Session 18: Cellular and molecular neurobiology

Lectures

L18.1

Novel activity-driven architectural remodeling of chromatin architecture in neurons

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Recent studies in neurons indicate that the large-scale chromatin architectural framework, including chromosome territories or lamina-associated chromatin, undergoes dynamic changes that represent an emergent level of regulation of neuronal gene-expression. This phenomenon has been implicated in neuronal differentiation, long-term potentiation, seizures, and disorders of neural plasticity such as epilepsy.

L18.2

Lost in translation; post-transcriptional regulation of gene expression in neuropsychiatric disorders

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Translation of mRNAs, also termed protein synthesis, relies upon an intricate molecular network, whose dysregulation can lead to pathological conditions like cancer, viral infections, neurodegeneration and neurodevelopmental diseases, such as Autism Spectrum Disorders (ASD). ASD encompass an ensemble of unique behaviors within three domains: social interaction, communication and restricted/repetitive/stereotyped behaviors. We have shown that exaggerated cap-dependent translation can lead to ASD-like behaviors in mice. These behaviors are concomitant with an imbalance of excitatory to inhibitory synaptic transmission and increased translation of a subset of brain-specific mRNAs. Using pharmacological blockade of translation or gene therapy targeting specific mRNAs, we were able to reverse ASD-like behaviors in mouse models of neuropsychiatric disorders. These results open new avenues for the development of strategies to modulate ASD-associated behaviors and further our understanding of the molecular underpinnings of neurodevelopmental disorders.

L18.3

Molecular and kinetic scenariosof GABAa receptor activiation: between agonist binding and channel opening

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GABAa receptor belongs to the cys-loop family and mediates rapid inhibition in the adult brain. Classic view on activation of ionotropic ligand-gated receptors postulates that following agonist binding, receptor undergoes a conformational transition to the open state. Thus, binding enhances the probability of opening directly from a closed bound state. In the past years this view has been challenged by studies indicating that, in the case of another member of cys-loop receptor family – glycine receptors, activation occurs via intermediate states named preactivation, flipping or priming (Lape *et al*., 2008, *Nature* **454**: 722-727). This finding appears consistent with structural aspects of these receptors. Namely, agonist binding site is located as far as approximately 5 nm from the pore where the channel gate is located. To investigate the preactivation phenomenon in the GABAa receptors we have expressed the wild type (WT) recombinant a1b2g2 receptors as well as the a1F64C mutants and studied their properties at the macroscopic and single channel level. Interestingly, mutation of a1F64 residue, which is located at GABA binding site, resulted not only in altered agonist affinity but also in severely changed kinetics of conformational transitions between fully bound states. Detailed kinetic analysis supported by the use of a variety of pharmacological agonists (GABA, muscimol, P4S) provided evidence that mutation of this residue mostly affected the preactivation transition and, to a smaller extent, also the receptor desensitization. Considering thus that preactivation plays a pivotal role in GABAa receptor activation, we asked whether mechanisms of GABAaR modulation by pharmacological factors involves preactivation transitions. We found that indeed such modulators as pH and benzodiazepines strongly affect the preactivation step in receptor activation. Altogether, our results provide the evidence that preactivation is strongly involved both in GABAa receptor activation and its modulation by considered compounds.

Posters

P18.1

Mechanisms of acute and chronic administration of bergapten induced improvement in memory and learning processes in passive avoidance test in mice

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Coumarins comprise a very large class of oxygenated aromatic natural products showing wide range of pharmacological activities. Bergapten (5-methoxypsoralen, 5-MOP), is a furanocoumarin found in many medicinal plants. Literature data have demonstrated that bergapten possess inhibitory activities on monoaminooxydase (MAO), butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE). BuChE and AChE degrade acetylcholine, which prevents the formation of senile plaques in Alzheimer's disease. Moreover, bergapten has been found to exert strong antioxidant properties. Therefore the aim of present study was to examine the effects of an acute and subchronic administration of bergapten on memory processes in the passive avoidance (PA) paradigm in male Swiss mice as well as to assess the effects of this drug on the level of oxidative stress in brain and its structures involved in cognitive processes (hippocampus, prefrontal cortex).
It was shown that bergapten administered acutely at the doses of 25 and 50 mg/kg improved acquisition of memory processes whereas at the dose of 25 mg/kg improved consolidation of cognitive processes in mice. Subchronic administration of bergapten at the dose of 12.5 mg/kg improved memory acquisition and consolidation of memory and learning processes observed in PA test. Biochemical studies including determination of total antioxidant capacity (TAC) and concentration of malondialdehyde (MDA), main product of lipids peroxidation, have proved antioxidant properties of bergapten administered acutely as well as subchronically in the whole brain, but also in its single structures: hippocampus and cortex.
In conclusion, the present studies demonstrate that acute and chronic administration of bergapten improved memory acquisition and consolidation in PA test in mice. Furthermore, we may conclude that observed procognitive effects of bergapten may be attributed to antioxidant, and therefore neuroprotective properties of that furocoumarine. Based on the present results we can speculate that bergapten could be an interesting therapeutical option, which will change the course of the disease and improve the quality of life of people with memory deficits. Additionally, antioxidant prophylactics may be beneficial for people with a genetic risk factor for neurodegenerative diseases occurrence.

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P18.2

Lactate: a fuel for aging brain?

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Over the last years growing body of evidence has accumulated that glycogen metabolism in astrocytes modulates synaptic plasticity and its abnormality is related to various pathologies. It was also shown, that lactate may play a role of novel signaling molecule in the brain. Results presented here reveals that L-lactate, derived from astrocytic glycogen and from extracellular source, differently regulates plasticity in young (one month) and old (24 month) rats.
As a model we used the CA3-CA1 pathway of glutamatergic transmission in hippocampal slices. We recorded the field excitatory postsynaptic potentials (fEPSP) in artificial cerebro-spinal fluid (aCSF) containing different glucose or lactate concentration and in the presence/absence of BAY-U6751 (glycogen phosphorylase inhibitor, BAY). We analyzed fEPSP to characterize: basal synaptic transmission; input output curves; paired pulse facilitation ratio; long-term potentiation (LTP) evoked by high frequency stimulation (HFS, 4x100 Hz) and measured for 90'.
According to literature [1], astrocytes-derived or extracellular lactate is crucial for LTP induction in young animals and increases the kinetics of fEPSP decay during HFS, but only in the presence of glucose. Nevertheless, lactate as a sole energetic source is not sufficient to support neuronal plasticity in one month rats.
In contrast to young animals, our results showed that LTP induction in hippocampal slices of aged rats was higher when glycogen phosphorylase activity was inhibited. When samples where incubated in aCSF containing glucose, glucose and lactate and only lactate, the fEPSP induction at 90' after HFS in the absence/presence of BAY was respectively: 18%/43%, 2%/25% and 52%/89%. The highest LTP potentiation was reported for HFS slices in the presence of BAY and lactate as the only energetic substrate. In such conditions an average long lasting fEPSP potentiation was significantly higher than LTP evoked in glucose-only medium (p0.05 from 10' to 90' post HFS). The changes in electrophysiological properties are associated both with morphological [2] and proteomic rearrangements.
Based on this results, we hypothesize that inhibition of glycogen and glucose metabolism in astrocytes can be used to stimulate neuronal plasticity in old animals and humans to attenuate aged-related impairment in memory and cognitive function.

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P18.3

Characterization of subcellular localization signals of neuronal PAS domain-containing protein (NPAS4)

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Homeostasis of neuronal networks is very important for normal behavior and cognition. For learning and memory pivotal is experience-dependent synaptic plasticity. The nervous system adapts to experience by inducing a transcriptional program that controls important aspects of synaptic plasticity, especially important on early stages of life when sensory experience is necessary for the development of cortical circuits. The main player of this regulation is neuronal PAS domain-containing protein (NPAS4)identified as brain–specific transcription factor which may serve a neuroprotective function. NPAS4 belongs to the family of bHLH-PAS (basic helix-loop-helix-Per-Arnt-Sim) transcription factors, critical regulators of gene expression networks. Functional activity of the bHLH-PAS proteins is often correlated with shuttling between nucleus and cytoplasm in accordance to the masking or unmasking subcellular localization signals by interacting partners as answer for environmental condition. Until now, no detailed research about the NPAS4 intracellular shuttling and cellular localization signals were performed. We present thorough analysis of distribution of nuclear localization signals (NLSs) and nuclear export signals (NESs) in NPAS4. In order to determine the sequences of NLSs and NESs in NPAS4, we prepared series of deletion and point mutants tagged by yellow fluorescence protein (YFP), expressed in COS-7 and HEK293T cells. Localization of expressed fusion proteins was observed with confocal microscopy system. Additionally we analyzed Leptomycin B effect on localization of full length NPAS4 and some of its deletion mutants. We found that NPAS4 possess multiple NLSs and NESs signals located mainly in N-terminal half of protein encompassing bHLH and PAS-domains. However, one NLS and one NES are located in NPAS4 C-terminal fragment. Interestingly, NLS and NES signals in NPAS4 are overlapping. We conclude that NPAS4 is a cytoplasm-nucleus shuttling protein with a complicated system which regulates final localization of this protein, enabling highly precise regulation of its activity.

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P18.4

HMG-CoA reductase inhibitor (pravastatin) improves cells' viability but not changes MMP-9 activity under chemical ischemia in GMK cell line model

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Ischemia is accompanies of a number of pathological conditions such as stroke or heart attack. The lack of oxygen and glucose initiates a series of biochemical processes, that changes the metabolism of the cells. The effect of which may be the increase of matrix metalloproteinases (MMPs) activity through their overexpression and secretion to extracellular fluid. Among the MMPs, the activity of MMP-9 grows in the early stages of many diseases, including inflammatory and ischemic events.

HMG-CoA inhibitors (statins) show pleiotropic effects beyond the cholesterol-lowering properties. By the blocking of the mevalonate synthesis, statins decrease the prenylation of selected proteins resulting in anti-inflammatory activity. *In vitro* studies revealed the extracellular, positive effect of water-soluble statin, pravastatin, on MMP-2 activity. The aim of current study was to analyze the effect of pravastatin on cells' viability and gelatinases secretion (MMP-2 and MMP-9) under chemical ischemic conditions. As a cell line model, GMK cells were used. GMK cells were maintained in Eagle's medium supplemented with fetal bovine serum and with antibiotics. 24 hours before experiment, cells were transferred into the serum replacement media, free of gelatinases. Next, the cells were divided into the 4 groups: 1) control 2) pravastatin group (5μg/ml) 3) group with ischemic conditions (induced by sodium azide (5 mM) and 2-deoxy-glucose (2 mM)) 4) chemical ischemia + pravastatin group. After 3 hours of ischemic conditions the cells and medium was transferred for the further analysis. Cellular viability of the cells was measured by MTT assay. Gelatinase activity in medium was measured with zymography.

Cells' viability was decreased under ischemic conditions (group 3). The incubation of ischemic cells with pravastatin (group 4) significantly improves viability to the values noticed in group 1 and 2. The media collected from studied groups showed two clear bands; ~110 kDa and ~90 kDa corresponding to MMP-9 forms. However, there was not significant difference between the MMP-9 activity evaluated in studied groups. The band corresponding to MMP-2 was not noticed.

The experiment indicated that pravastatin possesses the protective properties on GMK cells' viability under chemical ischemic conditions. However, any early influence of chemical ischemia or pravastatin on MMP-9 activity was not noticed in assumed cell model.

P18.5

The role of adaptor complex AP2 in the formation of dendritic arbors of hippocampal neurons

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The proper functioning of neurons is defined, among others, by dendritic branching or receptor composition within synapses. Previously, we identified b-adaptin as a modifier of dendritic tree. b-adaptin is a key component of AP2 complex, indispensable for clathrin-mediated endocytosis by which for instance AMPA receptors are believed to be internalized. Thus, the aim of this study was to find how AP2 complex contributes to shaping of dendritic arbors of developing hippocampal neurons.

To study role of AP2 complex in dendritic arborization we used primary hippocampal neurons expressing b-adaptin shRNA alone or in combination of functional rescue constructs (i.a. GluA2). We also tested b-adaptin knockdown *in vivo* by stereotactic injections of lentiviral vectors into the hippocampus of newborn rats. Moreover, GluA2 trafficking was assessed *via* internalization assay and GluA2 degradation and synthesis were investigated by e.g. cycloheximide treatment or qPCR. Currently, we are investigating the mTOR-dependent GluA2 synthesis upon b-adaptin knockdown.

We showed that knockdown of b-subunit led to a reduction in number of dendrites of hippocampal neurons *in vitro* and *in vivo* and this effect can be rescued by overexpression of functional GluA2 subunit. The knockdown of AP2 also led to decreased level of GluA2, what is probably a result of impaired mTOR-dependent GluA2 synthesis.

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P18.6

Different pattern of gelatinolytic activity in pituitary macro- and microadenomas

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Gelatinases (MMP-2 and MMP-9) are involved in several diseases including different types of tumors. Both enzymes are capable to damage extracellular proteins leading to the facilitation of tumor growth and invasion. Although both enzymes are studied in numerous aggressive cancers, the literature about their role in the pathogenesis of pituitary adenomas are scant. Our objective was to analyse MMP-2 and MMP-9 activities in serum and tumor tissue of pituitary adenoma patients.
Eighteen patients with pituitary macroadenomas and three with microadenomas, qualified to the endoscopic resection of tumors were enrolled into the study. Serum samples were obtained from venous blood before the surgery and stored at -30°C. The pituitary adenoma tissue was collected during the surgery and immediately frozen at -30°C. MMP-2 and MMP-9 analysis in both study materials were performed with gelatin zymography. Before analysis, serum samples were diluted 1:50 with deionized water. Tissue material was homogenized, centrifuged and next the protein content of supernatant was set to18 ug/sample. Protein concentration in the supernatant was evaluated with Bradford assay.
The zymography of serum revealed the presence of several bands of gelatinolytic activities corresponding to the latent form of MMP-2 (pro-MMP-2, 70 kDa) and MMP-9 (pro-MMP-9 90 kDa) as well as MMP-9/lipocalin heterodimer (~130 kDa). The proteolytically activated form of MMP were not observed in the analyzed sera. Activities of the observed forms did not differ between sera collected from patients with micro- and macroadenomas. The zymographic analysis of supernatant obtained from tissue material revealed comparable to serum pattern of gelatinolytic activities. However, the intensity of observed bands differed between micro- and macroadenomas. The analysis of material obtained from microadenomas showed lower activities of both forms of MMP-9 simultaneously with the increased activity of pro-MMP-2 as compared to macroadenomas. Furthermore, only in the case of microadenomas the presence of the active form of MMP-2 (molecular weight 65 kDa) was observed.

Acknowledgements:

The study indicated on different role of MMP-2 and MMP-9 in the development of pituitary macro- and microadenomas.

P18.7

mTORC1 regulates early endosome maturation and trafficking

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Early endosomes, marked by the small GTPase Rab5 and its effector EEA1, carry certain cargo internalized at the cell surface either for recycling via recycling endosomes, or for degradation in late endosomes/lysosomes, marked by Rab11 and Rab7, respectively. The lysosome is the main signaling platform for mTOR kinase complex1 (mTORC1), to which mTORC1 is translocated upon the addition of amino acids. mTORC1 integrates the permissive level of amino acid availability with growth factor signaling, subsequently controlling several biosynthetic pathways e.g. protein and lipid synthesis.
The aim of this study was to determine whether the activity of mTORC1 can also reciprocally regulate intracellular membrane trafficking. As a first step, potential interaction of mTOR with Rab5 was investigated in HEK293 and Rat2 cells with use of immunoprecipitation, pull-down, immunofluorescence colocalization and proximity ligation assay techniques. Immunofluorescence analysis was also performed in cultured rat hippocampal neurons. All of these approaches revealed that mTOR coexists in a protein complex with Rab5. Further investigation revealed that N-terminal part of mTOR and intact structure of mTORC1 are needed for this interaction. On the other hand, neither mTOR nor Rab5 GTPase activities were required for mTOR-Rab5 interaction to occur. Yet functional analysis revealed that inhibition of mTORC1 activity with rapamycin increased the number of enlarged early endosomes, marked by Rab5 and EEA1, as well as colocalization between Rab5 and EEA1 and between mTOR and EEA1.
The results obtained during this study suggest that mTORC1 activity is needed for maintaining the proper morphology and flow of early endosomes. mTORC1 inhibition leads to the formation of enlarged early endosomes, marked by Rab5 and EEA1, which implies a role of mTOR in either controlling the early endosome fusion or in the process of early to late endosome conversion.

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P18.8

Effect of manganese and glutamine intake on antioxidant status and neurotransmitter amino acids levels in the brain of rats

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The biochemical mechanism of Mn-induced neurotoxicity due to excessive Mn exposure is still not fully elucidated. However, a potential mechanism for this process is manganese-induced oxidative stress, thereby leading to excessive production of reactive oxygen species (ROS). Furthermore the manganese-induced ROS overproduction could affect the brain glucose metabolism. Mn can easily inhibit GS activity – one of the astrocytic-specific enzyme. The consequence of this process could be the enhanced biosynthesis of neurotransmitters such as glutamate (Glu), aspartate (Asp) and γ-amino butyric acid (GABA). Glutamine (Gln) is the two-thirds amino acid in the brain. It plays an important role as a precursor of the neurotransmitter amino acids, including the excitatory Glu and Asp, and the inhibitory GABA and glycine (Gly). Some researchers suggest that Gln metabolites can prevent against hydroxyl-radical apoptosis in animal erythrocytes.
The aim of this study was to investigate whether the long term administration of manganese (alone or in combination with glutamine) in dose and time dependent manner could affect the selected parameters of oxidative-antioxidative status (superoxide dismutase and glutathione peroxidase activities, concentrations of vitamin C and malonic dialdehyde) and concentrations of excitatory (Asp, Glu) and inhibitory amino acids (GABA, Gly) in the brain of rats. The experiments were carried out on 2-months-old albino male rats randomly divided into 6 group : Mn300 and Mn 500 – received solution of $MnCl_2$ to drink (dose 300 and 500 mg/L, respectively**)**, Gln group – solution of glutamine (4 g/L), Mn300-Gln and Mn500-Gln groups – solution of Mn at 300 and 500 mg/L and Gln at 4g/L dose. The control group (C) received deionized water. Half of the animals were euthanized after three and the other half - after six weeks of experiment.
The exposure of rats to Mn in drinking water contributes to diminishing of the antioxidant enzymes activity and the increase in level of lipid peroxidation. Glutamine in the diet admittedly increases SOD and GPx activity, but it is unable to restore the intracellular redox balance. The most significant differences in the examined amino acids levels in comparison to both control and Gln group were observed in the group of rats receiving Mn at 500 mg/L dose alone or with Gln. It seems that Gln is amino acid which could improve antioxidant status and affect the concentrations of the neurotransmitters.

P18.9

Antioxidant properties of antipsychotic drugs used in the treatment of schizophrenia

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The aim of this study was to compare the antioxidant activities of six atypical antipsychotic drugs: clozapine (CLZ), quetiapine, olanzapine (OLA), risperidone, ziprasidone, aripiprazole (ARI), as well as a typical antipsychotic drug, haloperidol. Several tests of antioxidant activity were used: protection of thiol groups against oxidation by peroxynitrite (PN) and 3-morpholinosydnonimine (SIN-1, generator of PN), oxidation of dihydrorhodamine 123 by PN, SIN-1 and hypochlorite (NaOCl), bleaching of fluorescein fluorescence by PN, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH, generator of peroxyl radicals) and NaOCl, radical-scavenging activity with respect to 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical, 2,2-diphenyl-1-picrylhydrazyl free radical and the Ferric Reducing Antioxidant Potential. In most of the tests, OLA showed the highest antioxidant activity, followed by CLZ and in some cases ARI, other compounds being much less active or not active. OLA and CLZ exerted limited toxicity on mouse neuroblastoma Neuro-2A (N2A) cells and protected the cells against the toxic action of SIN-1, AAPH and NaOCl in the physiologically relevant concentration range of these oxidants. Both drugs reduced the PN-induced nitration of intracellular proteins. Given that schizophrenia is associated with oxidative and nitrosative stress, the direct antioxidant activity OLA and CLZ may contribute to the therapeutic action of these compounds.

P18.10

Dietary hyperhomocysteinemia up-regulates mTOR signaling and down-regulates autophagy in the mouse brain

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Elevated plasma homocysteine (Hcy), *i.e.* hyperhomocysteinemia (HHcy) is associated with brain disease in humans including cognitive decline (Oulhaj *et al*., 2010), vascular dementia (Hainsworth *et al*., 2016) and Alzheimer's disease(Seshadri *et al*., 2002). However, molecular basis of brain diseases associated with HHcy are not understood. Prior work from our lab has shown HHcy causes accumulation of *N*-homocysteinylated proteins, which are prone to aggregation (Jakubowski, 1999).
In the present study we examined a hypothesis that HHcy activates the mechanistic target of rapamycin (mTOR) signaling pathway, which inhibits clearance of damaged/misfolded proteins by autophagy, and thus leads to the accumulation of toxic protein aggregates.We induced dietary HHcy in 6 weeks old C57BL/6 female mice by providing 1% methionine (Met) in drinking water for 14 weeks. Urinary Hcy was elevated 14-fold in HHcy mice relative to control animals (mean tHcy=434±157 μM (n=4) and 30.5±23.5 μM (n=4), p=0.002 respectively). We quantified components of mTOR pathway and markers of autophagy in mouse brains by Western blotting. We found that levels of mTOR (0.039±0.004 (n=4) *vs*. 0.095±0.024 (n=3); fold change (FC)=2.47; p=0.005) and PRAS 40 (0,114±0.027 (n=4) *vs*. 0.220±0.023 (n=3); FC=1.94; p=0.0025) were significantly increased while AMPK (0.410±0,078 (n=3) *vs*. 0,223±0,075 (n=4); FC=-1.84; p=0.0237) and its phosphorylated form (0.447±0.075 (n=3) *vs*. 0.235±0.060 (n=4); FC=-1.90; p=0,0083) were decreased. AMPK directly inhibits mTOR and promotes autophagy. Beclin 1 and Atg 7 levels were significantly reduced (0.537±0.130 (n=3) *vs*. 0.310±0.092 (n=3); FC 1.73; p=0.0415 and 0.608±0.104 (n=3) vs. 0.300±0.071 (n=4); FC 2.03; p=0.013, respectively).
These findings show that dietary HHcy leads to up-regulation of mTOR pathway and down-regulation of autophagy and suggest that impaired autophagy can account for the neurotoxicity of HHcy.

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P18.11

Sleep-active neuron specification and sleep induction require FLP-11 neuropeptides to systemically induce sleep

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Sleep is an essential behavioral state that has been found in all animals that have a nervous system. Sleep homeostasis suggests that sleep is vital for animals life, and deprivation of this behavior typically has detrimental consequences. It is induced by conserved sleep-active neurons that express GABA. However, little is known about how sleep neuron function is determined and how sleep neurons change physiology and behavior systemically. Here, we investigated sleep in *Caenorhabditis elegans,* which is induced by the single sleep-active neuron RIS. We identified a gene regulatory system that determines the sleep-inducing cell fate of RIS. We found that the transcription factor LIM-6, which specifies GABAergic function, in parallel determines sleep neuron function through the expression of another transcription factor APTF-1, which specifies the expression of FLP-11 neuropeptides. Surprisingly FLP-11, and not GABA, is the major component that determines the sleep-promoting function of RIS. At sleep onset, RIS strongly activates, releases FLP-11, and induces quiescence through these peptides. Sleep induction through FLP-11 requires multiple receptors, including FRPR-3, NPR-22, and NPR-4, which are expressed in neurons and muscles that are mainly not postsynaptic to RIS. FLP-11 expression can turn wake neurons into sleep neurons suggesting that the major determinant for sleep-promoting neuron fate is not its position in the circuit, but the expression of FLP-11. This suggests a model of how sleep can be induced systemically by the single RIS neuron through FLP-11 release and its local diffusion to multiple effectors.

P18.12

Neuronal regulation of astroglial energy metabolism

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Glucose is the main energy source for the mammalian brain and neurons are the most energy-consuming cell type in the nervous system, however, they aren't the primary cells that take up glucose from blood. This role is accomplished by astrocytes, glial cells. In response to a neuronal signal, astrocytes release lactate, a glucose-derived metabolic substrate for neurons. Previous studies revealed that neurons can regulate expression of metabolic enzymes in astrocytes [1], however, the molecular bases of these changes are still unknown. Our present research is focused on identification of regulatory factors released from neurons and affecting astrocyte metabolism.

To address this issue, we carried out experiments which demonstrate that incubation of rat hippocampal astrocytes in neuronal conditioned medium (NCM) elevates the level of mRNA and the activity of crucial glycolytic enzymes (aldolase, pyruvate kinase) in astrocytes. For aldolase, the increase in mRNA level was observed only for one of the brain-expressed isozymes (ALDOA), although no rise in enzyme activity was noted. In contrast, for pyruvate kinase, a twofold increase in mRNA level was observed for both brain-expressed isoforms (PKM1, PKM2) and these changes translate into significant (10x) growth in enzyme activity. Simultaneously, the increase of ATP level is observed in cultured glial cells. Moreover, our data revealed that factors released from neurons are heat-unstable and differ on molecular mass (under 10 kDa or over 30 kDa).

Taken together, it may be presumed that neuronal modulation of astrocytic metabolism is based on the regulation of level of expression and enzymatic activity of pyruvate kinase, and is mediated by secretion of some molecules with distinct molecular mass.

Reference:

1. Mamczur P *et al* (2015) *Glia* **63**: 328-340.