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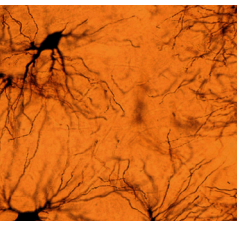

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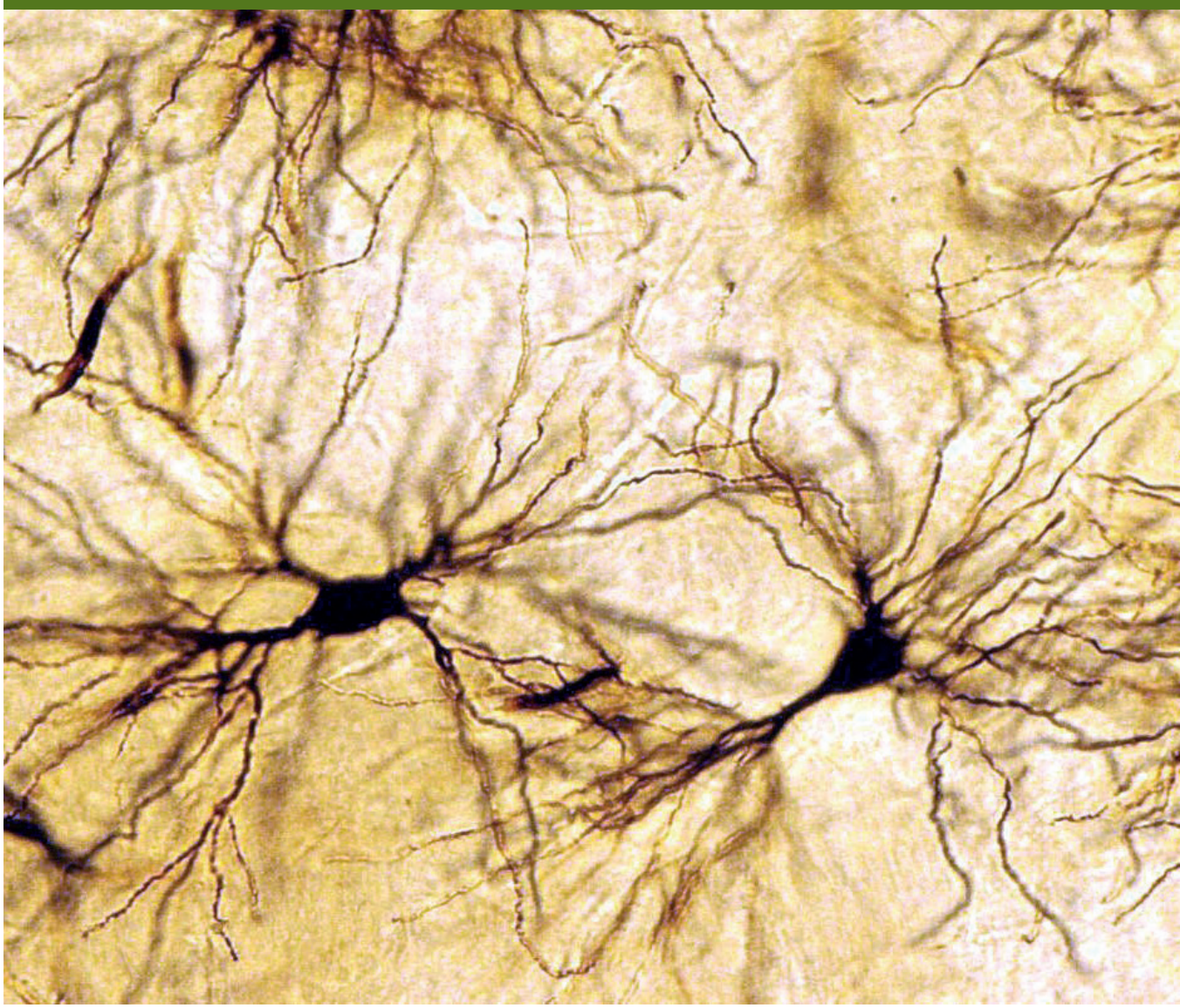

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LECTURES
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Pathophysiology of astroglia: noradrenergic hypothesis

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Astrocytes, abundant and functionally heterogeneous cell type of neuroglia in the brain, are essential for the maintenance of brain homeostasis. Although electrically silent cells, astrocytes respond to extracellular signals *via* receptors, by increasing intracellular levels of second messengers Ca^{2+} and/or cAMP, what affects astrocyte function. Astrocytes rich in adrenergic receptors were recognised as the main target of the *locus coeruleus* noradrenergic neurons. During attention, wakefulness, and stress *locus coeruleus* noradrenergic system by releasing noradrenalin activates brain to augment brain metabolism, memory formation and learning. Nucleus *locus coeruleus* is one of the first areas undergoing degradation in various neurodegenerative diseases, including Alzheimer's and Parkinson's disease. How this affects astrocyte function is unclear. We have shown recently by real-time imaging of intracellular fluorescent sensors for Ca^{2+} and metabolites that activation of astroglial adrenergic receptors *via* Ca^{2+} and cAMP signals within minutes attenuates exocytosis of secretory vesicles, alters astroglial morphology, preventing cytotoxic oedema, and augments cell metabolism, including lipid droplet accumulation and aerobic glycolysis with the production of L-lactate. L-Lactate is an important energy fuel transported from astrocytes to neurons to support neuronal functions, including learning and memory formation. However, adrenergic signalling, metabolism and L-lactate release were dysregulated in astrocytes that form intracellular protein inclusions, a hallmark of neurodegenerative diseases. This suggests that astroglial adrenergic activation and capacity to homeostatically support neurons are impaired. Thus, astrocytes may importantly contribute to the progression of the disease and cognitive decline, representing a novel target to treat neurological disorders.

Astrocytes in Parkinson's disease. The role in neurodegeneration and its functional compensation

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Astrocytes support neuronal function and closely interact with microglia. Glia directly influences neuronal functioning and survival. Astrocytes govern multiple essential brain functions such as energy metabolism or inflammation. Aberration in astroglia function and neuro-glia interaction could be the underlying cause of neurodegenerative diseases such as Parkinson's disease (PD).

In a validated animal model of progressive, partial dopaminergic neuron loss combined with prolonged astrocyte dysfunction we tested the influence of astrocyte death on the dopaminergic system potential to compensate behavioral deficits, dopamine turnover as well as multiple energy metabolism substrates, Krebs cycle, oxidative phosphorylation and mitochondrial function markers. Massive microglia activation was observed concomitant to the astrocyte death. Treatment with microglia activation inhibitor – minocycline or a substance shifting the microglia phenotype from toxic M1 to regenerative M2 – fasudil didn't act neuroprotective but interestingly influenced the compensatory potential of animal functional recovery after behavioral deficit caused by dopaminergic system lesion and astrocyte death.

Obtained results indicate that astrocytes are essential in the process of functional compensation. They allow neurons to enhance their energy metabolism required during the compensation. Astrocyte functioning is inseparably correlated with microglia state. Pharmacologic modulation of glia polarization states, rather than its inhibition, could become a valuable future treatment option in idiopathic PD.

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Astroglial chloride-homeostasis in health and disease

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Chloride is the most abundant anion in the central nervous system. While it plays an important and well characterized role in neuronal inhibitory synaptic transmission its function in astrocytes is less well defined. It is in general accepted that astrocytes have high intracellular chloride ($[Cl^-]_i$), even though conflicting data has been published (Ma *et al*. (2012), *Glia* 60: 1761-1772), reporting a broad range of $[Cl^-]_i$. Furthermore, it remains unknown how astrocytes accumulate and regulate $[Cl^-]_i$. The imaging technique FLIM (fluorescence lifetime imaging microscopy) is the first approach that allows for direct and absolute Cl^- measurements in living cells (Gensch *et al*. (2015), *Springer Series in Chemical Physics* 111: 189-211). The fluorescent properties of the Cl^- sensitive dye MQAE enables non-invasive recording of absolute $[Cl^-]_i$. By employing this technique to acute brain slices, it was shown that cerebellar Bergmann glial cells actively accumulate Cl^- to a level of around 35 mM in adult animals, while younger animals have even higher $[Cl^-]_i$ of around 50 mM (Untiet *et al*. (2017), *Glia* 65: 388-400). This age-dependent decrease in glial $[Cl^-]$ is correlated with an increase of anion conductance due to an increase of anion channel expression. The importance of maintaining a certain $[Cl^-]_i$ is demonstrated by the severe effect of abnormal $[Cl^-]_i$ and anion conductance. For example, glioma, a very aggressive form of brain tumors derived from astrocytes, sustain very high $[Cl^-]_i$ of around 100 mM (Habela *et al*. (2008), *J Neurophysiol* 101: 750-757) that enables their migration and tissue infiltration. Another example is the human genetic disorder episodic ataxia 6 that is characterized by ataxia and epileptic seizures (Jen *et al*. (2005), *Neurology* 65: 529-534). These severe symptoms are associated with a gain-of-function mutation that causes an increased anion conductance of EAAT1 (Winter *et al*. (2012), *Brain* 135: 3416-3425) that directly affects glial $[Cl^-]_i$. The role of $[Cl^-]_i$ is versatile and covers many other functions in astrocyte physiology. Understanding the role of chloride homeostasis in astroglial neuronal signaling is crucial for understanding neurological pathomechanism in which inhibitory signaling is impaired.

Glutamate receptors on astrocytes: is there something new?

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The astrocytes are the glial cells that exert homeostatic control over the brain function, on all levels of the central nervous system (CNS) organisation: molecular, metabolic, cellular, network, organ and systemic.

Astrocytes are endowed in a full set of functional ionotropic and metabotropic receptors (for glutamate, GABA, dopamine, adenosine, noradrenalin, ATP, acetylcholine etc.), what enables them to sense and qualitatively discriminate signals from neurons or other cells, and to react actively to the signals from the milieu by releasing the volume transmitters (e.g. trophic factors) and gliotransmitters (e.g. ATP, D-serine, glutamate). Therefore, the brain function arises from the coordinated activity of a network, comprised of both neurons and glia.

The traditional tripartite synapse idea has been challenged by the fact that astrocytic endfeet enwrapping the synapse are simply too thin to contain required molecules and organelles. The idea then evolved to the synaptic cradle, describing fine astrocytic endfeet, organelle free leaflets, being responsible for the maintenance of the synapse. Recently, however, the tripartite synapse and synaptic cradle concepts have been reconciled and described as the 'active millieu' – a complex inclusive system, comprised of abovementioned functional elements: multipartite synapse signalling, neuro-glio-vascular unit, extrasynaptic signalling and gliotransmission and volume transmission.

Here, the focus will be put on how astrocytic glutamate receptors, both metabotropic (mGlu Rs) and ionotropic (kainate, AMPA and NMDA) are engaged in the abovementioned processes. The least recognised are the astrocytic NMDA receptors, although their functionality is no longer contested. The hallmark of astrocytic signalling, the calcium influx or release from the internal stores, as a consequence of glutamatergic receptor activation, will be discussed. Further, it an overview will be presented of how the events downstream the astrocytic glutamate receptors activation are involved in the formation and modulation of neural circuits, as well as synaptic plasticity and behaviour. Moreover, the update on the involvement of astrocytic glutamate receptors in the pathological states of the CNS and glutamate receptors as potential targets for therapy, will be provided.

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Mechanism of cerebral edema in the acute liver failure simplified interplay of glutamine?

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Cytotoxic brain edema accompanying acute liver failure (ALF) reflects astrocytic swelling driven by excessive accumulation of ammonia and, subsequently, increased synthesis intra- and extracellular accumulation of glutamine. The excess glutamine in the mechanism of brain edema in ALF has been postulated to lead to i) intracellular osmotic imbalance and ii) dysfunction of mitochondria, followed by intra-mitochondrial release of ammonia, and oxidative/nitrosative stress (the "Trojan horse" hypothesis, Albrecht and Norenberg 2006). Glutamine trafficking is secured by active transport. The system N glutamine transporter SN1, which is preferentially localized on the astrocytic membrane, is thought to play a key role in glutamine egress from astrocytes. We hypothesized that SN1 transporter deficiency noted in mouse brain the azoxymethane (AOM) model of ALF (Hamdani *et al*. 2021) would promote changes in the morphology of astrocytic mitochondria. To test this hypothesis, we compared astrocytic mitochondrial parameters in mice with AOM-induced ALF and with vivo-morpholino (VM) knock down of SN1 protein in the prefrontal cortex. To unravel details of cytotoxic edema, astrocytes' morphology and ultrastructure changes of astrocytic mitochondria were analyzed in both models. Swollen astrocytes, a cardinal feature of cytotoxic brain edema, and an imbalance in concentrations of extracellular and total glutamine were observed in both models. Furthermore, in the AOM brain cortex, but not in the brain cortex of SN1 VM mice with depleted transporter, total area of astrocytic mitochondria was found increased. A significant number of astrocytic mitochondria presented features of edema and damage of outer and inner membranes. The results indicate that SN1 glutamine transporter decrease associated with ALF contributes to intracellular glutamine accumulation and cytotoxic edema development preferentially by a mechanism involving osmotic imbalance. Mitochondrial dysfunction is not likely to be related to intracytoplasmic glutamine accumulation due to SN1 glutamine transporter decrease.

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Neuronal death and autophagy in perinatal brain injuries

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The deregulation of (macro)autophagy, a physiological intracellular process of lysosomal degradation, appears to play a dual role in different neurological conditions, since autophagy is reduced and impaired in neurodegenerative diseases but excessively activated in acute brain disorders such as perinatal cerebral hypoxia-ischemia (HI). My presentation will focus on the involvement of autophagy in the pathophysiology of perinatal cerebral HI. Compiling evidence from my and other labs has shown that autophagy is enhanced in HI dying neurons both *in vitro* and in *in vivo* rodent models of perinatal brain injury. In these models, genetical and pharmacological modulation of neuronal autophagy revealed that HI-enhanced autophagy is deleterious by, depending the conditions, mediating apoptosis or being a death-promoting pathway by itself (independent of apoptosis and necrosis). Finally, and more clinically relevant, high neuronal autophagic activity is also observed in dying neurons (thalamus and basal ganglia) in autoptic brains of human asphyxiated babies with severe hypoxic-ischemic encephalopathy (HIE).

We are now investigating the molecular mechanisms by which autophagy could be involved in neuronal death. We provided evidence that neuronal apoptosis is, in many pro-apoptotic conditions, associated with enhanced autophagy. Genetic inhibition of autophagy is importantly reducing the apoptotic response, including cytochrome c and SMAC/DIABLO mitochondrial release, caspase-3 activation etc., induced by pro-apoptotic treatments.

Using the autophagy-inducing peptide Tat-BECN1, we also demonstrated that enhanced autophagy could kill primary cortical neurons without the involvement of other cell death pathways. Autophagic neuronal death (autosis) is dependent on the alpha 3 subunit of the NaK ATPase (ATP1A3) and mediated by ATP1A3-BECN1 interaction.

Altogether, these results cast light on a new mechanistic pathway in the pathophysiology of HIE and suggest that experimental neuroprotective strategies targeting autophagy should be considered for the development of future therapeutic approaches for perinatal brain injuries.

Autophagy in perinatal hypoxia-ischemia – oligodendrocytes

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Perinatal hypoxia-ischemia (HI) is a common consequence of diverse pathological conditions (infectious, vascular, traumatic...) occurring around the delivery of late preterm and term neonates. Disrupted blood circulation, a condition associated with limited oxygen and nutrients' supply, is particularly deleterious for the nervous tissue of infants born between 28 and 34 weeks of gestation, a period corresponding to the white matter formation in the brain. Thus, disruption in tissue homeostasis during this developmental period may result in altered differentiation or maturation as well as death of oligodendroglial lineage cells (OLs), resulting in white matter injury (WMI). The aim of this study is to investigate the role of autophagy (an essential degradation and recycling process) in OLs proliferation, differentiation, and maturation in both physiological and HI conditions.

The study was performed using an *in vitro* model of primary rat oligodendrocyte progenitor cell cultures. To mimic HI injury *in vitro*, cultures were subjected to oxygen-glucose deprivation (OGD). Autophagic flux, oligodendrocyte maturation processes and apoptosis were evaluated by fluorescent microscopy and Western Blot. Lentiviral-mediated knockdown of autophagic proteins were used to study the role of autophagy.

Our results indicate that an efficient autophagic flux is necessary for the proper differentiation of oligodendrocyte progenitor cells. BECLIN1 silencing resulted in 70% reduction in MBP (myelin basic protein) expression and induced activation of Caspase-3. Similar results were reported with ATG7 silencing, another important protein for autophagosome formation. OGD treatment affected autophagic flux and changed the expression of myelin proteins as well as morphology of maturing oligodendrocytes.

This study could contribute to define new pathophysiological mechanisms of HI-induced altered oligodendrocyte differentiation and to propose new protective strategies targeting autophagy to prevent WMI after perinatal HI.

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Autophagy-lysosomal pathway in transmission of misfolded alpha-synuclein in Parkinson's disease: regulation by neurotrophic signaling

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Accumulation of misfolded α-synuclein in the intracellular aggregates known as Lewy bodies and Lewy neurites is a histopathological hallmark of Parkinson's disease. Causative role of α-synuclein in Parkinson's disease was initially supported by genetic and neuropathological studies followed by intensive preclinical research which allowed to reconstitute α-synuclein aggregation *in vitro* and in animal models. Physiologically α synuclein is a soluble, intrinsically disordered protein involved in wide range of cellular processes. However, it is not its physiological function but capability of α-synuclein to assume pathological conformation with self-templating properties, which grow into cell-to-cell transmittable amyloid fibrils, that seems relevant for disease etiology. Extracellular α-synuclein fibrils are uptaken by cells putatively through endocytosis and subsequently are lingering in endocytotic pathway, where they might be partially processed before escaping. Once in cytoplasm, the fibrils grow by corrupting and incorporating endogenous α-synuclein. Ultimately this leads to formation of large multicomponent aggregates, while new fibrils are released from afflicted cells. Alpha-synuclein fibrils can be degraded by lysosomal proteases – either before reaching cytoplasm in endo lysosomal system or trough autophagy. However, their degradation is not efficient as misfolded α-synuclein can impair – processes.

Utilizing primary dopaminergic cultures and animal models, together with virally mediated transgene expression, CRISPR/Cas9 system and pharmacological tools, we have recently shown that neurotrophic signaling can reduce α-synuclein aggregate burden in neurons. Protective effects are mediated by Akt pathway and mimicked by overexpression of constitutively active AKT1 while blocked by inhibition of lysosomal proteases: Cathepsins B and D. Our data indicate that activation of neurotrophic signaling promotes efficient degradation of α-synuclein fibrils in endo-lysosomal or autophagic pathway, which might have implication for understanding neuronal vulnerability in Parkinson's disease and future therapeutic interventions.

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Mitochondrial dynamics and elimination during post-ischemic recovery in ischemia-resistant and ischemia-vulnerable gerbil hippocampal regions

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Transient ischemic attacks (TIA) are the common cause of disability and often precede full-scale stroke. Thus, considerable effort is put to understand intracellular mechanisms evoked by TIA, especially the role of mitochondria – one of a key elements determining cell fate.

The aim of this study was to evaluate the involvement of the mitochondrial network dynamics, mitophagy and biogenesis in two sections of gerbil hippocampus characterized by a different neuronal survival after ischemia-reperfusion (I/R).

Features of mitochondrial morphology and autophagy were investigated by transmission electron microscopy (TEM). The immunodetection of autophagy (LC3-I/II, SQSTM1/ p62, PINK1, Parkin) and mitochondrial biogenesis (PGC1α, NRF1, TFAM) markers was performed in a time course of reperfusion (3-96 hours), together with the analysis of mitochondrial dynamics (Mfn1/2, Opa1 and Drp1) and mtDNA content.

In I/R-vulnerable CA1 region, 24 and 48 hours post ischemia, mitochondria presented ultrastructural features of severe damage of both membranes, which preceded loss of pyramidal neurons. Mitochondrial fission and general autophagy were enhanced early after the I/R (3 hours), sustained during reperfusion and followed by decreased activity of citrate synthase, reduced Hsp60 and electron transport chain subunits (ETC), which together may indicate mitochondrial mass reduction.

Contrastingly, in I/R-resistant CA2-3, DG, the ultrastructure of mitochondria was only slightly altered. Enhanced mitochondrial dynamics was observed with the prevalence of mitochondrial fusion and sustained mitochondrial elongation. Features of autophagy activation were most pronounced 48 hours post ischemia (TEM) and accompanied by the enhancement of selective autophagy markers: PINK1 and SQSTM1/p62. Also, a higher protein level of transcription factors (PGC-1α, NRF-1, TFAM) was present during the reperfusion together with an increase of ETC.

Our study reveals that a greater mtDNA content in CA2-3, DG corresponds with a neuronal resistance to I/R. Also, an enhanced mitochondrial fusion, followed by late and selective mitophagy and mitochondrial biogenesis, may together contribute to reduced susceptibility to TIA.

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Thursday, October, 21st, 2022

Mitochondria as potential targets in neurodegenerative disorders therapy: the role of amyloidogenic proteins

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Sporadic Alzheimer's disease (AD), the most common age-related neurodegenerative disease, is characterized by initial memory impairment progressing toward total loss of mental and physical abilities. The key histopathological features are amyloid-beta (Aβ) containing plaques and microtubule-associated tau-bearing neurofibrillary tangles. In contrast to familial AD caused by a mutation in the amyloid precursor protein (APP), or the presenilin genes 1 and 2 leading to increased Aβ load, the main risk factor for sporadic AD is aging itself. Aging is associated with long-time exposure of our brain to oxidative stress, leading to accumulation of oxidized proteins, lipids, and nucleic acids. The mitochondrion, the major hub of cellular energy conversion, is a main source of reactive oxygen species (ROS). The majority of ROS derive from complexes I and III of the respiratory chain in the form of superoxide anion radicals. Importantly, complex I activity declines substantially during normal brain aging, whereas complex III activity is nearly unchanged, suggesting complex I as the major player of the brain aging scenario.

We demonstrated that complex I dysfunction associated with increased ROS production is a starting point and driving force of the amyloid cascade in AD which is linked to the ROS-dependent activation of BACE1. Aβ itself further accelerates mitochondrial dysfunction and oxidative stress, its formation is self-stimulated. Recent-

ly, we further investigated the molecular mechanism why complex I function is impaired in AD. We focused on post-translational modifications of mRNA, especially of one subunit if complex I, ND5. We were able to show that the methyltransferase TRMT10C, which modifies ND5 mRNA and thereby impairs protein translation, is upregulated in AD. This mRNA methylation of ND5 impairs mitochondrial function and might thereby also trigger formation of Aβ.

Taken together, a vicious cycle is initiated that originates from mitochondrial dysfunction, implying that AD can be viewed as an age-associated mitochondrial disorder.

Glycation of alpha-synuclein: a target for intervention in Parkinson's disease and related synucleinopathies

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Posttranslational modifications (PTMs) are major determinants of protein folding, localization and function. Glycation is a non-enzymatic PTM that is increased in the brains of hyperglycemic patients, such as those affected by type 2 diabetes (T2D). Since it typically leads to irreversible changes in protein properties and behavior, it has attracted a great deal of attention in the last years. Alpha-synuclein (aSyn) can be glycated at lysine residues, thereby reducing fibril formation *in vitro* and modulating aggregation in cells. However, the molecular basis for these effects is unclear. To elucidate this, we are investigating the effect of different physiologically-relevant glycating agents on the aggregation of aSyn. The dicarbonyl methylglyoxal (MGO) and the sugar ribose modify aSyn to the greatest extent, and these glycation agents are the most efficient inhibitors of fibril formation. Glycation primarily inhibits elongation rather than nucleation of aSyn, and has only a modest effect on the level of oligomerization. Furthermore, glycated aSyn is not significantly incorporated into fibrils. *In vivo*, MGO-glycation alters dopaminergic pathways, consistent with alterations observed in Parkinson's disease. Our results are not only relevant for other aSyn PTMs, but also for understanding PTMs affecting other fibrillogenic proteins, and may thus open novel avenues for therapeutic intervention in protein aggregation disorders.

alpha-Synuclein, parkin and mitochondria dysfunction: a deleterious trio in Parkinson's disease pathogenesis

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A plethora of studies indicate mitochondrial dysfunction as a prominent, early and chronic event that contributes to selective neuronal degeneration in Parkinson's disease (PD). Abnormal mitochondria signaling may constitute an important link between major PD-initiating factors: misfolding and deposition of α-synuclein (α-syn) and loss of parkin function. Thus, our study aimed to find the interrelationship of mitochondrial dysfunction with α-syn and parkin in the pathogenic processes underlying PD. Our recent studies from cellular models of PD showed that α-syn, through acting on various extracellular receptors and calcium channels, induces a decrease in the mitochondria membrane potential, the elevation of reactive oxygen and nitrogen species production as well as disturbances of redox homeostasis. Moreover, in wild-type non-transgenic mice a single intrastriatal inoculation of synthetic α-syn oligomers led to an increase in nitric oxide synthase activity, triggered an inflammatory response in the midbrain and striatum followed by a decrease in striatal dopamine level and significant behavioral impairment. In turn, oxidative/nitrosative stress evoked by α-syn led to parkin S-nitrosylation, auto-ubiquitination, and degradation either in cellular or in the animal PD models. While parkin plays an important role in mitochondrial biogenesis and turnover, including mitochondrial fission/fusion as well as mitophagy we observed a protective effect of parkin overexpression on α-syn-induced mitochondrial dysfunction. α-Syn-dependent disturbances of mitophagy were also shown to be directly related to reduced parkin levels in mitochondria and decreased ubiquitination of mitochondrial proteins. Finally, loss of parkin function as a result of α-syn treatment induced an overall breakdown of mitochondrial homeostasis that led to the accumulation of abnormal mitochondria. These findings may thus provide the first compelling evidence for the direct association of α-syn-mediated parkin depletion to impaired mitochondrial function in PD. Establishing strategies involving efforts to protect cells at the mitochondrial level by stabilizing or restoring parkin function appears to be a challenging, but very promising route to slow down the progression of the PD.

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Dysregulation of mitochondrial function by maternal immune activation. Relevance to the pathophysiology of autism spectrum disorders

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Maternal immune activation (MIA) *via* infection during pregnancy is known to increase risk for autism spectrum disorders (ASD). However, it is unclear how MIA affects mitochondria function and how mitochondrial abnormalities might interact with other physiological disturbances associated with autism, such as oxidative stress. In the current study we used the MIA model evoked by a single intraperitoneal injection of lipopolysaccharide to pregnant rats at gestation day 9.5 to examine the relationship between immune response, mitochondria function and oxidative stress in the brain of adolescent offspring. We demonstrated that MIA evoked oxidative stress (elevated level of free radicals and decreased GSH/GSSG ratio) and mitochondria failure (lower mitochondrial membrane potential and ATP level) in the brain of adolescent offspring. Looking for the free radicals sources, we discovered that NADPH oxidase (Nox) activity was gravely elevated in the hippocampus, while not changed in the frontal cortex of MIA animals. Further brain region-dependent differences were found in mitochondrial dynamics. Lower expression of mitochondrial fusion genes was observed in both cerebral cortex and hippocampus. However, up-regulation of fission machinery of the outer mitochondrial membrane regulated by dynamin-related protein1 (Drp1) and phosphorylation of this protein on Serine 616 what promotes mitochondrial fragmentation, was observed exclusively in the cerebral cortex. Although mitochondrial fission plays an important role in the removal of damaged organelles by autophagy, there were no signs of elevated mitophagy in our model, suggesting accumulation of disturbed mitochondria. This was supported by transmission electron microscopy (TEM) images revealing many small, ultrastructurally changed mitochondria. Alterations in mitochondrial dynamics are correlated with changes in bioenergetic capacity. Activity of key electron transport chain (ETC) complexes (CI and CIII) significantly decreased in both cerebral cortex and hippocampus of MIA animals. These findings indicate that MIA may confer increased risk for ASD by dysregulation of mitochondria function and activation of free radicals generation that are relevant aspects in pathophysiology of ASD.

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Tracking the potentials of saliva for detection of molecular-biomarkers of neurodegeneration in Parkinson's disease

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Neurodegenerative disorders are characterized by the accumulation of misfolded proteins in different cells types of the nervous system, leading to the activation of different molecular pathways – including autophagy, neuroinflammation and apoptosis – which finally converge in neuronal death and synaptic loss. Clinical diagnosis and treatment of neurodegenerative disorders are hampered by the progressive deterioration of target neuronal circuits and by the mismatch between clinical and neuropathological onset. Molecular biomarkers are of unreplaceable importance to couple neuropathological and clinical features and to monitor disease progression from a molecular point of view, also at the light of the development of disease modifying therapies.

Saliva is an easily accessible biofluid, whose collection is free of pain and discomfort for the patient. In our recent studies we have used saliva as a source of molecular biomarkers for the diagnosis of Parkinson's disease (PD) and atypical parkinsonisms. ELISA analysis of different PD cohorts have demonstrated reduced levels of total α-synuclein (α-syn) and increased levels of α-syn aggregates in the saliva of PD patients in comparison to healthy subjects. More interestingly, by using Real-Time Quaking Induced Conversion (RT-QuIC) assay, we have detected that seeding competent species of α-syn are present in the saliva of PD patients and that the RT-QuIC kinetic parameters correlate with disease severity. Finally, we have recently investigated the presence in saliva of additional biomarkers, targeting different molecular pathways involved in neurodegeneration and we have found that both autophagic markers and inflammatory markers are increased in the saliva of PD patients and that they determine the molecular clustering of PD patients presenting with different clinical features.

Saliva represent a key biofluid candidate for the detection of biomarkers in PD and could be also used for clustering different PD subtypes, improving molecular diagnosis and follow-up. Further studies are needed to detect the potential application also in other neurodegenerative disorders such as dementias and motor-neurons diseases.

Mouse models of *Gba1*-associated Parkinson's disease

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Objectives: Parkinson's disease is the most common motor disorder, estimated to affect one in twenty individuals by the age of 85. The most frequent mutations associated with Parkinson's disease are in glucocerebrosidase 1 (*GBA1*) gene, which encodes an enzyme involved in glycolipid metabolism, with an estimated 7-10% of Parkinson's individuals carrying a *GBA1* mutation. In this project, we aimed to gain novel molecular understanding of *GBA1* pathology by assessing the effect of *Gba1* mutations in mice, before investigating the effect of ambroxol treatment on glucocerebrosidase activity and on α-synuclein and phosphorylated α-synuclein protein levels in mice and non-human primates.

Methods: First, we analysed two aged (24-month-old) *Gba1* mouse models, one carrying a knockout mutation and the other a L444P knock-in mutation in the murine *Gba1* gene to determine glucocerebrosidase activity, α-synuclein accumulation and dopaminergic neurodegeneration. Next, we studied the effect of overexpression of human α-synuclein (achieved by intraparenchymal injection) in the substantia nigra of L444P *Gba1* mice. Then, we assessed the effect of injection of mouse α-synuclein fibrils into the striatum of L444P *Gba1* mice. Finally, we investigated the effect of ambroxol treatment in wild-type mice, knock-in L444P *Gba1* mice, transgenic mice overexpressing human α-synuclein and wild-type non-human primates.

Results: We observed that L444P *Gba1* mice showed significant reduction of glucocerebrosidase activity and associated increase in α-synuclein accumulation, but no changes in the number of nigral dopaminergic neurons. Interestingly, stereological counts of nigral dopaminergic neurons following overexpression of human α-synuclein revealed significantly greater cell loss in L444P *Gba1* than in wild-type mice. Further, a single injection of mouse α-synuclein fibrils resulted in significantly greater formation and spread of α-synuclein inclusions in L444P *Gba1* mice compared to controls. Finally, we demonstrated that ambroxol treatment resulted in increased brain glucocerebrosidase activity in wild-type mice, knock-in L444P *Gba1* mice, transgenic mice overexpressing human α-synuclein and wild-type non-human primates. Furthermore, in the mice overexpressing human α-synuclein, ambroxol treatment decreased both α-synuclein and phosphorylated α-synuclein protein levels.

Conclusions: Together, these results indicate that *GBA1* mutations can enhances neuronal vulnerability to neurodegenerative processes triggered by increased α-synuclein expression and can accelerate α-synuclein pathology and spreading, making *GBA1* a promising target for drug treatments. Further, our data suggests that ambroxol is an exciting potential new drug for treatment of Parkinson's disease, capable of increasing glucocerebrosidase enzyme activity and decreasing α-synuclein protein levels.

Carotid body modulation prevents cognitive deficits and regulates levels of neurodegenerative markers in dysmetabolic rats

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Introduction: Type 2 diabetes is a risk factor for neurodegenerative disorders, having insulin resistance a role in these pathologies. Carotid body (CB) overactivation was identified in dysmetabolic animal models and prediabetic patients and the abolishment of CBs activity, through the denervation or neuromodulation of its sensitive nerve, the carotid sinus nerve (CSN), prevented and reversed dysmetabolic states. We hypothesize that the abolishment of CBs activity could ameliorate the neurodegenerative processes associated with dysmetabolism.

Material and methods: Male Wistar rats were fed a high fat-high sucrose (HFHSu) diet (60% lipid rich diet plus 35% sucrose in drinking water), or a normal chow diet (NC). Fourteen weeks post-diet, groups were randomly divided and submitted to CSN resection or to a sham surgery and followed-up for 7 weeks. Metabolic and behavioural parameters were evaluated. Hippocampus and the prefrontal cortex were collected for protein analysis.

Results: HFHSu animals showed an increase in weight, glucose intolerance, insulin resistance, and in hypoxic responses, effects attenuated and reverted with CSN denervation. HFHSu animals at 20 weeks of diet developed cognitive alterations, decreasing the time spent in the novel arm in the y-maze test by 62.1% in comparison with the NC group, an effect prevented by CSN denervation. In hippocampus and prefrontal cortex HFHSu animals exhibited an increase in α-synuclein (23.93%, 20.1% respectively) and APP (30.29%, 29.8% respectively) levels, effects prevented by CSN resection.

Conclusions: Modulation of CBs activity prevents cognitive dysfunction and decreases the levels of α-synuclein

and APP in prefrontal cortex and hippocampus, suggesting therapeutic potential for the neurodegenerative process associated with dysmetabolism.

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The role of cytomegalovirus in glioblastoma growth and therapy

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Several reports suggest a connection between the presence of human cytomegalovirus (HCMV) in the brain tumor glioblastoma (GBM) and poorer patient prognosis. Clinical trials targeting HCMV in GBM have yielded exceptional long-term survival rates. To study the role of CMV in GBM progression, we have developed a mouse model GBM in which murine CMV (MCMV) is used to infect the host prior to tumor implantation. GBM growth in these mice us significantly accelerated and is associated with a striking increase in tumor-associated blood vasculature, which can be reversed by treatment with anti-viral drugs. In our recent studies, we set out to investigate the relative contribution of CMV infection in GBM growth using multiple syngeneic mouse GBM-tumor models exhibiting distinct mutational profiles.

Tumor growth and overall survival were evaluated in four syngeneic murine GBM models (GL261, CT-2A, M005 and Mut3) in MCMV infected or naïve C57BL/6 mice. Histological analysis was performed for blood vessel morphology, microglia infiltration and reactive astrocytes. Tumor growth was also examined in the context of age-dependent MCMV infection and during replication-deficient MCMV mutant Dgl infection.

GBM growth was accelerated in MCMV infected mice compared with sham controls in all four GBM models tested. RT-PCR and immunostaining showed that MCMV was detectable at low levels intratumorally but not in other organs or in normal brain tissue in MCMV infected hosts. Enhanced angiogenesis, microglia infiltration and astrocytic immunoreactivity were observed in tumors of MCMV infected mice. GBM growth was independent of age mice acquired MCMV infection. Markedly, productive viral replication was critical for the observed effect as infection with the non-replicating Δgl-MCMV strain led to no increase of tumor growth rates.

These data suggest that CMV induces glioblastoma progression in mutationally diverse tumor models. These findings provide further evidence supporting a role for CMV in driving more aggressive GBM growth which could be targetable therapeutically.

Preclinical studies to support PK- and PD-driven trials in GBM

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There are multiple challenges to glioblastoma (GBM) drug development, including inter- and intra-tumoral heterogeneity, unknown or poor tumor pharmacokinetics (PK) and pharmacodynamics (PD), and unpredictive preclinical models, and few combined therapy studies. Preclinical development of drugs often excludes brain pharmacokinetics assessment and PK-PD-efficacy correlation. We have established a PK- and PD-based pipeline to screen novel drugs and drug combinations using patient-derived xenograft models. The first step is to screen the drugs for protein binding properties (human and mouse matrices), brain penetration properties, and the presence of unbound drug levels above the biochemical IC50 concentrations. If the drugs achieve 5 × IC50 concentration or above in the normal brain, PK-PD evaluation is conducted in tumor-bearing mice after acute treatment. The immediate target is analyzed for target modulation and PD effect. Multiple PDX models are tested to address tumor heterogeneity. Survival studies provide further confirmation of the mechanism of action. Drugs with optimal PK properties and stability are then evaluated in a Phase 0 clinical trial setting.

Cargo of glioblastoma cell-derived extracellular vesicles for tumor classification

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Objective: Extracellular vesicles (EVs) represent a population of lipid bilayer nanoparticles released by all cell types, including tumor cells, and have recently attracted attention as mediators of intercellular communication. EVs harbor tumor-specific nucleic acids and proteins, cross

the blood-brain barrier, and therefore can serve as a non-invasive source for liquid biopsy. To date, MRI images have been the established method for monitoring treatment efficacy in brain tumor patients. Given the urgent need for a reliable biomarker for treatment monitoring of glioblastoma patients, we investigated the potential of EVs and their cargo for diagnosis, prognosis, and treatment monitoring in gliomas.

Methods: We collected EVs from glioblastoma cell cultures (n = 9) as well as from plasma samples (n = 101). Plasma samples were taken before, as well as on the first and fourth day after, microsurgical tumor resection. Follow-up samples were obtained every 3 months. Additionally, we analyzed a group of healthy donors (n = 29). Cell culture derived as well as plasma EVs were isolated by Ultracentrifugation and size exclusion chromatography (SEC) and the plasma concentration was measured by single EV technologies (NTA/IFCM). EV cargo was analyzed by mass-spectrometry, epigenetic as well as genetic profiling. Tumor burden was measured on T1-weighted and FLAIR MRI images. Clinical characteristics were prospectively recorded and retrospectively analyzed.

Results: GSCs secrete diverse EVs as measures by IFCM and multiplex EV assay that are high for common EV markers (i.e. CD9, CD63 and CD81). The range of EVs was 120-150 nm measured by NTA. Genome-wide methylation profiles of GSC EVs in addition to copy number alterations and mutations matched their parental GSC and original tumor sample, being Glioblastoma, IDH wildtype or mutant, with additional subclass analyses. Specifically, MGMT methylation statuses could be obtained through EV DNA. In plasma samples prior to surgery, the level of circulating EVs in glioblastoma is elevated, distinguishing them from healthy controls (5-fold increase in GBM; $p < 0.0001$). Circulating EVs counts correlated only with FLAIR hyperintensity and with no other MRI or blood-based parameter. Dichotomization of GBM patients in EV-high and low revealed a significant overall survival and progression free survival benefit for EV-low patients ($p = 0.004$). After surgery, circulating EVs decreased significantly (5-fold, $p < 0.0001$). A massive drop in EVs was associated with a more radical surgical resection ($p < 0.05$). Interestingly, at the time of tumor recurrence, the number of circulating EVs increased again in all patients during a follow-up period of 9 months.

Conclusions: Our findings highlight the potential of circulating EVs as a biomarker tool for diagnosis, prognosis and treatment monitoring in GBM patients, as they seem to reflect the presence of a tumor mass and thus may assist in clinical decision making.

Non-coding RNA in the brain tumor microenvironment

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Aggressive brain cancers – glioblastomas, are highly heterogeneous at the chromosomal, epigenetic, transcriptional, and phenotypic levels. This variability thwarts therapeutic efficacy and is further compounded by a plethora of microenvironmental gradients that both respond to and drive the adaptation of cancer cell subpopulations. These include fluctuations in oxygen availability, glucose flux, or acidity. Adding yet another layer of complexity, we decided to take a closer look at the previously underappreciated class of molecules with a still hard-to-grasp role in shaping cellular phenotypes and adaptations: non-coding RNAs. These RNAs are an astonishingly large and diverse group of molecules with one overarching characteristic: they do not encode proteins. They thus do not directly affect the protein-orchestrated output of any cell. Yet, as the research progressed, we learned how they could have multi-level influence over cell transcriptomes, proteomes, interactomes, etc.

This talk focuses on the role of diverse sub-classes of non-coding RNAs in shaping adaptations and responses to often unpredictable fluctuations in the levels and availability of the two most crucial compounds that any living cell depends on – oxygen and glucose. These discoveries also shed more light on fundamental differences in cellular signaling between *in vivo* and *in vitro* scenarios that offer the explanation for the often-observed therapeutic failure *in vivo* despite promising results obtained *in vitro* and bring about new potential avenues for improving therapeutic efficacy.

How to transform an immunosuppressive glioblastoma microenvironment into an immunostimulatory one? Transcriptome and cargo analysis of extracellular vesicles after oncolytic virus infection

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Glioblastoma is the most common primary brain tumor in adults which is classified as stage IV malignancy according to the WHO. The median survival rate for patients from the first diagnosis of the tumor is 14 months. It is a difficult disease to treat due to the fact that it is very aggressive. The tumor is heterogeneous, composed of different subpopulations of cells, forms infiltrations into surrounding tissues and is characterized by diffuse growth. What is more glioblastoma is characterized by an environment that dampens immune response activation.

Thus, strategies that would transform the "cold" immunosuppressive microenvironment into a "hot" immunostimulatory one holds promise. One of them is oncolytic virus (OV) – the modified herpes simplex virus 1 tested in clinical trials, targeting cancer cells while triggering immune cells.

Patient-derived glioblastoma stem-like cells (GSCs) (n = 6) were infected *ex vivo* with OV. The cellular transcriptome and cells secretome were then analyzed by gene microarray (n = 21,393) and mass spectroscopy (n = 3,569), respectively.

We showed that the response of all GSCs for infection was associated with the immune response. GSCs after OV infection increase the expression of immune response genes encoding their secretome, which translated into cargo of extracellular vesicles secreted by infected cells.

Analysis of the data showed that infection of GSCs with oncolytic virus not only alters the transcriptome of GSCs but also affects the content of EVs by turning them into immunostimulatory molecules, which may result in better infiltration of the tumor by immune cells.

0001

Protective effect of bacterial melanin against rotenone-induced neuronal dysfunction

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Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder with nigrostriatal and globus pallidus (GP) neuronal dysfunction. In PD GP neurons are damaged, and as a result disruption of dopamine (which is responsible for motor functions in the organism) production occurs. The aim of this work was to study the effect of bacterial melanin (BM) on the morphofunctional state of GP in a rotenone model of PD. Studies were carried out on GP of intact rats, in a PD model 4 weeks after rotenone injection and with BM injection for 4 weeks. Morphohistochemical studies were carried out by the method for detecting the activity of Ca^{2+}-dependent acid phosphatase, developed by Meliksetyan I.B. Here, we describe a drug-free rotometr test that was used to evaluate the effects of unilateral rotenone lesions, nigral disfunction and recovery following bacterial melanin treatment. Neuronal damage of GP is accompanied by lysis of the Nissl substance, and a decrease in phosphatase activity is observed in the cytoplasm. Against the background of normal cells, various types of cellular atrophy have been revealed. Rotenone caused abrupt morphological changes in intracellular structures, and metabolic and morphological disorders. Behavioral tests showed that rats display a form of behavior inherent in PD and under action of BM restoration of lost functions occur. Positive changes in the structural properties of neurons are observed in GP in comparison with PD model after treatment with BM. The morphological picture of neurons were normal, the shape and size of the cells were preserved. In the cytoplasm of cells, an increase in phosphatase activity is observed, which indicates the activation of metabolic processes. Thus, BM showed protective activity against neurodegenerative changes in a rat model of PD.

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0002

Metabotropic glutamate receptors group II (mGlur2/3) agonists reduced apoptosis and up-regulated BDNF, GDNF, and TGF-β levels in hypoxic-ischemic injury in neonatal rats

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Introduction: Birth asphyxia occurs when the brain blood flow is impaired (ischemia) and oxygen supply is arrested (hypoxia). Hypoxia-ischemia (HI) results in damage of the central nervous system leading to neonatal death or developmental disorders. A recent study has shown that group II metabotropic glutamate receptors (mGluR2/3) activation can provide neuroprotection against HI but the mechanism of this effect is still not clear.

Aims: This study aimed to establish the anti-apoptotic effect of mGluR2/3 agonists in an experimental model of birth asphyxia.

Methods: The animal model of HI on 7-day old rat pups was used. Specific agonists of mGluR2 (LY 379268) and mGluR3 (NAAG) were injected intraperitoneally 1 h or 6 h after HI. The weight deficit of the ischemic brain hemisphere was measured and the expression of Bax, Bcl-2, HTR/OMI was examined. The expression of trophic factors GDNF, BDNF, TGF-beta was also measured.

Results: The application of each agonist decreased brain tissue weight loss in the ischemic hemisphere independently on the time of application (from 40% in HI to 15-20% in treated). Both agonists applied 1 h or 6 h after HI increased expression of Bcl-2 and decreased expression of Bax and HtrA2/OMI proteins compared to untreated HI. mGluR2/3 agonists decreased expression of TGF-β and GDNF and increased BDNF in the ischemic hemisphere compared to HI.

Conclusions: The results show that activation of mGluR2 or mGluR3 in a short time after H-I insult triggered neuroprotective mechanisms and reduced apoptotic processes initiated by HI in the developing brain.

0003

Search for typical 5-HT7R ligands – virtual screening studies

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Serotonin receptors, representatives of G protein-coupled receptors, participate in the wide range of physiological and pathological processes in the organism. One of the representatives of serotonin receptors is its 5-HT7 subtype. Although its presence is not limited to the central nervous system (it was also proved to be localized e.g. in stomach, intestines, heart, kidneys, and adrenal glands), it constitues target mostly for CNS-related diseases.

There are numerous ligands of 5-HT7R, which were already reported in the publications. The great majority of them possesses basic nitrogen atom in their structure, which undergoes protonation in physiological conditions

and interacts *via* hydrogen bond with the aspartic acid from the third transmembrane helix. Such basic nitrogen in the ligand structure can be however the source of toxic effects displayed by a ligand, as it is often related to the blockage of hERG potassium channels and resulting cardiotoxicity.

In the study, we focused on the search of new atypical ligands of 5-HT7R, characterized by low basicity. We screened the commercial compound library, ChemBridge, and selected non-basic ligands with the highest potency of 5-HT7R activity. The compound evaluation was carried out mostly on the basis of docking studies. During screening, we used 5-HT7R homology models; however, in the meantime, the cryo-EM structure of 5-HT7R was released and therefore, the previously obtained docking results were confronted with the outcome of the studies with the use of the experimentally determined 5-HT7R structure. Experimental verification of compound activity proved micromolar affinity to 5-HT7R of selected ligands.

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0004

Yolkin – a polypeptide complex isolated from chicken egg yolk upregulates the expression of brain-derived neurotrophic factor (BDNF) in PC12 and H19-7 cells

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Introduction: The progressing aging of the population entails a significant increase in the number of people suffering from dementia, a chronic and progressive process mainly affects the elderly. It leads to a gradual, irreversible impairment of brain function providing to impairment of cognitive processes. Neurons are particularly susceptible to damage resulting in downregulation of neurotrophins (regulating neuronal survival and outgrowth and influencing synaptic plasticity), disturbances in neurotransmission, and finally development of neurodegeneration. Biologically active nutraceuticals exhibiting multidirectional activity may constitute an important therapeutic aspect in preventing and/or inhibiting the development of dementia processes and the development of neurodegenerative diseases.

Aim: The aim of the present study was to evaluate whether yolkin, a polypeptide complex isolated from hen's egg yolk, is able to upregulate PKA/CREB-dependent BDNF expression/production.

Methods: Rat neuron-like PC12 Tet-On cell line and rat primary hippocampal neurons of H19-7 cell line were used as *in vitro* models. Western blotting was used to determine changes in phosphorylation of transcription factor CREB and PKA kinase. BDNF and cAMP levels were measured by ELISA.

Results: It was found that yolkin, when added at concentrations higher than 1 µg/ml, upregulates the cAMP production and PKA activation, in both PC12 cells and H19-7 cells, providing increased CREB factor phosphorylation and activation of BDNF expression.

Conclusions: Obtained results indicate that yolkin might play an important role in the protection of neuron function by its ability to regulate intracellular mechanisms connected with activation of cAMP/PKA/CREB – dependent signaling, resulting in BDNF expression/production.

0006

Respiratory plasticity in experimental research and clinical trials of neurological and neuromuscular disorders

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In the search for effective therapies/rehabilitation of patients with respiratory dysfunction, the use of intermittent chemical respiratory stimuli is very promising, and among them, special attention has been paid to the acute intermittent hypoxia (AIH) exposure model. Both the author's research and the review of experimental and clinical studies, especially recent findings document that, indeed, humans and animals with respiratory dysfunction/failure associated with nervous system injury/disease can develop rapid plasticity compensation under such conditions. Additionally, they indicate that compensation associated with systemic hypoxia is a strong manifestation of the plasticity of respiratory neurons and related structures and organs. The hypoxia-induced plasticity phenomena affect the nervous system (NS) at all its levels, including those regulating (motor) muscle activity, which is manifested in the plasticity of respiratory muscles, or limbs. Recent studies on the effects of intermittent hypoxia (IH) in both the acute AIH exposure model and the chronic CIH

model have made important discoveries that IH improves NS function in animal models of spinal cord injury and patients with spinal injuries. The relevance of the AIH phenomenon/stimulus exposure model as a therapeutic intervention still requires in-depth research and understanding of the mechanisms and effects but has already been shown to be well-tolerated and safe for use in humans. Moreover, the beneficial respiratory effects of exposure to AIH appear quickly and persist for several hours, raising the possibility that this type of intervention could serve as an initial mechanism to facilitate conventional respiratory therapy after neurological trauma in humans. And it seems to be equally promising in neuromuscular diseases, such as amyotrophic lateral sclerosis, as support for the respiratory therapy undertaken in these patients.

0007

Virtual and biological parameters/markers in neuroscience

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Over the past decade, Extended Reality (XR) technologies, along with the globalization of the Internet, have become increasingly important in people's daily lives and professional activities. The highly attractive XR and its classical components such as VR (Virtual Reality), AR (Augmented Reality), and MR (Mixed Reality) represent a promising perspective for contemporary neuroscience, for example, neurobiology, neuropathology, neuropsychology, neuropsychiatry, neurogeriatry or (bio)neuroinformatics. In this study, we compare the effective use of novel virtual indicators and biological parameters in basic and clinical neuroscience. Our model-based non-immersive VR research and the interesting results to date on the application of VR/AR/MR environments for various groups of patients (e.g. stroke, neurodegenerative and neuromuscular disorders or the elderly) indicate their statistically significant high accuracy, sensitivity, and specificity, similar to some traditional/real tests/markers [Brain Res 2021; 1766: 147537; Symmetry 2021; 13(10): 1810; Folia Neuropathol 2020; 58(4): 401-402; Folia Neuropathol 2019; 57(4): 391; Neuroinformatics 2019, DOI: 10.12751/incf.ni2019.0053]. However, these innovative approaches are still not standardized, and cybersickness or Internet addiction is sometimes observed. Overall, this rapid development of novel digital technologies calls for defining safe norms and standardized studies with healthy participants and testing virtual proposed methods/systems for screening/monitoring, diagnosis, and treatment in modern clinical neuroscience and medical practice, including prophylactics/prevention.

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0008

Influence of serotonergic system stimulation on hypercapnic respiratory dysfunction in a bilateral model of Parkinson's disease

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Patients with Parkinson's disease (PD) exhibit respiratory impairments in addition to motor dysfunction. In this study, we used a bilateral model with injection of 6-hydroxydopamine (6-OHDA) into the striatum to model the advanced stage of PD. We analyzed respiratory disturbances in response to hypercapnia following stimulation of serotonergic 5-HT1A and 5-HT2A receptors in awake male Wistar rats. Our experiments were conducted in a plethysmographic chamber, where respiratory tests were done.

Neurotoxin-treated rats showed decreased serotonin (5-HT), dopamine (DA), and noradrenaline (NA) contents in the striatum and brainstem. This affected the respiratory response to hypercapnia, since PD rats displayed reduced minute ventilation (VE) during hypercapnic ventilatory response, which was mainly due to tidal volume (VT) decrease.

Stimulation of serotonergic 5-HT1A receptors by injection of 8-OH-DPAT increased respiration under normocapnia and hypercapnia in both neurological conditions. It showed no effect on the responsiveness of the respiratory response to hypercapnia.

In sham and 6-OHDA rats, the 5-HT2A receptor agonist NBOH-2C-CN also augmented minute ventilation during normocapnic and hypercapnic breathing. However, in sham rats, hypercapnic reactivity after NBOH-2C-CN administration was suppressed in contrast to rats treated with 6-OHDA, which may be due to the increased basal ventilation of sham rats prior to 5-HT2A receptor activation compared with PD rats. Agonists of 5-HT1A and 5-HT2A receptors elevated respiratory response to level similar to those in untreated sham animals, compensating for the abnormalities observed in the hypercapnic respiratory response in PD rats.

We conclude that serotonergic stimulation may have a positive stimulatory effect on the respiratory deficits occurring in PD and may become a therapeutic target for the treatment of respiratory abnormalities present in PD.

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0010

Method for isolation of oligodendrocyte progenitor cells using magnetic microbeads for cell culture

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Oligodendrocyte progenitor cells (OPCs) have been gaining interest in recent years in the context of therapies for various neurodevelopmental or neurodegenerative diseases. These cells can differentiate into mature oligodendrocytes that are responsible for producing myelin sheaths. In response to the pathophysiological conditions they can also proliferate and produce trophic factors and other compounds to stimulate neurorepair processes. Thus, they can be a target of therapy in different neurological conditions.

In order to obtain good *in vitro* model to conduct research on OPCs, we evaluated two methods of neonatal rat OPCs isolation – classical method with intermediate step of mixed glial culture and direct isolation with A2B5 magnetic microbeads. Obtained cells were exposed to OGD (oxygen-glucose deprivation), which mimics one of the most prevalent injury to the neonatal brains' white matter – neonatal hypoxia-ischemia. We evaluated the maturation of oligodendrocytes, expression of selected proteins and screened for the presence of microglia and astrocytes.

Microscopic analysis indicates that with the classical method of OPCs isolation we obtain contamination in the form of macrophages (10.4% ±1.33%) and astrocytes (4.28% ±0.93%). The isolation with magnetic microbeads is shorter procedure (time required reduced from 12 days to 4 hours), and involves 3 days of culture in medium that favor the differentiation of A2B5+ neural progenitors into OPCs. It also allows the elimination of microglia, but cells expressing EAAT1, GFAP, S100B ale still observed. Regardless of the method of isolation, cells exposed to OGD have impaired maturation which might be involved in the pathophysiology of white matter injury.

Microbeads-based method is efficient and cost-effective and it will be used in further experiments to study HIF (hypoxia-induced factor) pathway regulation after OGD.

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0011

Serum metabolomics and metallomics of patients with multiple sclerosis and patients with neuromyelitis optica spectrum disorders

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Neuromyelitis optica spectrum disorders (NMOSD) is the disease misdiagnosed with multiple sclerosis (MS). We hypothesized that serum metabolic profile could be helpful in the differentiation of both diseases in an early stage. A metabolic profile was obtained using 1H-NMR spectroscopy of serum hydrophilic and hydrophobic compounds. Serum metal levels were measured using ICP-MS. For statistical analyses, we used ANOVA and multivariate (MVA) tests. The observed changes in the metabolic profiles covered the same compounds, but their changes were greater in NMOSD than in MS, as compared to controls.

The MVA analysis of both patient groups indicated the most differentiating compounds: phospholipids, ω-6 fatty acid, formate, urea, glutamine, pyruvate, and methionine. In the metal analysis, we observed significantly increased Zn level only in MS, while Co, Cr, and Ba levels were significantly increased only in NMOSD, as compared to controls. The results of our studies point out the occurrence of oxidative stress and inflammation. We concluded that it is possible to distinguish patients with MS and NMOSD from control subjects. However, the differentiation of both diseases between themselves can be performed only using the built MVA model.

0012

Biomarkers of oral cavity homeostasis (dental, mitochondrial and genetic) as predictors of ischemic stroke risk

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Periopathogens (responsible for periodontal diseases) due to their strong pro-inflammatory and pro-atherogenic properties can lead to a development of many systemic multifactoral pathologies, including cardiovascular, cerebrovascular and neurodegenerative diseases.

Oral health biomarkers related to inflammatory processes in oral cavity were analyzed to evaluate their usefulness in assessing of stroke risk in 150 patients after ischemic stroke and 150 controls, in the following steps: 1) dental (number of teeth, DMFT, edentulism) and periodontal (BOP, PD and CAL) status examination, 2) profiling of the mitochondrial respiration in gingival epithelial cells (Oroboros respirometer) and 3) genotyping of the Defensin 1β gene variability in the 5'UTR region of the promoter including two SNPs: DEFB1 G[-20]A (rs11362) and DEFB1 C[-44]G (rs1800972).

Periodontal tissues condition in patients were significantly worse than in controls. The CAL values (OR = 2.64) and the BOP index (OR = 1.15) were much higher among patients. The increase in the number of missing teeth was associated with a higher risk of stroke, particularly in individuals with the number of teeth ≤ 10 (OR = 3.46) and edentulism (OR = 6.85). In the analysis of the intensity of mitochondrial respiration in all phases of the protocol: ROUTINE, LEAK, ETS and ROX, higher values were found in the group of patients, but significant differences (p = 0.029) occurred only in the LEAK phase associated with an uncoupling process. It is possible that these changes could be caused by an oxidative stress within inflamed gingival tissue. There were no differences in the distribution of the analyzed genotypes DEFB1 G[-20]A (rs11362) and DEFB1 C[-44]G (rs1800972) in the groups of patients with ischemic stroke and controls.

Patient's poor dental/periodontal status increases stroke risk certainly. While the explanation of the impact of both mitochondrial respiration alterations in oral mucosa epithelial cells and β-defensin 1 levels determined by the *DEFB1* gene variability on stroke occurrence requires more extensive research.

0014

Neuroprotective and regenerative properties of WJ-MSCs transplanted in platelet lysate-based hydrogel scaffolds in the experimental model of stroke

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Stroke is one of the most common causes of death in humans. The lack of effective treatments for ischemic stroke requires alternative therapies. Thus, in regenerative medicine applications, precise protocols for therapeutically competent stem cells are needed. Hydrogels composed of human plasma-derived proteins are a promising carrier for cell transplantation that can promote neuroprotection and stimulate recipient tissue for regeneration.

The aim of this study was the assessment of the neuroprotective properties of human mesenchymal stem cells isolated from Wharton's jelly (WJ-MSCs). WJ-MSCs were cultured in different oxygen conditions (21% and 5% O_2), then transplanted into injured rat brains in saline or platelet lysate hydrogel scaffolds (3DPL). Transplant distribution, area of lesion site, water diffusion in the brain, and the expression of neurotrophins were analysed.

The signal from WJ-MSCs labelled with iron oxide nanoparticles was observed in the injection site (striatum) 1-, 7-, 14-, and 21- days post-transplantation. No

cell migration to the other brain structures was observed, except to the striatum. In addition, after the WJ-MSCs transplantation in saline or scaffolds, a signal was detected in the injured hemisphere during diffusion-weighted imaging, which was not observed in sham groups or after the focal injury without cell transplantation. After the WJ-MSCs injection, a decreased size of the brain-damaged area and increased mRNA expression of rat BDNF and GDNF has been observed, especially after transplantation of 3DPL WJ-MSCs preconditioned in 5% O_2. The concentration of neurotrophins in rat cerebrospinal fluid significantly increased after transplantation, particularly in the 3DPL group compared to WJ-MSCs injected in saline.

The most preferred therapeutic approach (neuroprotection and regeneration) was obtained after preconditioning the cells under physiological normoxia (5% O_2) followed by their transplantation as encapsulated in hydrogel scaffolds which reflects the conditions found in the brain.

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0015

Adenosine A1 receptor agonist, (±)-5'-chloro-5'-deoxy-ENBA reverses harmaline-induced tremor and thalamic apoptosis in the rat model of essential tremor

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Essential tremor (ET), one of the most common movement disorders, is often modeled in rats by systemic harmaline administration. The main cause of harmaline-induced tremor is abnormal activation of olivo-cerebellar glutamatergic climbing fibers resulting in excessive glutamate release in the cerebellum (CER), motor thalamus and cerebral cortex. Animal studies show that harmaline induces apoptosis in the inferior olive and CER. Our recent studies proved that adenosine A1 receptors stimulation inhibits harmaline tremor. Moreover, *in vitro* data show that the A1 agonist exhibits neuroprotective effect inhibiting apoptosis in astrocyte and cerebellar granule cell lines. Therefore, the aim of the research was to check whether selective adenosine A1 receptor agonist, 5'Cl-ENBA, apart from reducing tremor, also inhibits harmaline-induced apoptosis in different brain structures.

The intensity of tremor was evaluated in Force Plate Actimeters and the expression of caspase-3, -8 and -9, as markers of apoptosis, was measured 24 and 48 h after acute harmaline administration using qRT-PCR in brain structures associated with harmaline-induced tremor: CER, thalamus (THAL), and motor cortex (mCTX).

Harmaline (30 mg/kg ip) induced generalized tremor which was inhibited by 5'Cl-ENBA (0.5 mg/kg ip). Harmaline also increased caspase-3 and -9 expression in CER and THAL 24 h after administration, which was not observed 48 h post-harmaline. Harmaline had no influence on caspase-8 expression in none of the structures and time points, and did not alter the expression of caspases in mCTX. 5'Cl-ENBA, given 30 min before harmaline, blocked harmaline-induced changes in caspase-3 and -9 expression in THAL, but not in CER.

5'Cl-ENBA, a selective adenosine A1 receptor agonist, in addition to its anti-tremor effect, also showed neuroprotective activity by blocking harmaline-induced apoptosis in THAL. Further studies are needed to check whether inhibition of apoptosis in this structure could be sufficient to treat ET.

Study was supported by National Science Center grant (2021/05/X/NZ7/00057).

0016

The influence of HDAC inhibitor, Givinostat on CX3CL1/CX3CR1 axis in a rat model of neonatal hypoxic-ischemic brain damage

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One of the crucial pathogenic factor in the hypoxic-ischemic brain injury is inflammation. The initial inflammatory response after HI is mediated by rapidly activated resident microglia. In recent years, crucial role of neuron-microglia connections through specific transmembrane receptors and their ligands has been emphasized. The interplay between the neuronal ligand CX3CL1 (fractalkine) and the microglial chemokine receptor CX3CR1 enables precise and efficient communication between neurons and microglial cells, which is important for coordinating many aspects of brain function. Moreover neuron-glia cross-talk is one of the mechanisms which can regulate the activation of microglia. Recent data shows that histone deacetylase inhibitor (HDACi), Givinostat (ITF 2357), provides protection associated with reduction of

inflammation after brain injury in adult rats, however its action after neonatal HI is still undefined.

The main purpose of this study was to examine the effect of Givinostat treatment on microglia-fractalkine interactions after neonatal hypoxia-ischemia.

Seven-day-old rat pups were subjected to unilateral carotid artery ligation followed by 60 minutes of hypoxia (7.6% O_2). Givinostat (10 mg/kg b.w.) was administered in a 5-day regime with the first injection given immediately after hypoxic exposure. To determine the levels and interactions of fractalkine and its receptor-CX3CR1 after HI and Givinostat treatment the immunohistochemical and WB analyses were performed.

In the control rat brains about 80% of cells expressing fractalkine colocalized with its receptor CX3CR1, suggesting that under normal conditions, both proteins remain in close contact. Hypoxia-ischemia reduced the number of fractalkine/CR3CR1 interactions to about 50% compared to the control. Moreover after HI the number of CX3CR1 localized on microglia decreased. Givinostat administration did not influence the number of double-stained CX3CL1/CX3CR1 and Iba1/CX3CR1 cells after HI.

The reduced colocalization of the receptor with its specific ligand may imply the impairment of contact between neurons and microglia after neonatal HI, however Givinostat/ITF2357 did not impact on these interactions.

0017

The evaluation of neuroprotective effect of the HDAC inhibitor - sodium butyrate - on the activation of the complement system in rat model of perinatal asphyxia

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Perinatal asphyxia remains one of the most important causes of morbidity and mortality in new-borns. One of the key pathogenic factors in hypoxic-ischemic (HI) brain injury is the complement system – which plays an important role in inflammatory response. Complement usually protects against infection and promotes tissue repair, but it can cause tissue damage if over-activated. The histone deacetylase inhibitor (HDACi) – sodium butyrate (SB), provides reduction of inflammation by reducing the expression of the proinflammatory factors. SB could become a part of a future therapeutic approach to prevent or at least reduce the effects of neonatal asphyxia.

The main purpose of this study was to examine the effect of SB treatment on complement activation and synapse elimination after HI.

Cerebral HI was induced in Wistar rats pups by permanent unilateral ligation of the common carotid artery followed by 60 min hypoxia (7.6% O_2). Sodium butyrate (300 mg/kg b.w.) was administered on a 5-day regimen with the first injection administered immediately after hypoxia exposure.

We observed increased expression of C1qa, C3, C9 and C3aR, C5aR genes of the complement system after HI, which decreased significantly after SB treatment. We also noted increased level of C5 protein of the complement after HI, which decreased significantly after SB treatment. Additionally, expression of some of the synaptic proteins (PSD95, synaptophysin and synapsin) decreased after HI, and returned to the control level after SB administration.

These results suggested the neuroprotective effect of SB by reducing the activity of complement system proteins as well as the protection of synaptic connections. Therefore pharmacological modifications of complement activation could create new therapeutic approaches to reduce brain damage.

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0018

The generation of motor neurons from donor-derived induced pluripotent stem cells: an experimental approach and clinical perspective

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Objectives: Amyotrophic lateral sclerosis (ALS) is currently untreatable neurodegenerative disease characterized by progressive loss of motor neurons. Generation of motor neurons from donor-derived induced pluripotent stem cells (iPSCs) for cell replacement therapy can be a promising strategy to treat motor neuron diseases. The aim of this project was to establish the methodology for the generation of iPSCs and their safety validation, as well as further differentiation into motor neurons in Good Manufacturing Practice (GMP)-compliant conditions.

Methodology: Fibroblasts were isolated from skin samples and reprogrammed to the induced pluripotent state using cell-reprogramming technology. The pluripotent

phenotype of donor-specific iPSC clones was confirmed by immunocytochemistry and quantitative real-time PCR analysis of Sox2, Nanog and Oct3/4, as well as by alkaline phosphatase activity. Cell lines obtained from selected clones were differentiated into motor neurons according to the protocol established in our group. The expression of neural markers was analyzed at two different time points after neural induction – day 15 and day 22. Tumorigenicity test evaluated the tumor-forming potential of transplanted motor neurons *in vivo*.

Results: The expression of pluripotent markers and reactivity of alkaline phosphatase confirmed the pluripotent phenotype of reprogrammed fibroblasts into iPSCs. Cell population directed into motor neurons phenotype expressed neural and spinal motor neuron markers such as: Nestin, B-III tubulin, NeuN, NF200, DCX, ISL1, ISL2 and ChAT. Tumorigenicity test (performed in Foxn1nu mice) revealed no tumor formation at the site of the transplantation.

Conclusions: The established method for the generation of iPSCs and their differentiation into motor neurons may be considered as a potential therapeutic strategy for motor neuron diseases during medical intervention.

The work was supported by Medical Research Agency grant no. 2020/ABM/01/00014 and statutory funds to MMRI PAN.

0019

Analysis of upconverting nanoparticles in hippocampal tissue: Insights into cellular uptake, biodistribution and cytotoxicity

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The dynamic development of nanotechnology created opportunities to apply nanomaterials in various fields including neuroscience. Nanomaterials' ability to across the cell membranes and manipulate their physicochemical properties holds great promise for improving the nanostrategies efficacy for bioimaging and therapy of central nervous system (CNS) diseases. Currently, among the most popular inorganic nanoparticles used in biomedicine, a new generation of luminescent upconverting nanoparticles (UCNPs) based on rare-earth elements deserves special attention.

This study aimed to evaluate the biological interactions between the NaYF4:20%Yb3+,2%Er3+ UCNPs and *ex vivo* model of organotypic hippocampal slice cultures (OHSCs). Analysis includes determination of the possibility of NaYF4:20%Yb3+,2%Er3+ UCNPs internalization by hippocampal cells and cytotoxicity evaluation after exposure to NaYF4:20%Yb3+,2%Er3+ UCNPs.

In order to confirm UCNPs internalization by hippocampal cells, transmission electron microscopy (TEM) and confocal microscopy analysis were performed. TEM results showed that UCNPs were easily internalized by hippocampal cells and co-localized with selected organelles inside neurons and glial cells. Research on the distribution of UCNPs in hippocampal slices showed that the ability to penetrate hippocampal cells and the efficient uptake of the UCNPs are their natural features to enter cells quickly and accumulate only in selected intracellular organelles – endosomes and lysosomes. Moreover, the internalization of UCNPs proceeded by clathrin- and caveolae-mediated endocytosis. The UCNPs in the cytoplasm, other organelles or in the cell nucleus were not observed. Confocal microscope observations also showed the biodistribution of UNCPs within tissue (Z-stack analysis) and their spectroscopic properties in tissue.

The internalization of NaYF4:20%Yr3+,2%Er3+ UCNPs by hippocampal cells and their localization in specific cell structures indicate their possible use in neurotoxicity studies and their therapeutic potential for CNS disorders. The NaYF4:20%Yb3+,2%Er3+ UCNPs exhibit effective luminescence *in vitro* tissue, making them functional infrared imaging nanosystems.

0020

Effect of resveratrol on kynurenine pathway in rat tissue – a chemometric study

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Resveratrol (RSV) is a natural poliphenolic phytoalexine occuring in plants from Vaccinium, Vitis and families Cyperaceae or Gnetaceae. RSV displays antioxidant properties and potential pharmacology effects including anticancer, neuroprotective and anti-inflammatory.

The kynurenine pathway plays a crucial role in the tryptophan metabolism. Kynurenine (KYN) and kynurenic acid (KYNA) are the successive steps in kynurenine pathway. KYNA is known as an endogenous antagonist of ionotropic

glutamate receptors which is associated with neuroprotective activity, whereas KYN is a precursor of KYNA and acts as a aryl hydrocarbon receptor (AhR) agonist, being involved in inflammation, cancerogenesis and neurodegeneration.

The aim of this study was to evaluate the effect of increasing doses of resweratrol on TRP and its metabolites in rat tissues and to analyse the results in mulitivariate way.

The research was carried out on Male Wistar rats. RSV was injected intraperitoneally for 14 days. Brain cortex, striatum, hippocampus and liver was collected and the amount of KYNA, KYN and TRP was determined by HPLC method with fluorescence and UV detection.

The analysis of interaction between resveratrol and content of TRP, KYNA and KYN was carried out using principal component analysis (PCA). Two first principal components were chosen for the analysis-the first explains 22.75% and the second 17.65% of the whole variance. The PCA showed the separation of control groups and the lowest of RSV dosage.

Projection of the variables onto the plane showed positive correlation between TRP concentrations in brain cortex, striatum and liver, but not in hippocampus. KYNA concentration in hippocampus, striatum and plasma were also intercorrelated and negatively correlated with KYNA amount in brain cortex. KYN concentrations were dependent in complex way on these two compounds: hippocampus KYN was correlated with cortex KYNA, plasma KYN was correlated with TRP in each organ except hippocampus.

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0021

The synthetic ligand of PPAR-α regulates the expression of genes related to mitochondria function in an animal model of Alzheimer's disease

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Peroxisome proliferator-activated receptors (PPARs) are very potent transcription factors and regulators of lipid metabolism and energy homeostasis. PPAR-α affects fatty acids level and inflammation. It has been suggested that PPAR-α together with PPAR-γ plays a significant role in neuropsychiatric disorders.

This study aimed to investigate the effect of PPAR-α synthetic ligand, the GW 7647, on the transcription of genes encoding proteins for crucial enzymes involved in mitochondria fission and biogenesis in the brain cortex of the AD mice model.

The 12-months-old female mice with V717I "London" mutation of APP, FVB-Tg (Thyl1; APPLD2/B6), the AD model, were used in this study. The AD Tg mice (APP+) were treated s.c. with GW 7647 (5 mg/kg b.w.), solved in DMSO, and the control animals (APP–), treated with the DMSO, both for 14 days before decapitation. The brain cortex was used and quantitative PCR was performed.

The data presented that GW 7647 decreased mRNA level for Drp-1, the key gene in mitochondria dynamic. Moreover, GW 7647 maintains the expression of this gene in APP+ animals, on a similar level to APP– control mice treated with GW 7647. However, the PPAR-α ligand does not affect the transcription of gene encoding Fis1, another important protein for mitochondria fission. Additionally, our results presented that GW 7647 enhanced the mRNA level of genes encoded several proteins of mitochondria biogenesis, such as TFAM, PGC-1α, and NRF1 in the brain cortex of AD Tg mice vs. DMSO control, but has no effect on gene expression coding NRF2. Our data indicated that the ligand of PPAR-α, GW 7647, may exert a neuroprotective effect in AD Tg mice through the regulation of transcription of genes coding proteins engaged in mitochondria dynamic and biogenesis.

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0022

Expression of selected chemokines by glia in an *in vitro* rat model of neonatal asphyxia

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Hypoxia-ischemia (HI) in newborns leads to brain damage and the development of neurological disorders. Unfortunately, the only available therapy is hypothermia, which is not always effective and the mechanisms of the disease are not fully understood. The purpose of our research is to analyse the effects of HI on glial cells, in which a shortage of oxygen and trophic support triggers a cascade of metabolic alterations. An *in vitro* rat HI model allows us to study the secretory profile of glial cells subjected to a procedure of temporary oxygen and glucose deprivation (OGD). Analysis of the chemokines as factors which affect chemotaxis and proliferation may bring us closer to understanding the disease mechanisms. The mixed glial cultures were established from the brains of 2-day-old Wistas rats. After 12

individual fractions of glial cells (microglia, oligodendrocyte progenitors, astrocytes) were isolated and cells were subjected to the OGD procedure as monocultures or co-cultures and cultured under serum-free conditions in physiological normoxia for up to 5 DIV. The cells and supernatants were then analysed with ELISA tests. A expression of CXCR2 was found to significantly decrease in co-cultures, both in the OGD and control groups. In the case of CXCR4, an increase in expression in microglial cells and a decrease in expression in OPCs was observed after the OGD. In addition, a markedly different expression was noted in co-cultures compared to monocultures. CXCL12/SDF-1 levels were also examined and an increase in expression in the OGD group was observed. The studies conducted allowed to identify a targets for potential therapies. The expression of the chemokines CXCR2 and CXCR4 is crucial in terms of studying the migratory potential of cells, which is essential for modulating the resulting inflammation and for the proper regeneration of damaged tissue.

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0023

The effect of PPAR-α activation on transcription of genes encoding enzymes of amyloid β homeostasis and death signaling in the brain cortex of an animal model of Alzheimer's disease

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Peroxisome proliferator-activated receptors (PPARs) are very potent transcription factors and regulators of lipid metabolism and energy homeostasis. PPAR-α affects fatty acids level and inflammation. It has been suggested that PPAR-α together with PPAR-γ plays a significant role in neuropsychiatric disorders.

This study aimed to investigate the effect of PPAR-α synthetic ligand, the GW 7647, on the transcription of genes encoding proteins for crucial enzymes involved in mitochondria fission and biogenesis in the brain cortex of the AD mice model.

The 12-months-old female mice with V717I "London" mutation of APP, FVB-Tg (Thyl1; APPLD2/B6), the AD model, were used in this study. The AD Tg mice (APP+) were treated s.c. with GW 7647 (5 mg/kg b.w.), solved in DMSO, and the control animals (APP–), treated with the DMSO, both for 14 days before decapitation. The brain cortex was used and quantitative PCR was performed.

The data presented that GW 7647 decreased mRNA level for Drp-1, the key gene in mitochondria dynamic. Moreover, GW 7647 maintains the expression of this gene in APP+ animals, on a similar level to APP– control mice treated with GW 7647. However, the PPAR-α ligand does not affect the transcription of gene encoding Fis1, another important protein for mitochondria fission. Additionally, our results presented that GW 7647 enhanced the mRNA level of genes encoded several proteins of mitochondria biogenesis, such as TFAM, PGC-1α, and NRF1 in the brain cortex of AD Tg mice vs. DMSO control, but has no effect on gene expression coding NRF2.

Our data indicated that the ligand of PPAR-α, GW 7647, may exert a neuroprotective effect in AD Tg mice through the regulation of transcription of genes coding proteins engaged in mitochondria dynamic and biogenesis.

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0024

The role of sphingosine kinase 1/ sphingosine-1-phosphate receptors signalling under ceramide toxicity in alpha-synuclein transduced cells

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Disturbed homeostasis between pro-survival sphingosine-1-phosphate (S1P) and pro-apoptotic ceramide may be an important trigger of neurones death. Simultaneously, S1P receptor stimulation is considered promising in research models of neurodegenerative disorders, like Parkinson's disease (PD).

The interplay between sphingolipid imbalance and α-synuclein, which is a protein overexpressed in PD is barely explored. The current study aimed to investigate the role of sphingosine kinase 1 (Sphk1) – essential enzyme controlling S1P/ceramide ratio in the PD cellular model,

using SH-SY5Y cells transduced with lentiviruses carrying a gene for the human α-synuclein, called further SH-SNCA.

Flow cytometry and microscopic analyses confirmed the increase in the α-synuclein immunofluorescence in SH-SNCA cells compared to their untransduced counterparts. Moreover, SH-SNCA cells at the beginning of the sub-culture showed reduced mitochondrial activity measured by the MTT assay. Flow cytometry analysis of Sphk1-positive cells indicated no changes between control and SH-SNCA cells. Despite the lack of alterations in Sphk1 immunofluorescence, its pharmacological inhibition by SK1-I, an agent specific against Sphk1, significantly increased the α-synuclein-positive cell number in SH-SNCA and control cells. Furthermore, cytotoxicity assayed both directly by propidium iodide staining analysis of dead cells, as well as indirectly by MTT assay indicated a significant survival reduction in SH-SNCA and control cell populations after treatment with Sphk1 inhibitor or C2 cell-permeable ceramide. Simultaneous incubation with SK1-I and C2-ceramide was highly toxic to cells, inducing death in almost all studied populations. On the other hand, preincubation with siponimod (selective agonists of S1P1/S1P5 receptors) protects cells against C2-ceramide-mediated stress.

Our results show a significant role of Sphk1 under ceramide toxicity in α-synuclein transduced cells and point to S1P receptors activation as a valuable pharmacological target in these conditions.

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0026

The potential anticancer properties of mephedrone on human glioblastoma LN-18 cells and human glioblastoma multiforme T98G cells

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Introduction: Mephedrone (4-methylmethcathinone) is one of the most popular synthetic cathinone derivative that is structurally and pharmacologically related to the psychostimulants (3,4-methylenedioxymethamphetamine (MDMA) and other amphetamines). Animal studies have shown that mephedrone causes persistent memory impairment. The underlying mechanism is unknown, but neurotoxic effect of mephedrone cannot be ruled out. Such a possibility is substantiated by our previous results, which indicate that mephedrone resulted in a modest reduction of cell viability and proliferation of normal brain cell lines. The aim of the present study was to assess whether mephedrone affected the proliferation of brain tumor cells *in vitro*.

Methods: Human glioblastoma multiforme cells (T98G) and human glioblastoma cells (LN-18) were used. Cell viability was assessed using lactate dehydrogenase (LDH) assay, cell proliferation by yellow tetrazolium salt (MTT) assay and ELISA BrdU assay.

Results: Our study revealed that proliferation of both studied cell lines was decreased in a concentration-dependent fashion as measured by means of the MTT assay and BrdU assay. Furthermore, exposure of T98G and LN-18 cell cultures to mephedrone resulted in moderate cytotoxicity as measured by means of the LDH assay.

Conclusions: Mephedrone possess potential anticancer activity against human glioblastoma LN-18 cells and human glioblastoma multiforme T98G cells. Elucidating the mechanism of this action and assessing its potential therapeutic significance requires further research.

0027

Disruptions of glutamine translocation in tau-related pathology is crucial for neurodegeneration and astrocyte-neuron integrity *in vitro*

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Astrocytes, abundantly present in the brain, are crucial regulators of brain functions. Their metabolism is tightly coupled to that of neurons, supporting neuronal function and survival. However, recent findings show that astrocytes can acquire neurotoxic properties in several brain disorders. A previous study on tau-dependent neurodegeneration suggests that disruption of astrocyte-neuron interactions may be a result of impaired function of glutamate (Glu)/glutamine (Gln) cycle (GGC). Tau-dependent neurodegeneration is accompanied by astrocytosis in a mouse transgenic models, which replicates the neuropathological characteristic of tauopathy and other human

neurodegenerative disorders. Astroglia derived from tg mice model expressing human tau, exhibit changes in cellular markers of astrocyte neuroprotective function related to the GGC, sustaining a key part of astrocyte-neuron integrity In our study, we focused on the investigation of the functional properties of the crucial GGC components involved in in the astrocyte-neuron network associated with induced tau pathology. We established an *in vitro* model of tau pathology by using neuronal cultures, ACM and selected doses of mutant recombinant tau (rTau), to study Gln translocation through GGC. Especially, we wanted to find the most specific system transporting Gln that is activated or deregulated upon rTau exposition. Here, we demonstrated that under tau pathology condition neurons degenerate, while astrocytes releasing factor(s) are neuroprotective. Overall, our study uncovers the novel mechanisms underlying tau-related diseases, associated to disruption of glutamine GGC, strengthening our hypothesis that Gln recycling and translocation in necessary for neuronal-astrocytic integrity.

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0028

Study of the influence of STIM proteins on NMDA receptor trafficking in dissociated rat cortical neurons

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Stromal interaction molecules (STIMs) are single-pass transmembrane proteins involved in store-operated calcium (Ca^{2+}) entry (SOCE). SOCE is described as Ca^{2+} influx into cells to replenish Ca^{2+} stores in the endoplasmic reticulum (ER). Apart from the participation in SOCE, STIMs also have non-canonical functions, e.g. regulation of Ca^{2+} influx *via* N-methyl-D-aspartate receptors (NMDARs). NMDARs belong to ligand-gated ion channels, which mediate excitatory synaptic transmission which is important for the development of synapses and neuronal plasticity.

In previous studies we showed that STIMs can interact with NMDARs in the primary rat cortical neurons in culture. Thus, we aimed to further explore the STIM-NMDAR relationship by investigating possible regulation of NMDAR maturation and trafficking by STIMs.

With the WB method, we have shown that silencing of STIM1 or STIM2 protein (shRNA) does not result in a change in the total amount of NMDAR subunits (GluN1, GluN2A and GluN2B) in neurons *in vitro*, but changes the immunoreactivity of proteins involved in the transport of these subunits from the ER and anchoring in the plasma membrane (e.g. SAP102, SAP97, KIF17). Furthermore, by examining the localisation of NMDAR subunits in the synaptosomal fraction by WB and immunofluorescence staining of neurons, we have shown that silencing of STIM2 (but not STIM1) causes an increase in GluN2B and a marker of early endosomes (EEA1) in synaptosomes and their co-localisation in dendrites. In addition, based on the presence of mannose residues in NMDAR subunits (EndoH-mediated de-glycosylation assay), we can suggest that silencing of STIM2 increases the presence of immature GluN2B, presumably residing in the ER. Therefore, our results indicate that STIM2 may regulate the process of GluN2B endocytosis and the trafficking and maturation of this subunit.

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0029

Zebrafish gives insight into mitochondrial Ca^{2+} homeostasis both *in vitro* and in living animal

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Mitochondrial dysfunctions, including impairment of Ca^{2+} homeostasis, are found in neurodegenerative disorders like Alzheimer's, Parkinson's and Huntington's diseases. There is a need for development of tools allowing studying neuronal mitochondria, not only in cells but also in alive animals. Zebrafish larvae are ideal for this as they are translucent and allow the visualization and quantification of various parameters. Moreover, components of zebrafish mitochondrial Ca^{2+} toolkit are similar to human. Here, we established methods to study mitochondrial Ca^{2+} fluxes in zebrafish.

The expression of genes encoding mitochondrial proteins in zebrafish larvae and tissues from adult fish

was studied with qPCR. Mitochondrial Ca^{2+} uptake and mitochondrial membrane potential (MMP) were quantified in isolated mitochondria using fluorescent dyes and microplate reader. To analyse mitochondrial Ca^{2+} *in vivo*, a transgenic zebrafish line with a genetically-encoded Ca^{2+} sensor targeted to mitochondria, CEPIA2mt and lightsheet microscopy were applied. Changes in MMP in alive larvae were tracked after TMRE microinjections into zebrafish embryos.

All of the analyzed components of MCU complex were found to be expressed in all samples. As in mammals, Micu3 transcript reached high level in the brain, while Micu2 dominated in the liver. Mitochondria isolated from zebrafish larvae were able to uptake Ca^{2+}, that was inhibited by dissipation of MMP with CCCP. Larvae treated with CCCP showed Ca^{2+} efflux from mitochondria and reduction in MMP *in vivo*, while treatment with glutamate resulted in a quick increase of mitochondrial Ca^{2+}.

These results show that zebrafish can be used to investigate mitochondrial Ca^{2+} homeostasis and successfully combine *in vitro* and *in vivo* studies.

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0030

The role of somatostatin interneurons in emotional contagion regulation

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To study how negative emotions are transferred in mice, particularly in the amygdala and prefrontal cortex, the Remote Transfer of Fear behavioural paradigm was employed. This involved housing pairs of mice (Observer and Demonstrator) for three weeks before the Demonstrator was removed from the home cage and subjected to adverse stimuli (10 foot shocks – 1 s long, 0.6 mA).

Once the Demonstrator had been returned to the home cage, the first ten minutes of interaction were recorded. After another eighty minutes, the mice were sacrified for immunohistochemical staining purposes. Sst-IRES-Cre mouse strains were used, as they expressed fluorescence marker (dTomato) through viral tagging. Combined with immunochemistry against c-Fos (a standard neuronal novelty marker), this enabled checking for somatostatin interneuron activity.

The first ten minutes of interaction show higher levels of anogenital sniffing, body sniffing and self-grooming behaviour for Observers (Control group). Meanwhile, exploratory behaviour was higher for both Demonstrator and Observer mice (Experimental group).

This altered behaviour within the Experimental group, combined with increased neuronal activation (higher c-Fos levels for both amygdala and prefrontal cortex), confirms that emotional contagion occurred. Changes in somatostatin cell activity within amygdala region (Observers; both groups) likewise indicate their role in the emotional contagion regulatory circuit.

0031

Inhibition of bromodomain and extraterminal (BET) proteins modulates inflammation-induced sickness behavior; the relevance to Alzheimer's disease-related depression

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Epidemiological and molecular studies link inflammation with Alzheimer's disease (AD), cognitive decline, and depression. The stimulated immune system releases several inflammatory cytokines which significantly impact cognition and sense of well-being, leading to symptoms known as sickness behavior. Sickness behavior plays an important role in inflammation-associated depression, but its components and mechanism are not fully understood. Our aim was to analyze the role of bromodomain and extraterminal (BET) proteins, the readers of histone acetylation code, in controlling inflammatory cytokines and sickness behavior during systemic inflammation.

In vitro, we used lipopolysaccharide (LPS)-stimulated murine microglial cell line BV2. *In vivo*, systemic inflammation was evoked in adult male mice C57BL6 by intraperitoneal injection of LPS. JQ1 was used as the BET proteins inhibitor. The expression and level of selected cytokines were analyzed using qPCR method and immunoassay up to 12 h post-treatment. In parallel, sickness behavior was estimated using a modified murine sepsis score (MSS).

JQ1 significantly reduced the expression of pro-inflammatory genes in LPS-stimulated BV2 microglia. Moreover,

it prevented an increase in the serum level of some specific cytokines (e.g. IL-1α, IL-5, IL-6, IL-12p40, eotaxin, GM-CSF, RANTES) both 3 and 12 h after peripheral administration of LPS. In the hippocampus, the inhibitory effect of JQ1 on the expression of inflammation-related cytokines was observed after 3 h, but not after 12 h. Surprisingly, JQ1 reduced sickness behavior after 12 h, but not after 3 h, suggesting the important role of systemic cytokines.

Our results demonstrated that pharmacological inhibition of BET proteins affects the expression of some inflammation-related genes and, in consequence, modulates the activity of the immune system, including microglial phenotype and sickness behavior. We suggest that inhibition of BET proteins may be a strategy for the treatment of inflammation-related sickness behavior/dementia in AD.

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0032

Deregulation of the microtubule cytoskeletal network in the cerebellum of adolescent rats prenatally exposed to valproic acid. Relevance to autism spectrum disorders

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Autism spectrum disorders (ASD) comprise a group of heterogeneous neurodevelopmental conditions characterized by impairments in social interactions, communication and the presence of restricted and/or stereotyped patterns of behaviours. The pathogenesis of ASD is not yet fully understood, but increased research efforts have provided evidence that abnormal brain development (synaptic dysfunction, anomalies in the cytoskeleton, and thus compromised neuronal connectivity) may underlie the aetiology of ASD. On the other hand, a growing body of evidence supports the hypothesis that abnormal cerebellar development may be a primary risk factor for ASD development. However, the links between this brain structure's abnormalities, developmental deficits and the neurological dysfunctions underlying ASD are not entirely understood. Therefore, in this study, we used a valproic acid (VPA)-induced rodent model of autism to investigate the alterations of key cytoskeletal components in the cerebellum of offspring. Especially, we analysed the protein level (Western blot) of α/β-tubulin and the major neuronal microtubule-associated proteins (MAP) such as MAP-Tau and MAP1B, MAP2, MAP6 (STOP) along with actin-cross-linking αII-spectrin and neurofilament light polypeptide (NF-L). Our study revealed that embryological exposure to VPA promotes a significant reduction in the protein levels of α/β-tubulin, MAP-Tau, MAP1B, MAP2, and αII-spectrin. In addition, we observed Tau hyperphosphorylation at (Ser396) along with the up-regulation of key Tau-kinases. Additionally, immunohistochemical staining revealed histopathological abnormalities in the cell body of neurons (chromatolysis) together with a significant loss of Purkinje cells in the cerebellum of autistic-like rats. In summary, the observed deregulation of key cytoskeletal protein homeostasis may lead to a disturbance of synaptic plasticity and connectivity, contributing to dysfunctional, autism-related behaviours.

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0033

Effect of the selective inhibitor of dipeptidyl peptidase-4 (DPP-4) enzyme, linagliptin, on short-term and long-term memory impairments observed during morphine withdrawal in mice

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Morphine is considered one of the most effective analgesic drug. However, long-term administration of morphine induces the morphine addiction and morphine withdrawal is associated with memory impairment. Linagliptin, inhibiting a dipeptidyl peptidase-4 (DPP-4) enzyme, increases the glucagon-like peptide-1 (GLP-1 peptide). Apart from involvement in the blood glucose levels, it is known that the GLP-1 peptide also play an important role in food intake, in the rewarding effect of addictive substances. Considering the literature data, the wide distribution of GLP-1 receptors in the brain and the interactions between the GLP-1 peptide and other neurotransmitters, the GLP-1 peptide is suspected to be an important factor affecting memory and emotions. Therefore, the aim of this study was to evaluate the effect of a DPP-4 inhibitor on memory impairment induced by morphine withdrawal in mice assessed in the novel object recognition (NOR test) test. The conducted experiments have shown that long-term withdrawal of morphine causes memory impairment

in animals, and administration of linagliptin reduces these disturbances.

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0034

Developmental abnormalities in the motor cortex architecture of the spontaneously hypertensive rats (SHRs) – an animal model of attention-deficit hyperactivity disorder (ADHD)

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Introduction: Attention-deficit/hyperactivity disorder (ADHD) is one of the most frequent condition in children, and it is characterized by age-inappropriate hyperactivity, impulsivity and inattention. The current literature also indicates that the development of this ADHD is accompanied by several morphological and functional brain abnormalities.

Aim: The purpose of this study was to analyze the neuron density abnormalities in the primary (M1) and secondary (M2) motor cortices during postnatal development in the spontaneously hypertensive rats (SHRs) – an animal model for ADHD and Wistar Kyoto rats (WKYs) used in the present study as a control group.

Material and methods: Brains were carefully isolated from SHRs and WKYs in seven age stages (from 4 to 10 weeks), cut and subjected to DAB staining. To quantify the neuron density in the particular layers (II, III, IV, V and VI) of M1 and M2 the optical dissector method was used. Next, data obtained were statistically compared using the GraphPad Prism Version 6.0.

Results: Neuron density changes across postnatal development in both SHRs and WKYs had unique pattern in each of the M1/M2 layers. SHRs had significantly lower neuron density than WKYs at 6 weeks of age in all layers of M1 and M2. However, with age the difference was gradually attenuated. Furthermore, in 10-week-old animals neuron density became significantly higher in SHRs. The reported differences were the most significant in the right hemisphere.

Conclusions: Generally, the abnormalities observed in M1/M2 of SHRs may indicate a delay in brain maturation in ADHD. Whereas significant, increase in the neuron density in adult SHR might be caused by the enlargement of the brain ventricles, which characterize ADHD individuals.

Key words: motor cortex, neuron density, immunostaining, ADHD, rat.

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0035

Serum microRNA (miR) in acute liver failure and hyperammonemia rat models. Role in blood-brain barrier impairment?

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Acute hepatic encephalopathy (aHE), resulting from liver failure-evoked toxic metabolites (mainly ammonia) accumulation that cross the blood-brain barrier (BBB), and enter the CNS. Hyperammonemia, massive systemic (eventually central) inflammation, and severe intracranial hemodynamic alterations, contribute to aHE pathogenesis. ALF is associated with fatal cerebral edema in approximately 50% cases. We analyzed miRnome by NGS in plasma of rats with ALF (thioacetamide; TAA), and hyperammonemia (ammonium acetate; OA) to define miRs contribution to poorly explored structural and functional BBB changes.

Total RNA was isolated from serum (miRNeasy Serum/Plasma Advanced Kit; Qiagen), quantified and used for library preparation. miRNA sequences, were identified using the miRDeep2 Quantifier analysis. NGS of plasma miRNA in rat models that differ with plasma biochemistry (ammonia, liver enzymes, cytokines) and grade of BBB alterations, were performed using DNBSEQ G400 system. We identified 488 miRs (129 significantly changed) and 473 miRs (36 significantly changed) in TAA and OA group, respectively. Using three databases (miRDB, TargetScan and TarBase), we evaluated the possible biological impact of miRNAs on BBB-related targets.

As a result, nine miRs targets, were obtained in TAA, but no overlapped targets in the OA group. Prediction scores in MiRDB, were determined by computational target prediction algorithm with a target score < 60 as less relevant. We selected miR-122-5p and miR-183-5p targeting occludin and integrin 1, respectively, as the most changed miRs having the highest target score represented more statistical confidence in the prediction results. The involvement of selected miRs in KEGG Pathways using String, a tool for the retrieval of genes interaction, was shown. Finally, selected miRs and targets were evaluated by qPCR and immunohistochemically.

Results implicate miR-122-5p and miR-183-5p by modifying occludin and integrin B1 protein expression, respectively, may contribute to the BBB dysfunction and provide a reference for potential novel therapy by controlling BBB permeability after liver failure.

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0036

S100B protein as a systemic biomarker of brain impairment in patients with inherited hyperammonemic disorders

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Specific inborn errors of metabolism (IEM) are associated with elevated blood ammonia. A high ammonia level triggers astrocyte swelling, encephalopathy, cerebral edema, coma, and eventually death. The level and duration of hyperammonemia appear to be critical determinants of cognitive outcome. The present study focused on searching for peripheral markers in the course of congenital hyperammonemias other than ammonia. Plasma samples were collected from patients suffering from various IEM (hyperinsulinism-hyperammonemia syndrome; methylmalonic acidemia; propionic acidemia; ornithine transcarbamylase deficiency). Inflammatory cytokines, chemokines, 3-nitrotyrosine (3-NT), and the astrocyte-derived S100 calcium-binding protein B (S100B) were tested. There were no significant increases in the pro-inflammatory cytokines and chemokines that would correlate with ammonia or the type of congenital hyperammonemia. Furthermore, 3-NT levels were elevated substantially in some patients but were unrelated to ammonia levels. On the contrary, a linear correlation of S100B protein with plasma ammonia concentration was revealed, indicating that analysis of this protein can be valuable in estimating the risk of brain injury in patients with congenital hyperammonemias. S100B released across the blood-brain barrier may constitute a prognostic or treatment biomarker for cases of IEM, especially since it is stable and relatively unaffected by storing or hemolysis. The presented results suggest that S100B testing in the peripheral bloodstream may provide diagnostic benefits at relatively low expenses.

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0037

Analysis of Parkin, PINK1 and ZNF746 proteins in the plasma of Parkinson's disease patients

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Objectives: Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. Genetic and environmental factors are responsible for the development of PD. Both the genetic variants PARK2 (PRKN), PARK6 (PINK1), ZNF746, and their protein products are considered parameters related to the onset and development of PD. Moreover, there is an interdependence between Parkin, PINK1, and ZNF746 (PARIS) proteins. Parkin and/or PINK1 proteins, by influencing the concentration of the ZNF746 protein, are responsible for maintaining the proper level of dopaminergic neurons. Inactivation of the Parkin or PINK1 protein leads to an increase in the neurotoxic level of ZNF746 (PARIS) and thus the loss of dopaminergic neurons and the development of neurodegeneration.

There are no reports on the regulation of the peripheral levels of Parkin, PINK1, and ZNF746 (PARIS) proteins in PD patients.

Methodology: The study included 38 PD patients, mean age of 52.2 years, and 44 controls, mean age of 51.8 years. The concentration of the Parkin, PINK1, and ZNF746 proteins in the plasma was determined by the ELISA method. The obtained results were analyzed statistically.

Results: A statistically significantly lower level of Parkin ($p < 0.05$) was demonstrated in PD patients (80.70 ±125.84 ng/ml) compared to the controls (267.00 ±292.20 ng/ml). At the same time, the decreased level of Parkin was accompanied by a statistically significant increase in the concentration of the ZNF746 protein ($p < 0.05$) in PD patients (52.80 ±141.55 pg/ml) compared to the control group (38.60 ±51.60 pg/ml). In contrast, the PINK1 protein tended to be elevated level in PD patients. In these patients, however, no statistically significant differences were found between the PD patients (54.08 ±244.85 pg/ml) and the control group (30.21 ±152.26 pg/ml).

Conclusions: Parkin protein appears to be more strongly involved in regulating the level of the ZNF746 protein. Monitoring the level of analyzed proteins may become a new diagnostic and prognostic factor in PD in the future. Analysis of the concentration of Parkin, PINK1 and ZNF746 proteins in the peripheral blood brings new information to the pathogenesis of PD.

0039

Zingiber officinale rhizoma as a source of new anticonvulsant agents – *in vivo* studies

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Objectives: The aim of the study was to evaluate the effect of Zingiber officinale (ginger) rhizoma extract and its active constituent – 6-gingerol, in the pentylenetetrazole (PTZ)-induced seizure model in larval zebrafish. Moreover, some possible mechanisms of 6-gingerol's anticonvulsant activity were determined.

Methodology: Larval zebrafish of AB strain up to 7 days post-fertilization were used. The larvae were bathed in the extract or solutions of 6-gingerol for 24 h. Subsequently, seizures were induced by the acute application of PTZ. Locomotor activity was measured using tracker (Zebrabox, Viewpoint, France). Local field potential (LFP) recordings were obtained from larval optic tectum (Axon Instruments, USA). To determine neurotransmitters levels in fish, HPLC-ESI-QTOF-MS/MS analysis was conducted. The expression of genes was measured using RT-qPCR.

Results: Zingiber officinale rhizoma extract exerted anticonvulsant activity in PTZ-evoked hyperlocomotion seizure assay in larval zebrafish. Furthermore, 6-gingerol in a dose-dependent manner attenuated PTZ-evoked hyperlocomotor behaviour. LFP recordings confirmed these observations. In PTZ-treated larvae, 6-gingerol decreased glutamic acid (GLU) level as well as GLU to GABA ratio, compared to only PTZ-incubated fish. 6-gingerol decreased the expression of grin2b (encoding glutamate N-methyl-D-aspartate receptor 2B subunit) in PTZ-bathed larvae. A molecular docking study implied that 6-gingerol might act as an inhibitor of NR2B-containing NMDA receptor.

Conclusions: Ginger rhizome extract is a valuable source of new anticonvulsant agents, as shown in the example of 6-gingerol.

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0040

Analysis of distribution patterns of polyamine-related enzymes and mitochondrial calcium uniporter suggests differential mitochondrial Ca^{2+} fluxes in hippocampal subregions

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Introduction: Polyamines (PAs), polyvalent cations involved, among others, in neurotransmission and neuroprotection, are found at high levels in the hippocampus. Their distribution and roles in this structure are not well described. One of cellular functions of PAs is the regulation of mitochondrial fluxes of Ca^{2+}, what can affect numerous cellular pathways in neurons. Here, we aimed to determine the precise localization of hippocampal PAs metabolic machinery and a major regulator of mitochondrial Ca^{2+} homeostasis, mitochondrial calcium uniporter (MCU), a protein controlled by PAs.

Methods: Spatial distribution of mRNAs of PAs metabolism-related genes was studied with Allen Brain Atlas. The experimental work was performed using WT mice and mouse model of loss of Arginase 2 (Arg2), an enzyme supplying ornithine (Orn) for PAs synthesis. Localization of selected proteins within the hippocampus was analyzed using immunohistochemistry, and their levels were determined with western blotting. The content of amino acids related to PAs metabolic pathways was measured by HPLC.

Results: According to the Allen Brain Atlas, region CA2 features a unique profile of PAs-related gene expression. Distribution of PAs-related enzymes at the level of mRNA and

proteins suggests that hippocampal PAs synthesis is a process that takes place in pyramidal neurons, particularly in CA2. Immunohistochemical studies demonstrate that MCU colocalizes with the enzymes involved in PAs synthesis. Amino acid measurements in Arg2 KO mice establishes CA2-specific Arg2 as a major regulator of hippocampal Orn levels.

Conclusions: My data suggest a distinct PAs metabolismin in hippocampal subregions. Arg2 expressed at high levels in CA2 seems to be a main supplier of Orn for intensive production of PAs. Locally synthesized PAs, through interactions with CA2-enriched MCU, may control mitochondrial Ca^{2+} fluxes, constituting specific phenotypes of CA2 e.g. low synaptic plasticity and high resistance to neurotoxic insults.

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0041

Rise of EEG energy of initial pilocarpine-induced seizures and its attenuation by MSO pretreatment is correlated with extracellular accumulation of taurine, but not of glutamate, acetylcholine or GABA

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We recently showed that pretreatment with a non-convulsive dose of glutamine synthetase (GS) inhibitor, methionine sulfoximine (MSO), delays the onset and mitigates the intensity of initial seizures in the juvenile rat Li-Pilo model of temporal lobe epilepsy, by both a canonical (GS inhibition) and non-canonical mechanism (PMID: 33422530; PMID: 34681786). Here we examined simultaneously EEG profiles, as a measure of seizure intensity, and extracellular (hippocampal microdialysate) levels of Tau, whose release to the extracellular space is considered to be a marker of cell swelling. To assess the potential relation of the seizure onset to Gln synthesis and excitatory or inhibitory neurotransmission, the extracellular concentrations of Gln and the neurotransmitters Glu, GABA and ACh were recorded. The experiments were performed in free-moving Li-Pilo treated rats, subjected or not to MSO pretreatment; microdialysate content was measured by HPLC. Rapid and gradual accumulation of Tau was noted upon the onset of seizures, and the effect was significantly less pronounced in animals pretreated (for 2.5 h) with MSO. Relative EEG energy recorded during the initial seizure, was significantly reduced in MSO-pretreated rats ($p < 0.0001$), and a change of energy over time strongly and positively correlated in time with levels of Tau, both in MSO-treated and untreated rats ($r = 0.9524$, $p < 0.0001$ and $r = 0.8972$, $p < 0.0001$, respectively). MSO reduced Gln, but did not affect the contents of GABA, Glu, and ACh in the microdialysates. The results indicate that MSO delays and alleviates initial pilocarpine-induced seizures by relieving brain edema, but much less so by correcting the imbalance between the inhibitory and excitatory neurotransmission. Given the high abundance of Gln and its contribution to the pool of brain osmolytes, attenuation of edema could be related to the MSO-evoked decrease in Gln content.

0042

Arginase 2 is a striatal enzyme controlling arginine levels in medium spiny neurons

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Introduction: Arginase is an enzyme responsible for the conversion of arginine (Arg) to ornithine (Orn). Two arginase isoenzymes exist – cytosolic Arg1 (enriched in the liver) and mitochondrial Arg2 (enriched in the kidney). Arg1 is absent in the brain in physiological conditions, when Arg2 is found only in selected localizations. Striatum is strongly enriched with Arg2, but the role and exact localization of this protein is unknown. Here, we aimed at determining detailed Arg2 localization within the striatum and establishing the importance of Arg2 for striatal Arg homeostasis.

Methods: The studies were performed using control WT C57Bl/6J mice and genetic model of Arg2 loss (Arg2 KO mice; Arg2tm1Weo/J). Protein levels were studied using western blot. Protein localizations were visualized with immunohistochemical staining. Amino acid content was measured by HPLC. Nitrates and nitrites (NOx; cellular nitric oxide (NO) indicator) were analyzed with the use of fluorimetric microplate test.

Results: Arg2 is widely expressed in the striatum, where it is present in a population of medium spiny neu-

rons (MSNs), localizing mostly in cell bodies, but also, to some extent, in the processes. Arg2 is absent in striatal interneurons, glia and some other subpopulation(s) of MSNs. As expected, Arg2 is found in the mitochondria and its loss results in the significant accumulation of Arg in the striatum. Arg2 absence doesn't affect the levels of neither NOx nor other amino acids.

Conclusions: Striatal Arg2 is a neuronal protein, limited to a defined population(s) of MSNs. This enzyme controls striatal Arg levels, but its absence doesn't appear to influence the content of Orn, what may suggest that some compensatory mechanisms are activated in the striatum. Arginase share common substrate (Arg) with NO synthase, therefore it may potentially control NO production ratio, although, in physiological conditions, Arg2 appears not to affect striatal synthesis of NO.

0043

Is neuronal dysfunction an early sign of diabetic retinopathy? The interactions between multiple retinal cell-types forming the retinal neurovascular unit

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Diabetic retinopathy (DR) is one of the most common complications of diabetes mellitus. It contributes to vision loss among people of working age, as approximately 40% of diabetics suffer from diabetic retinopathy. The majority of studies in this field focus on vascular damage in DR or treat the vascular and neuronal injury as a parallel process. Therapies used in the diabetic patient are limited only to alleviate vascular alteration. There are only few recent publications showing that injury of different types of neuronal cells of the retina may appear ahead of vascular changes.

The aim of the study is to examine the interactions between multiple retinal cell-types forming the retinal neurovascular unit, the basic functional unit in the retina, whose impairment plays the key role in development and progression of DR.

Our studies set itself the task, what happens to the interactions between all structural components of retinal neurovascular unit in hyperglycemic environment and how retinal neurons, glia and vascular cells affect to each other.

Our preliminary electrophysiological studies using the streptozotocin (STZ)-induced diabetic mice, have shown alteration of oscillatory potentials (decreased amplitudes) four weeks after diabetes development, simultaneously the number of retinal ganglion cells did not decrease statistically significantly in diabetic group as compared to the healthy group. Moreover, after eight weeks, the increase of retinal blood vessels permeability and the structural changes in endothelial cells were detected in EM analysis. Those results indicated that functional alteration in retina preceded the vascular changes what confirms that DR is rather a neuronal dysfunction than the vascular disease.