-101 and NLX-204 caused significant reversal of the CMS-induced deficits from day 1, similar to ketamine. These effects were maintained or enhanced over subsequent 2-weeks period of treatment, and were maintained for several weeks following treatment discontinuation, suggesting that the drugs had sustained effects on neuronal networks. NLX-101 and NLX-204 also exhibited anti-anhedonic activity from day 1 in WKY rats, a strain which is considered to be resistant to classical antidepressant treatments.

The results show that the antidepressant-like activity of NLX-101 and NLX-204 in the rat CMS model was as pronounced and as rapid as that of ketamine, suggesting that biased agonist targeting of cortical 5-HT1A receptors is a promising strategy for development of novel, safer RAADs.

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10 **Learning drives alternations in the intrinsic excitability of GABAergic interneurons in the somatosensory cortex of mice.**

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The inhibitory (GABAergic) system in the cortex consists of many heterogeneous types of interneurons. In general, inhibitory cells can be divided based on the expression of molecular markers like e.g. somatostatin (SST), parvalbumin (PV), and vasoactive intestinal polypeptide (VIP). Neurons of each above type are various in number, distribution manner in the cortex, synaptic connectivity, morphology and electrophysiological properties. Accumulating evidence implies their role in information flow thru the neuronal circuits, as well as memory consolidation and different forms of learning. During our studies, we examed learning-evoked plastic changes at the electrophysiological level in the GABAergic circuitry. To study these changes we applied a simple mode of conditional learning in mice where tactile vibrissae stimulation as a conditioned stimulus was coupled with an electric tail shock as unconditioned one. Subsequently, we performed in vitro whole-cell patch-clamp recordings of layer 4 GABAergic interneurons in the cortical representations of stimulated vibrissea of the I-order somatosensory cortex. We found that conditional learning in mice drives the rise of intrinsic excitability in low-threshold spiking SST-expressing interneurons Fast-spiking PV-expressing interneurons showed a decrease of intrinsic excitability after the pseudoconditioning procedure. Changes in intrinsic excitability of VIP-expressing cells were dependent on the neuronal firing patterns and forms of learning. Intrinsic excitability of accommodating VIP neurons decreased in pseudoconditioned mice in relation to conditioned ones. In contrast, there were no differences in intrinsic excitability of the low-threshold spiking VIP cells between two types of learning. These findings imply that changes in intrinsic excitability of GABAergic cells are a regular form of learning-evoked plasticity in mice. The direction of these alternations is specific for the form of learning and class of neurons.

11 **Ubiquitination of Arc regulates alcohol-induced synaptic plasticity in central nucleus of amygdala and alcohol seeking**

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The activity-regulated protein Arc/Arg3.1 (Arc) is one of key molecules that regulate synaptic plasticity and memory processes. The role of Arc in psychiatric disorders, including alcohol use disorder (AUD), remains less understood. However, our data

show that Arc protein is downregulated in the amygdala during alcohol withdrawal, suggesting its function in alcohol-induced plasticity and behaviours. The expression of Arc protein at synapses is tightly controlled: it rapidly increases following enhanced network activity and quickly declines due to protein ubiquitination. To investigate the role of Arc ubiquitination in AUD-related behaviours, we used Arc ubiquitination-deficient mutant mice (Arc KR) (Wall et al. 2018) with disrupted temporal control of Arc degradation. Arc KR and wild-type littermates (WT) underwent alcohol self-administration training in the IntelliCages: free access to 8% ethyl alcohol (30 days) followed by 7-day withdrawal (7DW) and presentation of alcohol-predicting cue light (cue relapse) in the reward corner. Next, we analyzed synaptic transmission and plasticity in the central nucleus of the amygdala (CeA) following 7DW: evoked local potentials were recorded in medial part of CeA after electrical stimulation of basolateral amygdala (BLA). There was decreased synaptic strength, measured as input/output, and decreased GluN1, but increased GluN2B, NMDAR subunits levels in Arc WT following 7DW compared to water, yet, no difference in Arc KR mice. Furthermore, theta burst stimulation-induced LTP was significantly boosted in Arc WT mice following 7DW compared to water group. We did not observed, however, such plasticity in Arc KR littermates. Finally, we found less alcohol seeking by Arc KR mice during alcohol withdrawal and cue relapse compared to Arc WT littermates. Together, our data suggest that temporal control of Arc degradation plays a crucial role in alcohol-induced synaptic plasticity in CeA and alcohol seeking during withdrawal.

12 **Neuronal correlates of Imminent Transfer of Fear in mice**

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The aim of the study was to assess the role of somatostatin interneurons in the prefrontal cortex in emotional contagion – the simplest form of empathy in mice. Empathy is a complex phenomenon occurring in humans and other animals. It is considered to have several levels of complexity. According to the popular de Waal's theory these include: state matching (at the core), sympathetic concern and perspective taking. State matching is expressed via emotional contagion, a phenomenon of sharing an emotional state by two individuals.

To study empathic abilities in mice we employed a behavioural paradigm - Imminent Transfer of Fear (ITF, also referred to as Observational Fear Learning), which allows the Observer mouse to directly witness a familiar Demonstrator mouse being

subjected to aversive stimuli. In this test mice are put into two-chambered cage with a grid floor, with only one of the chambers connected to the shocker. During the test the Demonstrator is subjected to ten 0.6mA 1s long foot shocks, while the Observer stays in the adjacent chamber. The Observer mouse can see, smell and hear his cage mate, but direct interaction is not possible. Behaviour of both animals during test is recorded. To check the activity of somatostatin cells we used SOM-IRES-Cre strain combined with immunochemistry against c-Fos, a standard neuronal novelty marker.

Our results confirm the occurrence of emotional contagion in the ITF model and the involvement of somatostatin interneurons in prefrontal cortex in this phenomenon. We observed that Observers were more interested with stressed Demonstrators which coincided with increased expression of an immediate early gene (c-Fos) in somatostatin cells.

This study suggests that the prefrontal cortex and somatostatin interneurons within this structure play an important role in emotional contagion. To functionally confirm the nature of that involvement and pinpoint the exact mechanism of this phenomenon we need further studies.

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13 **Epileptiform GluN2B-driven excitation in hippocampus as a therapeutic target against temporal lobe epilepsy**

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NMDAR is an ionotropic glutamate receptor that forms the foundation of excitatory synaptic transmission. One of the NMDAR subunits is GluN2B, which displays restricted expression in the mature brain. GluN2B-containing NMDARs are present in the hippocampus – the structure playing a major role in temporal lobe epilepsy (TLE) We report here, in animal models of TLE, a profound change in the nature of glutamatergic transmission mediated in hippocampus by GluN2B-containing NMDAR receptors. Based on these results, we investigate here the effects of GluN2B-oriented antagonism on pathophysiology of hippocampal circuitry in TLE and on conditioning of chronic seizures.

Satisfactory control of chronic seizures in TLE is still impossible for about 40% of patients. Therefore, new therapeutic approaches against the condition are desired. In that regard, our study presents the potential of ifenprodil for altering the course of epileptogenesis and to suppress chronic seizures, thus providing a rationale for clinical studies on ifenprodil as aimed against TLE.

14 The role of αCaMKII activity in alcohol seeking behavior

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Regular alcohol abuse leads to physical and mental dependency described like an alcohol addiction. The neuronal basis of this process are still poorly understood, thus, we investigated the behavioral and molecular consequences of the alcohol treatment.

Mice injected (i.p) with ethanol (2 g/kg) for 7 days, developed context-independent sensitization of locomotor response observed after 7-day withdrawal; and it was further enhanced after 30-day incubation. Interestingly, this short protocol resulted in higher motivation for alcohol and increased alcohol seeking during withdrawal measured close-to-ethologic conditions in the IntelliCages. Alcohol sensitization was accompanied by structural (analyzed with Serial Block-face scanning Electron Microscopy (SBEM)) and functional (whole-cell patch-clamp) changes of the central nucleus of the amygdala (CeA) glutamatergic synapses and decreased levels of the autophosphorylated form of αCaMKII. In agreement with this observation αCaMKII autophosphorylation-deficient heterozygote mutant mice (αCaMKII-T286A+/-) showed enhanced sensitization as well as increased alcohol consumption in the IntelliCages, and surprisingly lower alcohol seeking during withdrawal and cue relapse.

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15 **Neurodegeneration in the npc2-deficient zebrafish model of Niemann-Pick type C disease**

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Niemann-Pick type C (NPC) is a rare neurodegenerative autosomal recessive disease that is caused by a mutation of the intracellular cholesterol transporters, NPC1 or NPC2 gene. Notwithstanding which of the two NPC genes is affected, cholesterol will accumulate in late endosomes and lysosomes damaging patients' cells. The molecular mechanisms underlying NPC pathology are far from being understood. Zebrafish, a small tropical fish that are used as laboratory animals have been proven to be an excellent model for biomedical studies, also concerning lipid metabolism (Anderson et al., 2011), and neurodegenerative diseases (de Araujo Boleti et al., 2020). Hence, we decided to establish the zebrafish model for NPC and use it to study the impact of disease on the brain function. Using CRISPR/Cas9 technology we have introduced in fish a small deletion which impairs the function of the homologue of NPC2. Adult npc2-/- fish shows typical hallmarks of NPC disease - the morphological and histopathological changes in the central nervous system including e.g., reduced myelination, and loss of Purkinje cells. The signs of neurodegeneration correlate with inflammatory responses (upregulation of il1, nfκβ, and mpeg) and locomotor impairment. Five-days-old npc2-/- zebrafish, although morphologically indistinguishable from wildtype larvae, exhibited a high anxiety-related response and low mobility and correlating with impaired myelination indicated by downregulation of mbp, mpz, and plp1. Thus, npc2-/- fish seems to be an excellent model to uncover molecular mechanisms underlying early changes in NPC and search for chemicals correcting the phenotype.

16 **Brain size, gut size and cognitive abilities: experimental evolution of energy trade-offs**

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The enlarged brains of homeotherms bring behavioural advantages, but also incur high energy expenditures. The 'Expensive Brain' (EB) hypothesis posits that the energetic costs of the enlarged brain and increased cognitive abilities (CA) were met either by increased energy turnover or reduced allocation to the gut. We tested the trade-offs between energy expenditures, brain, gut and CA using an experimental evolution model. We subjected line types of laboratory mice to artificial selection on high basal (BMR) or maximum (VO2max) aerobic metabolism - traits being pre-requisites for the encephalisation and exceptional CA of mammals, including humans. High-BMR mice had bigger guts, but not brains. Yet, they performed better on the cognitively demanding tasks. Furthermore, thy had higher neuronal plasticity than their counterparts. The evolutionary increase of CA in mammals was initially associated with increased BMR and brain plasticity, fueled by an enlarged gut, which was not traded off for brain size.

17 **The role of hippocampal cofilin 1 in the regulation of alcohol addiction – related behavior**

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Actin filaments are an important structural component of synapses. Hence, regulators of actin dynamics take a central role in mediating neurotransmitter release, synaptic plasticity and ultimately in modulation of behavior. Cofilin-1 is an actin-binding protein that regulates the reorganization of actin filaments and contributes to pre- and postsynaptic physiology. Studies have shown that drug administration regulates the remodeling of actin filaments in the nucleus accumbens, however, still little is known about the mechanisms underlying these changes and whether they also occur

in the brain affected by alcohol. The aim of this study was to understand the function of cofilin-1 in the regulation of alcohol-related behaviors.

I used IntelliCages to induce addictive behavior in mice and identify the transcriptomic changes in the hippocampus characteristic for addicted and non-addicted alcohol drinkers. I found changes in the expression of the genes related to the actin cytoskeleton and synaptic transmission, including cofilin 1. To confirm these results, immunofluorescent labeling was performed. I observed higher levels of cofilin in the DG (dentate gyrus) in addicted mice during withdrawal compared to addicted mice sacrificed during free alcohol access. I also observed increased colocalization of synaptotagmin 1 and PSD-95 with cofilin 1 in addicted mice during withdrawal as compared to addicted mice during free alcohol access. Electrophysiological recordings revealed reduced presynaptic activity of synapses in DG of addicted mice compared to non-addicted mice. To further investigate the role of cofilin 1 in addiction-related behaviors I performed experiments on mice with local overexpression of cofilin 1 in PoDG. Mice overexpressing cofilin were more persistent in alcohol-seeking during withdrawal than mice in the control group, eGFP. Overexpression of cofilin 1 in PoDG also contributed to reduced presynaptic activity of synapses in the molecular layer of DG

18 **Factors influencing the interplay between neurochemical and social processes in the ants: ecology of the species, worker behavioural status and previous experience of the tested individuals**

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We present a review of selected findings illustrating how the interplay between neurochemical and social processes in the ants is influenced by the ecology of the species, worker behavioural status, and previous experience of the tested individuals. In particular, we discuss four findings reported in papers / conference reports co-authored by the members of our research team. (1) Queen removal was followed by the increase of brain GABA levels in workers of the red wood ant Formica polyctena (dominant, aggressive ants known to adopt alien queens if orphaned), but no such effect was observed in Myrmica ruginodis (a submissive ant species).(2) Experiments with two species of carpenter ants (Camponotus fellah and C. herculeanus) showed that abdominal injections of the biogenic amine octopamine were followed by partial suppression of trophallactic exchanges between a dyad of nestmates put together after a period of social isolation. However, in the experiment with another formicine species (F. polyctena) dyadic encounters of nestmate workers were not preceded by social isolation, and octopamine injections had no effect on trophallaxis. (3) Intra-tracheal administration of inotocin, an insect orthologue of the neurohormone oxytocin, was followed by shortening of the latency from the start of the test to the

onset of the so called company keeping behaviour shown by foragers of Formica cinerea during dyadic confrontations with a trapped nestmate. However, no such effect was observed in foragers of that species that switched to staying in the nest chambers. (4) GABA and Glu levels in brains of workers of F. polyctena depended strongly on worker behavioural status, but that effect was entirely masked if the ants participated first in aggressive dyadic encounters with allospecific workers (Formica fusca). All these findings taken together demonstrate crucial importance of the specific context for two-way information flow between neurochemical and social processes in the ants.

19 **3,3'-Diindolylmethane as a novel treatment for perinatal asphyxia**

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Perinatal asphyxia is the third leading cause of newborns' death worldwide. Moreover, oxygen deprivation in the perinatal period can also lead to permanent brain damage, with hypoxic-ischemic encephalopathy as the most serious consequence. The current gold-standard treatment involves moderate hypothermia and oxygen therapy, however, those methods are not devoid of serious side effects. Hence, there is an urgent need for novel neuroprotective strategies development in this field. In light of the results obtained by our team, administration of 3,3'-diindolylmethane (DIM), appears to be a promising approach to treat ravages of such events. This compound has properties of a selective aryl hydrocarbon receptor modulator and can be found in cruciferous vegetables such as broccoli, cabbage and kale. Utilization of the rat model of perinatal asphyxia and triple administration of DIM in a posttreatment paradigm showed that the tested compound effectively rescues neural cells from hypoxia/ischemia-induced brain damage. It was demonstrated in terms of restoration of the ipsilateral hemisphere weight. DIM administration also reduces hypoxia/ischemia-induced cell loss in the hippocampal CA1 region and cerebral cortex, as measured using cresyl violet. Furthermore, DIM inhibits the hypoxia/ischemia-induced increase in the expression of Hif1a/HIF1A and/or Bnip3/CALPAIN-1, as assessed with qPCR and ELISA. Therefore, according to our data, DIM shows neuroprotective properties in the treatment of the rat in vivo model of perinatal asphyxia.

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20 **Posttreatment with amorfrutin B protects mouse neurons from hypoxia and ischemia**

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Stroke evoked by a deficiency of oxygen and glucose in the brain is the second leading cause of mortality and the major source of disability worldwide. Another important issue related to the loss of oxygen supply is perinatal asphyxia, which is the third leading cause of neonatal death. Main treatments for these two medical conditions are thrombolysis with rtPA and hypothermia, respectively. However, the limitations of current therapies such as narrow therapeutic window and severe side effects (e.g., cerebral edema, hemorrhage), prompts scientists to look for new strategies against hypoxia- and ischemia-induced brain injuries. To assess the effects of amorfrutin B on hypoxic-/ischemic-evoked cytotoxicity and neurodegeneration, we measured the levels and/or activities of LDH, MTT and Fluoro-Jade C. A 6-h posttreatment of mouse neurons with amorfrutin B (5 µM) decreased LDH activity, unraveling anti-necrotic properties of the compound. The MTT test demonstrated that amorfrutin B posttreatment improved the mitochondrial activity and increased the number of viable cells. Moreover Fluoro-Jade C test showed that amorfrutin B reduced the extent of hypoxia- and ischemia-induced neurodegeneration. Our study points out that amorfrutin B applied even 6 h after hypoxia/ischemia, protects mouse primary neurons from cytotoxicity and degeneration, that positions amorfrutin B as a promising tool with a relatively wide therapeutic window to treat stroke and perinatal asphyxia.

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21 **Increased matrix metalloproteinase-9 activity affects recuperation following ischemic stroke**

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Cerebral ischemia is one of the most common causes of mortality worldwide. Ischemic brain displays enhanced activity of different proteins including an extracellularly acting protease – matrix metalloproteinase-9 (MMP-9). MMP-9 activity can have both beneficial and detrimental effect on brain's milieu. The aim of the present study was to evaluate the MMP-9 activity changes after ischemia and verify the impact of MMP-9 genetic modifications on long term survival and recovery process after cerebral ischemia. We followed focal middle cerebral artery occlusion (fMCAo) protocol in mice, after which long term survival and recovery process were studied. The impact of stroke on the brain structure was verified by TTC staining. MMP-9 activity was assessed by gel zymography. For evaluation of the influence of MMP-9 on recovery rate, mice lacking MMP-9 (MMP-9 KO) and mice with overexpression of MMP-9 (MMP-9 OE) were used. Animal weight, focal neurological deficits and motor function were evaluated during 30 days post-stroke. We have observed a prominent neuronal cell loss in the ipsilateral cerebral cortex after ischemia. Pronounced increase in MMP-9 activity was detected during first 24 hours after fMCAo not only in ipsi- but also contralateral hemisphere. Lack of MMP-9 has not diminished while MMP-9 overexpression increased mortality of animals as a result of ischemic stroke. Moreover, MMP-9 KO survivors showed lower weight loss and focal neurological deficits after fMCAo. In MMP-9 OE survivors' focal neurological deficits after fMCAo were intensified. Changes in MMP-9 level affected also nest building rate. Our study indicates that MMP-9 plays a role in post-stroke recovery time, affecting the recovery rate and reduces long- term detrimental effects of the ischemic stroke.

22 **Diverse roles of the various neuronal classes in the prelimbic cortex in social bonding**

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Although much is known about the neuroanatomy of social attachment, still its functional circuitry is poorly understood. Many studies indicate a crucial role of the prefrontal cortex (PFC) activity in sociability, however, the involvement of various neuronal classes in that process remains unexplored. We investigated the effects of the artificial manipulation of the activity of : pyramidal cells, parvalbumin- (PV+) and vasoactive intestinal peptide- (VIP+) expressing interneurons in the prelimbic part (PL) of the PFC, and evaluated its influence on the interest in spontaneous social interactions with conspecifics and interest in social stimuli.

Animals were tested in Eco-HAB, an ecologically relevant, RFID-based system for assessment of sociability in group-housed mice, which enables continuous, individualized measurement of voluntary behavior. Using genetically modified mice selectively expressing Cre protein combined with the PSAM/PSEM-based chemogenetics approach, we performed a time-constrained, cell-specific manipulation of the PV+, VIP+ and pyramidal neurons activity in the PL and tested subjects social behavior during the 90 minutes following the systemic administration of the drug (PSEM) activating virally introduced artificial ligand-gated ion channels (PSAM).

We show that PL-constrained inhibition evoked by the chemogenetics activation of the PV+ interneurons and inhibition of pyramidal neurons attenuates sociability by decreasing the time voluntarily spend together and diminishing interest in familiar social stimulus. However, exploration of novel social stimulus is intact in PV+ mice, but enhanced due to inhibition of pyramidal neurons. On the other hand, selective activation of the VIP+ neurons has no robust impact on murine social behavior.

Taken together, our data point to the diverse roles various neuronal classes play in the regulation of social bonding and thus lays the foundation for understanding the neural underpinnings of social bonding.

23 **Expected values and prediction errors are represented in the human amygdala in appetitive but not aversive instrumental learning.**

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Extensive studies on animals have led to development of models of information processing in the amygdala during learning. The most influential model of fear conditioning postulates that associations between neutral cues and fearful events are formed in the lateral nucleus of the amygdala belonging to the basolateral amygdala complex (BLA) and defensive responses are mediated by the central nucleus of the amygdala belonging to the centromedial amygdala complex (CMA). However, the relevance of this model to other types of learning has not been sufficiently explored. Here we used a computational model of learning and MRI to investigate contribution of the BLA and CMA to outcome prediction and response of surprise during operant appetitive and non-fear aversive learning in humans.

Subjects (N=33) performed a reversal learning task, trying to maximize the reward (in appetitive sessions) and minimize the punishment (in aversive sessions) by learning the probabilistic relationship between a neutral cue and a relevant outcome. Using behavioral responses and Rescorla-Wagner rule we calculated expected values and prediction errors, which were further applied to modulate the neural signal at the time of cue presentation and at the time of occurrence of an outcome, respectively.

During appetitive learning, significantly elevated BOLD signal to the expected values was found in the left and right CMA. The analysis of the BOLD signal generated to prediction errors showed activation of the bilateral CMA and the left BLA. Surprisingly, no activity in the amygdala was observed during aversive learning.

Thus, the CMA appears to be involved in both processes, outcome prediction and production of a response of surprise. On the other hand, the left BLA is recruited specifically to surprise. These results shed new light on the information processing during appetitive learning in the amygdala.

24 **Inflammation during the critical stage of synaptogenesis influences the behavior of adult mice**

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The nervous system development is strictly organized in time. One of the critical stages is exuberant synaptogenesis (synaptic formation). In humans, it occurs during the second trimester of pregnancy. Due to the complexity of this process, its disruption may lead to abnormal brain functioning. Collectively, disorders that affect the nervous system development are called neurodevelopmental disorders (NDDs). NDDs are characterized by altered psychosocial behavior. Those diseases, such as Autism spectrum disorder, ADHD, schizophrenia, or Tourette`s syndrome are considered to have a multifactorial etiology, which is not fully understood. It is believed that one of the causes may be inflammation during key stages of development.

The aim of this study was to evaluate changes in psychosocial behavior of adult mice treated with lipopolysaccharide (LPS) on postnatal day 7 (P7). LPS injections are commonly used to mimic bacterial infection in animal models. In terms of onset of exuberant synaptogenesis, P7 in mice corresponds with 16. week of pregnancy in humans. On P7 mice pups were injected either with LPS (dose?) or physiological saline. After 3 weeks, a wide range of behavioral tests evaluating activity, sociability, and learning abilities of mice was conducted. Animals after LPS treatment were more social within familiar animals but repelled by unknown animals, less active, and

needed more time to learn new. Therefore, early-life immune activation in mice induces alterations of behavior that resemble symptoms observed in NDDs human patients. Our results are promising but need further investigation into molecular mechanisms behind behavioral changes resulting from LPS injection.

25 **stim2 double knock-out affects gene expression, neuronal activity and behavior of zebrafish larvae**

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Stromal interaction molecules (STIM) are endoplasmic reticulum resident proteins, which are ubiquitously expressed and are present also in neurons. STIMs regulate Ca2+ homeostasis inside the cells by store-operated calcium entry (SOCE). Many attempts have been done in the cell culture in vitro model to study the role of STIM2. However, its role in in vivo is unclear and should be clarified. In this study, we investigated the stim2a and stim2b double knockout (DKO) zebrafish. Interestingly, DKO zebrafish did not have any morphological phenotype, were fertile, and had a regular lifespan. However, qPCR results for the SOCE genes showed the significant downregulation of ryr3, trpc1, and trpm2 genes which are involved in Ca2+ signaling. RNA-sequencing revealed a total of 443 genes that were differentially expressed (more than 2-fold change) in which 307 genes were upregulated and 136 genes were downregulated. Among these differentially expressed genes, the CaTK genes, anxa3a(5.1x), pvalb7(4.4x), cacnb3b(2.3x), gria2a(2x) were upregulated and plch1(-2.2x) and fkbp1ab(-2.6x) were downregulated. Using lightsheet microscopy, we measured in vivo neuronal Ca2+ activity in GCaMP5G transgene of (stim2a;stim2b)-/- double knockout zebrafish. We found a significant increase in the Ca2+ oscillation frequency in the neurons in the optic tectum region in the zebrafish larvae brain in double mutants as compared with the Tg:(GCaMP5G) line. We also observed abnormal behavior of DKO larvae, such as increased thigmotaxis, indicating increased anxiety and disrupted phototaxis as well as visual-motor response. These data suggest that the Stim2 is an important factor in the regulation of gene expression and neuronal activity and the lack of both isoforms significantly affects zebrafish larvae behavior. The lack of stim2 affects CaTK genes expression, which might be responsible for increased Ca2+ spiking frequency and anxiety.

26 **Dendritic spine plasticity – role of MMP-9**

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Matrix metalloproteinase 9 (MMP-9) plays a major role in synaptic plasticity, although the exact mechanisms of its action still remain unclear. Here we present results on MMP-9 importance for volume change of dendritic spines in the first minutes following induction of long-term potentiation (LTP), by means of glutamate uncaging in hippocampal organotypic slices. We applied optical induction of plasticity protocol for single spine stimulation and registered changes in volume of stimulated spines and level of activation of TrkB receptor for BDNF. To define MMP-9 role in LTP we used two types of inhibitors: broad spectrum inhibitor GM6001 and MMP-9 Inhibitor I. Moreover, we used MMP-9 KO neurons to confirm pharmacological MMP-9 inhibition results. Currently, we are identifying the time-course of MMP-9 release from the dendritic spine, using MMP-9 pH-luorine construct and sensor for MMP-9 activity. Our results suggest involvement of MMP-9 in synaptic plasticity from the onset of stimulation. Lack of MMP-9 activity results in small initial and sustained volume change of stimulated dendritic spines. Moreover, TrkB activation in the presence of Inhibitor I was significantly diminished as compared to control condition, which supports the notion of MMP-9 involvement in BDNF – TrkB pathway activation.