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MOLECULAR and CELLULAR NEUROSCIENCE

Molecular Control of Neocortical Projections

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Neocortical projection neurons form two major types of connections: cortico-cortical that connect two hemispheres and cortico-fugal that project to subcortical regions of the brain. Satb2 transcription factor is required for proper development of cortico-cortical axons that build corpus callosum. In this study, we show that the migration and axonal specification of late-born neocortical neurons is controlled cell-autonomously by Satb2. We provide evidence that Satb2 mediates these two processes by inducing the expression of GPI anchored protein Sema7A. Restoration of Sema7a in Satb2 deficient neurons rescues defects in both migration and axon specification. Additionally, we provide evidences that Sema7A, which is traditionally considered a ligand, exerts its effect by hetero-dimerization with Sema4D. Sema7A interaction with Sema4D increases serine phosphorylation of the latter and its assymetric distribution in the growing neuritis. Additionally, this interaction attenuates Cdc42 activity.

Sodium Signalling in Astrocytes and Astrocyte Networks

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The sodium concentration of astrocytes is classically viewed as being kept under tight homeostatic control and at a relatively stable level under physiological conditions. Indeed, the inward electrochemical gradient for sodium, generated by the Na^+/K^+ -ATPase, is a prerequisite for their highly negative membrane potential and a central element in energizing membrane transport. As such it is tightly coupled to the homeostasis of other ions and to the reuptake of transmitters such as glutamate. Consequently, it might be expected that sodium ions entering astrocytes are directly expelled again, protecting the cells from a reduction in the sodium driving force and an attenuation of sodium-dependent processes. Recent studies, however, have demonstrated that this picture is far too simplistic.

Research from our and other laboratories has provided compelling evidence that the intracellular sodium concentration in astrocytes undergoes significant changes with neuronal activity. The amplitude and spatial distribution of glial sodium transients depend on the level of activity and the number of activated synapses: it can be local and restricted to single processes, but also include global sodium changes that invade the soma and even neighboring astrocytes. Activity-induced sodium transients are surprisingly long-lasting and show properties that are distinctly different from those of calcium signals. Because sodium is not buffered, sodium transients generated by sodium-dependent glutamate uptake are linearly related to a wide range of neuronal activity. Sodium transients hence represent direct and unbiased intracellular indicators of neuronal glutamatergic activity, which are transmitted to the astrocyte network.

From these studies, it emerges that sodium homeostasis and signaling of astrocytes are two sides of the same coin: sodium-dependent transporters, primarily known for their role in ion regulation and homeostasis, also generate relevant ion signals during neuronal activity. The functional consequences of activity-related sodium transients are manifold and are just coming into view, enabling new insights into astrocyte function and neuron-glia interaction in the brain. The talk will highlight current knowledge about the mechanisms that contribute to sodium homeostasis in astrocytes, present recent data on the spatial and temporal properties of glial sodium signals and discuss their functional consequences under physiological and pathophysiological conditions.

Neuronal Circuits in the Hindbrain that Control Breathing and Vocalization

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Vocalization in young mice is an innate response to isolation or mechanical stimulation. Neuronal circuits that control vocalization and breathing overlap and rely on motor neurons that innervate laryngeal and expiratory muscles, but the brain center that coordinates these motor neurons has not been identified. I will present recent work from the laboratory demonstrating that the hindbrain nucleus tractus solitarius (NTS) is essential for vocalization and active expieration in mice. By generating genetically modified mice that specifically lack excitatory NTS neurons, we showed that they are both mute and unable to produce the expiratory drive required for vocalization. We observed that neurons of the NTS directly connect to and entrain the activity of spinal (L1) and nucleus ambiguus motor pools that control active expiration and laryngeal tension, respectively. In addition, I will present data on a frameshift mutation (FS) in the human/mouse LBX1/Lbx1 gene that alters the C-terminus of LBX1 without disturbing its DNA-binding domain. The mutation causes severe hypoventilation in humans and in mice. Interestingly, the mutant Lbx1FS protein functions correctly in most contexts but not during development of small neuronal subpopulations in anterior rhombomeres of the hindbrain. The affected neurons are essential for breathing. Thus, our work revealed an unusual pathomechanism where a mutation selectively interferes with a small subset of the functions of a transcription factor and its ability to correctly cooperate with other factors during neuronal development.

Synaptic Dysfunction is a Likely Cause of Cognitive Impairment in Ciliopathy, Bardet-Biedl Syndrome

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Bardet-Biedl syndrome (BBS), a rare genetic disorder characterised by early-onset retinal degeneration, obesity, renal dysfunction and cognitive impairment, was one of the first human multi-system conditions recognised to be caused by dysfunctional primary cilia 1. The majority of individuals with BBS experience cognitive disabilities ranging from mild impairment to severe mental retardation 2-5. The frequency of neuro-psychiatric disorders and autism in the BBS group also exceeds the prevalence rate of these disorders in the general population4. Although there are extensive data on the role of Bbs proteins in the regulation of primary cilia function, the molecular basis of the cognitive impairment phenotype in BBS remains elusive. Here we show that the BBS cognitive impairment phenotype is probably caused by defects in principal neuron dendritic spine formation and maintenance. We found that loss of Bbs proteins results in significant dendritic spine loss in the dentate gyrus of Bbs5 and Bbs4 knock out and Bbs1 M390R knock in murine models, that loss of spines occurs only postnatally and is spine sub-type dependent. Moreover, we show that dendritic spine deficiency is correlated with impaired IGF-1R signalling and increased autophagy, processes that are vital for the formation and maintenance of dendritic spines 6,7. Finally, our mass spectrometric analysis of synaptosomal fractions demonstrated for the first time that a number of Bbs proteins are expressed in the postsynaptic densities of hippocampal neurons. Based on our data, we propose a model in which Bbs proteins, similar to their BBSome role in primary cilia 8-10, regulate receptor trafficking to the membrane of dendritic spines potentially providing novel interventional targets for several neuropsychiatric disorders.

We have also found that wheel-based exercise partly reverses loss of spines of DG neurons in Bbs mice. On the back of these findings we have initiated a successful clinical trial on the effect of physical exercise on hippocampal volume (MRI) and learning in BBS children age 7-15. We found that an exercise session once per week for 6 months resulted in significantly increased hippocampal volume (Voxel-based morphometry analysis) and increased hippocampal blood flow (Perfusion MRI scan) in BBS children indicating an enormous effect of aerobic exercise on the bran plasticity.

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Human Midbrain Organoids for *In Vitro* Modeling of Parkinson's Disease

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The progressive degeneration of midbrain dopaminergic neurons (mDANs) is the hallmark of Parkinson's disease (PD). Modeling PD using mDANs differentiated from human induced pluripotent stem cells (iPSCs) is a powerful tool to study disease mechanisms and to identify novel therapeutics. However, the currently available differentiation protocols yields are relatively low with considerable variability, which has been an impediment. Here, we demonstrate that inhibition of BMP and TGFβ signaling in combination with activation of SHH and WNT signaling enables the generation of expandable midbrain floorplate neural progenitor cells (mfNPCs). mfNPCs can be propagated over multiple passages and cryopreserved in large quantities while maintaining a sustainable marker expression of midbrain markers. They can be differentiated into functional mDANs with an efficiency of 60-80 %, making them a valuable resource for disease modeling and drug discovery. To better model the complex structure of the human midbrain, we also applied 3D differentiation to mfNPCs, which yielded into organoids that resemble the complexity of the human midbrain. Those midbrain-specific organoids are composed of multiple cell types, including neurons, astrocytes and oligodendrocytes, secrete dopamine and form functional networks. We show that 3D midbrain organoids can be obtained reproducibly in large numbers of high quality. Importantly, midbrain-specific organoids derived from LRRK2 G2019S iPSCs recapitulate disease relevant phenotypes making them ideal for studying developmental and degenerative processes in 3D. Thus, we provide a robust method to reproducibly generate high quality mDANs in 2D and 3D with an enormous potential for investigating disease mechanisms, drug discovery, and studying developmental processes.

Pharmacological Modulation of Axon Conduction with Microfabricated Culture Device

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Aim

Neurons are interconnected through axons. Conventionally, axon is regarded as a cable for conducting action potential, digital impulse. However, recent study reported that waveform of action potential is modulated during propagation [1], meaning that conducting action potential is analogue signal, but not digital one. After the report, modulation during axonal conduction attracts great interest. We previously fabricated a microtunnel structure on a microelectrode array (MEA) for recording propagating action potential along axons, and found that conduction velocity slowed down during high frequency firing [2]. Here, we aim to improve the previous device to realize selective pharmacological treatment to axons in microtunnels.

Methods

Our culture device is composed of a culture chamber and an MEA, which has three compartments (two neuron culture compartments and a center compartment for pharmacological treatment). Compartments are connected with 30 microtunnels (3 μm wide, 5 μm high). In the center chamber, cell adhesion reagent was patterned as a stripe manner for guiding axon elongation. Two electrodes are aligned in a microtunnel for recording propagating action potential. The culture chamber was fabricated of polydimethylsiloxane using a SU-8 master mold, and aligned on the MEA coated with polyethylene imine. Cortical neurons were dissected from mouse embryos and seeded into the neuron culture compartments. Spontaneous activity was recorded using an MEA recording system previously described [3]. For a proof of concept experiment, various concentrations of tetrodotoxin (TTX) was applied to the center chamber, and conduction delay between two electrodes on a microtunnel was calculated.

Results

Neurons elongated their axons into the microtunnels, and the axons reached to the opposite compartment through the microtunnels by 10 days in vitro (DIV). Axon conduction was detected from 10 DIV. The conduction delay was 1.8 ± 0.5 ms at 20 DIV. Because the interval of two electrodes is 600 um, conduction velocity was 0.26-0.50 m/s, which is consistent with that of unmyelinated axons in a previous study [4]. Axons were then treated with various concentrations of TTX. As a result, the conduction delay significantly increased with more than 5 nM of TTX with a concentration dependent manner. The results suggest that we successfully observed the changes in axon conduction during partial blockade of Na+ channels.

Conclusion

We developed a culture device for recording axon conduction during selective pharmacological treatment to axons. TTX was used for blocking voltage sensitive Na+ channels on axons. As a result, TTX treatment increased conduction delay with a concentration dependent manner, showing that our device is suitable for evaluating changes in axon conduction properties during pharmacological treatment.

Acknowledgements

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Enzymatic Digestion of Hyaluronan-Based Brain Extracellular Matrix (Becm) Can Influence on Animal Behavior via Changes Synaptic AMPA Receptor Composition

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Aim

Synaptic plasticity often leads to alterations in in AMPA receptors (AMPARs) composition. GluA1-containing AMPARs are calcium-permeable so they may cause the appearance of seizure-like activity. We studied if enzymatic digestion of hyaluronan-based bECM in vivo influenced synaptic expression of GluA1-containing AMPARs.

Methods

C57BL/6J mice (pnd17) were used. Mice were divided among three groups: hyaluronidase-treated, control and intact. Hyaluronidase from Streptomyces hyaluroliticus (Sigma) was bilaterally injected into hippocampus (5 U/μl) using stereotaxic method to remove hyaluronan-based extracellular matrix. An equal amount of PBS was injected to control animals. All groups were tested in open field test, passive avoidance, social interaction and audiogenic seizures. Changes in expression of different AMPAR subunits were assessed by Western blot analysis at the time points 2, 48, 72 hours and 9 days after injection.

Results

We showed that enzymatic digestion of hyaluronan-based bECM influence behavior of animals. Hyaluronidase-dependent anxiety, deterioration of the formation of short-term memory and decreased sociability was identified in behavioral tests. 2% of animals had hyaluronidase-induced audiogenic seizures. Significant increase in synaptic GluA1-AMPARs compare to control animals was shown by Western blot analysis at the time point of 72 hours was detected.

Conclusion

Violation in AMPARs composition may lead to behavioral deviation and memory formation failure because of excessive calcium influx through GluA1-AMPARs. Enzymatic digestion of hyaluronan-based bECM leads to upregulation of GluA1-AMPARs in vivo. All the above mentioned results correlate with our previous studies in vitro.

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Adaptation of the Neurons in the Crab Stomatogastric Ganglion to Changes in Extracellular Potassium Concentration

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Neural circuits must be robust to various environmental perturbations. This is especially true for central pattern generators (CPGs) that are active throughout animals' lifetime and produce motor patterns such as breathing, walking, chewing. Stability over a range of perturbations requires multiple intrinsic and synaptic mechanisms that operate at various timescales in order for the neural network to maintain function. Adaptation mechanisms that operate on the intermediate time scale from minutes to a few hours are less well studied. To have a better understanding of regulatory mechanisms underlying robust output of neural circuits and operating on the intermediate timescale, we studied

the response of the pyloric neurons of the Cancer borealis stomatogastric ganglion (STG) to changes in extracellular potassium concentration. The pyloric network provides a good model for the study of neural adaptation because it produces a robust triphasic rhythmic pattern that drives filtering of the food in the crab stomach, which is active in the absence of sensory inputs.

By conducting intracellular electrophysiological recordings of the pyloric neurons in elevated potassium concentration, we observed that a 2.5-fold increase in [K^+] in the extracellular bath solution led to membrane potential depolarization by ~15 mV. This suppressed the activity of the pyloric dilator (PD) neurons for up to an hour followed by recovery of spiking and, in some cases, bursting activity, despite continued perfusion of 2.5x[K^+] saline. At a lower [K^+] neurons were more likely to continue spiking and recover bursting activity more quickly. To understand whether the adaptation to elevated [K^+] is largely synaptic, cell-intrinsic, or both, we isolated the pacemaker kernel consisting of the electrically coupled anterior burster (AB) and two PD neurons, using a chemical synaptic blocker, picrotoxin (PTX). PTX experiments revealed a few phenomena. First, in 2.5x[K^+] + PTX saline PD neurons did not cease activity like in the case of the intact circuitry, but instead switched to tonic spiking with a frequency higher than the burst frequency at baseline conditions. This indicates that the neuron in isolation and the neuron as a part of the intact circuit responds to the same perturbation differently. Second, in PTX, neuronal firing properties changed, gradually becoming more burst-like over the timescale of hours, suggesting that intrinsic cellular mechanisms are involved in restoring neurons' close to baseline activity.

Neuromodulation of circuits has been extensively studied for many years and has been shown to be change neural firing properties and alter effective synaptic strength. Further, many brain disorders, e.g. Parkinson's disease, schizophrenia, are associated with the dysfunction in the modulatory control systems. Thus, in order to rule out the role of neuromodulation in the recovery in response to changes in [K^+], we performed decentralized experiments, in which all the descending neuromodulatory inputs were removed. In decentralized experiments neurons behaved in a very similar way to the case of intact circuitry, i.e. quiescence period followed by the recovery of activity. These experiments further point to the intrinsic nature of the adaptation mechanism. Supported by NIH R35NS097343

Suppression of Incongruous Guidance Signals is Required for Axon Pathfinding

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Cells discriminate between relevant and irrelevant stimuli to control the spatial and temporal pattern of signaling events. This is common during wiring of neural connections, when neurons accommodate the need of rapid switching of axon-turning responses by co-expressing a core set of cell surface receptors that exceed the instructive signals present at each step of pathfinding. Incongruous directional cues must be filtered-out to avoid conflicting guidance instructions that would steer axons off-course. However, the mechanisms underlying this qualitative discrimination process are unclear. From a mouse mutagenesis screen, we identified the ubiquitous Rho GTPase antagonist p190RhoGAP as a critical determinant for discrete axon pathfinding decisions of spinal motor neurons. p190RhoGAP gates Netrin-1/DCC signaling as motor axons leave the CNS to connect to peripheral muscle targets. Rather than relaying signals, p190RhoGAP suppresses axon attraction to Netrin-1 at the spinal cord interface –a spurious guidance cue for motor neurons– independently of the conventional control of Rho GTPases. Thus, a multi-domain intracellular regulator functions as a qualitative filter for contextually-inappropriate signals, that axons would be otherwise competent to detect, to ensure congruency in neuron pathfinding decisions.

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A Closer Look at the Topography of Hippocampal Neural Stem Cells Indicates their Limited Self-Renewal

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Introduction: Hippocampal neural stem cells (NSCs) are maintained in their niche during the lifetime and can renew themselves through a process of symmetric or asymmetric division. The predominant asymmetric mode of division would lead to a limited renewal and gradual attrition of the stem cell pool, whereas symmetric division mode would lead to addition of new stem cells to the stem cell niche. Both modes have been suggested to occur in the subgranular zone of the dentate gyrus . Possible symmetrical divisions of stem cells are usually inferred from the clonal analysis or from the spatial analysis after labeling with thymidine analogs and focusing on closely residing pairs of labeled NSCs, with an assumption that these pairs of cell retain the label from their common precursor. Both types of analysis also include an assumption of random distribution of stem cells and of their dividing subset. However, it is possible that some of the pairs of closely residing cells appear as a result of close positioning of two unrelated dividing stem cells in the niche space. Therefore, to draw conclusions regarding symmetric divisions of the hippocampal stem cells it is crucial to evaluate the possibility of their non-random distribution and/or activation.

Methods: To estimate the frequency of symmetric divisions we took advantage of long-term labeling with BrdU and analyzed the topography of dividing NSCs within all (i.e., both dividing and non-dividing) NSCs in the dentate gyrus of young adult (3 months old) mice. We obtained the xyz positions of all NSCs and of the subset of dividing NSCs by combining a genetic marker (Nestin-driven GFP) and a marker of dividing cells (BrdU). We next determined the distribution of nearest neighbor (NN) distances between cells within the pool of dividing NSCs. We finally compared this distribution of dividing cells with the results of random resampling from a population of all existing NSCs. Further extending this approach, we estimated how random is the result of a continuous age-related disposal of NSCs. To this end, we compared spatial parameters of the stem cell niche of 14 days old and 7 months old mice.

Results and conclusions: Instead of estimating the degree of randomness in the distribution of the total set of stem cells and of its dividing subset, we compared the distributions of these sets to each other. Our data show that the distribution of dividing stem cells in adult hippocampus fits the random model that does not imply symmetric cell divisions. In other words, most of the bias observed in the distribution of labeled cells can be accounted for by the bias in the distribution of the entire set of cells. This suggests that some of the previously reports on close positioned pairs of dividing cells in the clonal or division analysis (implying their symmetric division) are in fact due to unaccounted spatial bias, present in the distribution of the entire set of NSCs. Our estimates indicates that at least 85-90% of divisions may be due to asymmetric divisions. We also found that in subregions of dentate gyrus the age-dependent cell elimination is different from random and is biased towards depletion of cell pairs at small distances. Along with recently reported live-imaging data, our findings support the predominance of the asymmetric mode of the hippocampal stem cell division and thus their inability to support or expand the neurogenic reserve. These constraints might be overcome by discovering new agents and stimuli for controlling the modes of stem cell division in the adult brain.

A Cellular Basis of Human Intelligence

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It is generally assumed that human intelligence relies on efficient processing by neurons in our brain. Although gray matter thickness and activity of temporal and frontal cortical areas correlate with IQ scores, no direct evidence exists that links structural and physiological properties of neurons to human intelligence. Here, we find that high IQ scores and large temporal cortical thickness associate with larger, more complex dendrites of human pyramidal neurons. We show in silico that larger dendritic trees enable pyramidal neurons to track activity of synaptic inputs with higher temporal precision, due to fast action potential kinetics. Indeed, we find that human pyramidal neurons of individuals with higher IQ scores sustain fast action potential kinetics during repeated firing. These findings provide the first evidence that human intelligence is associated with neuronal complexity, action potential kinetics and efficient information transfer from inputs to output within cortical neurons.

Presynaptic KV7 Channel Function in Hippocampal Mossy Fiber Boutons

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Kv7 channels underlie a slowly-activated and non-inactivating voltage-dependent K^+ current. These channels are highly localized to action initial segments of myelinated and unmyelinated axons where they influence action potential threshold and thereby action potential firing. Interestingly, immunohistochemistry shows that the KV7.2 and KV7.3 subunits are localized throughout hippocampal mossy fibers but it is unknown if these channels are present in hippocampal mossy fiber synaptic terminals and if they modulate neurotransmitter release here. By making electrophysiological recordings from mature mossy fiber boutons, we show that the KV7/M- current is present here, is active at rest and regulates the membrane conductance. Interestingly, the KV7 current restricts the input resistance and the number of action potentials elicited by depolarization as well as the amplitude of the afterdepolarization that follows a pre-synaptic action potential. Consequently, these channels decreased the release of the neurotransmitter, glutamate onto CA3 hippocampal pyramidal neurons. Hence, KV7 channels are also present on excitatory synaptic terminals where, by regulating their intrinsic excitability, they limit neurotransmitter release.

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New Synthetic Biology Approaches in Neuroscience: Thermogenetics and Metabolic Engineering

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Synthetic biology is an area of research where engineering principles are applied to biological systems to create living systems (e.g., cells, animals, plants) with desired properties. One of the central principles of synthetic biology is engineered trans-species or even trans-kingdom transfer of molecular blocks from "donor" to "acceptor" organisms to bring new properties to the acceptor organism. Many groundbreaking tools and technologies were developed using this principle such as fluorescent proteins for imaging, optogenetics, and CRISPR/Cas9 genome editing to name a few. In our studies we take advantage of the known selectivity of enzymes from prokaryotic and fungal species for substrates not present in higher eukaryotes. D-amino acids, for example, are present in vertebrates at very low levels and only in luminal compartments. In mammals, amino acids are almost exclusively L-type and the enzymes utilizing amino acids are highly stereospecific. Therefore, when placed in mammalian cells, the enzymes utilizing D-amino acids remain inactive in the absence of the D-amino acid substrate. For example, D-amino acid oxidases (DAO) catalyze deamination of a D-amino acid using oxygen as a second substrate and generates H2O2 as a reaction product. We have successfully utilized the yeast DAO for controlled production of H2O2 in mammalian cells including neurons. Another relevant example is the bacterial D-amino acid dehydrogenase (DAAD), which catalyzes the oxidative deamination of D-amino acid substrates using NADP+ as cofactor to generate 2-keto acids and NADPH. Our data show that when expressed in mammalian cells this enzyme enables control over NADP+/NADPH ratio dependent on administration of D-lysine.

Another synthetic biology approach we develop is thermogenetics. Using combination of heat-sensitive TRP channels from snakes, IR photonics and quantum thermometry we achieve non-invasive and robust neuronal control with the spatio-temporal resolution of classical optogenetics.

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A Role for MFN2 in Astrocyte Perivascular Repair Following Brain Injury

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Accumulating evidence suggests that major changes in the metabolic profile of astrocytes underlie their responsiveness to brain injury and disease, however which cellular compartments contribute to these changes is unclear. Here, we report that astrocyte perivascular end-feet, which are hotspots of Ca^{2+}-mediated signaling pathways co-regulating vascular tone and metabolic coupling, are characterized by a dense meshwork of elongated mitochondria and ER tubules forming extensive contact sites in vivo. Following acute brain injury however, these two organelles undergo a marked remodeling along with the emergence of typical hallmarks of astrocytic reactivity. On the one hand, this remodeling entails a net redistribution of ER membranes from peripheral processes towards end-feet. On the other hand, injury induces a time-dependent fragmentation of the mitochondrial network which is then re-established by three-to-four weeks, suggesting mitochondrial fusion dynamics to be critical during tissue recovery. Conditional deletion of Mfn2, which regulates mitochondrial fusion, prevents network re-establishment following injury, and leads to ultrastructural alterations in both mitochondrial and ER tubules at perivascular processes. Physiologically, two-photon Ca^{2+} imaging of Mfn2-deleted astrocytes expressing either mitochondrial or cytosolic GCamp6f in situ revealed significant alterations with respects to their Ca^{2+} buffering capacity, which were ultimately mirrored by a reduced frequency of spontaneous cytosolic events within end-feet. These results were corroborated by proteomic analysis of astrocytes isolated from adult mouse cortices, which showed a downregulation of Ca^{2+} signaling and other central signaling cascades. At the tissue level, selective ablation of Mfn2 (but not Mfn1) in reactive astrocytes hindered the recovery of injured cortices by preventing complete revascularization, indicating a key role for Mfn2 during perivascular repair (funding: ERC-StG-2015, grant number 67844).

Organization and Function of Cortical Microcircuits in Mouse and Human Brain

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A variety of inhibitory pathways encompassing different interneuron types shape activity of neocortical pyramidal neurons. While Basket cells (BCs) mediate fast lateral inhibition between pyramidal neurons, Somatostatin-positive Martinotti cells (MCs) mediate a delayed form of lateral inhibition. VIP interneurons preferentially inhibit other interneuron types, thereby disinhibiting pyramidal neurons. These circuits are under control of acetylcholine released from basal forebrain (BF) inputs, which is crucial for cortical function and cognition. Within cortical circuits, a sub-class of VIP interneurons can release acetylcholine as well, but how it affects cortical microcircuits is not known. I will discuss the role of inhibitory motifs in cortical microcircuits, their regulation by acetylcholine and what the role of local cholinergic modulation is in these circuits as well as in cognitive behavior. I will also discuss our recent experimental findings on how these mechanisms operate in human neocortical circuits.

Deficient WNT Signalling Triggers Synaptic Plasticity Defects and Synapse Degeneration: Implications in Alzheimer's Disease

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The structural and functional integrity of synapses is essential for brain function. In many neurodegenerative diseases, synaptic dysfunction and loss are early events before cell death is apparent. In Alzheimer Disease (AD), synapse loss strongly correlates with cognitive decline. A number of studies have demonstrated a pivotal role of Amyloid-ß (Aß) oligomers on synapse toxicity. However, the molecular mechanisms by which Aß oligomers promote synapse vulnerability remain poorly understood.

Increasing evidence suggests that deficiency in Wnt signalling plays a role in AD. Indeed, a potent and specific Wnt antagonist called Dickkopf-1 (Dkk1) is elevated in the human AD brain and in the brain of AD mouse models. We recently showed that Dkk1 expression is rapidly elevated by Aβ. Importantly, we have shown that Dkk1 is required for Aβ-mediated synapse degeneration. To address the role of Dkk1 in synapse vulnerability, we generated an inducible transgenic mouse that expresses Dkk1 in the adult hippocampus. These mice exhibit a significant synapse loss, long-term plasticity defects and memory defects as observed in AD mouse models that expressing Aß. Importantly, we demonstrated that reactivation of the Wnt signalling pathway leads to the full restoration of synapse number, synaptic plasticity and memory function.

It has been proposed that synapse degeneration in AD is triggered by early deficits in NMDAR-mediated synaptic plasticity. To begin to address how deficiency in Wnt signalling triggers synapse loss, we explore the role of Wnt in synaptic plasticity. We found that acute blockade of Wnts or loss of postsynaptic Frizzled-7 (Fz7) receptors impaired LTP-mediated synaptic strength, spine growth and AMPAR localisation to synapses. We also demonstrated that Wnt7a through Fz7 activates key plasticity pathways that regulate the synaptic localization of AMPARs. Our studies identify a critical role for Wnt-Fz7 signalling in LTP-mediated structural plasticity and AMPAR mobilization. Recently we found that Fz7 is down-regulated in AD as synapse degenerate. I will discuss how deficient Wnt signalling could contribute to synaptic plasticity defects resulting in synapse degeneration in AD.

OM&P

Diffraction-Unlimited Optical Imaging for Synaptic Physiology

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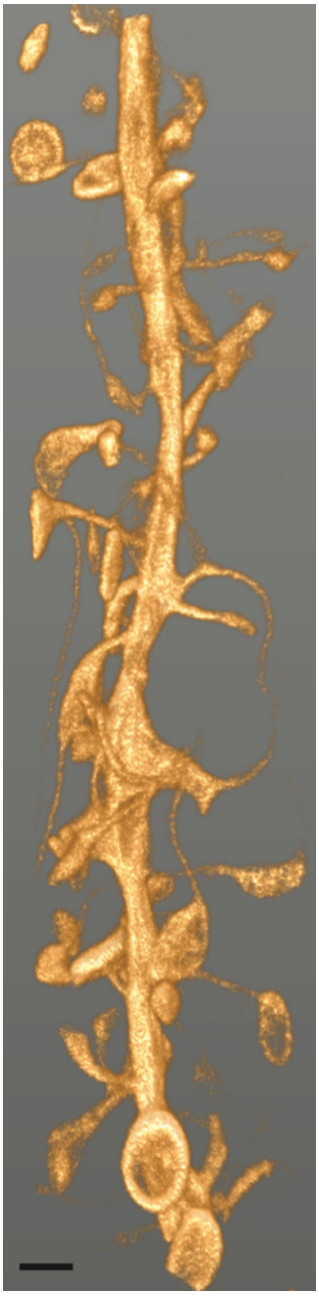

The human brain contains about 8x1010 neurons and trillions of synapses. The properties of individual neurons, their wiring, and the characteristics and plasticity of their connections are key determinants of brain function and such fundamental processes as memory formation.

Light microscopy is a powerful tool to study the nervous system, as it combines the ability to mark specific molecules with live-imaging capability, allowing to follow the dynamic response to specific stimuli, e.g. via Ca^{2+} imaging.

One major drawback of conventional light microscopy for analysing molecular arrangements in synapses or accurately counting the number of dendritic spines is its coarse spatial resolution, limited by diffraction of light waves to about half the wavelength of light or 200 nm. Far-field super-resolution microscopy or nanoscopy techniques "super-resolve" features residing closer than the diffraction-limit by transiently preparing fluorophores in distinguishable (typically on- and off-) states and reading them out sequentially.

I will discuss a specific example of how to improve the so-called coordinate-targeted super-resolution modalities, enabling e.g. to decode the detailed structure of living neurons in brain tissue with 3D diffraction-unlimited resolution, with a method that we dubbed "protected STED". This is exemplified by the 3D rendering of a stretch of a dendrite in a living hippocampal brain slice in the figure to the left (scale bar: 1 μm, taken from Ref. 1). Here, actin is labelled and peculiar ring-like structures are seen in the spine heads.

I will furthermore discuss progress of my recently founded group towards decoding the molecular architecture of single synapses via highly multiplexed super-resolution approaches.

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Human Brain Organoids on a Chip to Study Development and Disease

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Human brain folding has been implicated in neurodevelopmental disorders and yet its origins remain unknown. Here, we report the appearance of surface folds during the in vitro development and self-organization of human brain organoids in a micro-fabricated compartment that supports in situ imaging over a timescale of weeks. We observe the emergence of convolutions at a critical cell density and maximal nuclear strain, which are indicative of a mechanical instability. We identify two opposing forces contributing to differential growth: cytoskeletal contraction at the organoid core and cell-cycle-dependent nuclear expansion at the organoid perimeter. The wrinkling wavelength exhibits linear scaling with tissue thickness, consistent with balanced bending and stretching energies. Lissencephalic (smooth brain) organoids display reduced convolutions, modified scaling and a reduced elastic modulus. Although the mechanism here does not include the neuronal migration seen in in vivo, it models the physics of the folding brain remarkably well. Our on-chip approach offers a means for studying the emergent properties of organoid development, with implications for the embryonic human brain.

Modified GDNF has Stimulated the Neural Differentiation of Progenitor Cells and it May Be Used in the Treatment of Parkinson's Disease and Ischemic Stroke.

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GDNF therapy is effective for treating degeneration of dopaminergic neurons in Parkinson's disease. It is possible that pre-(α)pro-GDNF is needed for conventional neuron survival, and pre-(β) pro-GDNF serves as SOS system during traumatic injury of neurons or neurodegenerative diseases. To study 'pro' region function during fast transport and factor induction properties several versions of modified GDNF were made. Secretion of GDNF into medium has been shown. Then modified GDNF were introduced into HEK293 cells. Condition media after transgenic cell culturing was added into culture medium of rat embrional spinal ganglion explant. Deletion of 'pro' region essentially increases GDNF effects as neural inductor. Study of culture of dissociated spinal ganglion and calculation of neural sprouts yielded the same results. Deletion of both pre- and pro-regions enhances trophic activity of GDNF (mGDNF). Spinal ganglia cultured in the presence of medium conditioned by cells transfected with mGDNF exhibited active growth of β-3-tubulin-positive axons by day 4.

Then we demonstrate neurotrophic effect of mGDNF for PC12 cells in vitro and showed that on this model also the sprouts of beta-3 tubulin positive. To confirm the neuroinduced properties of mGDNF on human cells, we used the neuroblastoma cell line. It was found that the addition of mGDNF to the culture mediumwith neuroblastoma cell significantly increased the number of βIII tubulin positive cells in this culture. And we demonsrated positive effect on dopaminergic neurons in the substantia nigra pars compacta in vivo. A model of Parkinson's disease was used, which was obtained by subcutaneous injection of MPTP into C57Bl / 6 mice. Implantation of cells producing mGDNF in caudateum-putamen smoothed out symptoms of Parkinson's disease in motor activity tests and increased the number of tyrosine hydroxylase immunopositive cells in the substantia nigra pars compacta.

Research is supported by Grant from RNF and Program of the Presidium of the RAS "Fundamental Foundations of Technology of Physiological Adaptations"

The Study of Peculiarities in Morphology and Functional Activity of Primary Hippocampal Cultures Obtained from 5xFAD Mouse Embryos in Alzheimer's Disease Model

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Alzheimer's disease (AD) regarded as one of the most widespread neurodegenerative diseases. The exact cause of AD is still poorly understood. Currently it is known that accumulation of β-amyloid interferes and slows down signal transmission between brain cells. Calcium ions generate versatile intracellular signals that control key functions in all types of neurons. Therefore, calcium imaging will help determine a variety of neurons functions in biological organisms.

The aim of the study was to investigate morphological features and calcium activity of primary hippocampal cultures in Alzheimer's disease model.

Materials and methods: Primary hippocampal cultures was obtain from embryos (E18) of 5xFAD mice carrying wild and mutant genotype (mutant genes: APP and PSEN1). The embryos were preliminarily genotyped before surgery. Preparing and further cultures cultivation was carried out according to the standard protocol. Spontaneous calcium activity of primary hippocampal cultures was investigated on 10, 14 and 21 days of cultures development in vitro (DIV). For imaging studies calcium-sensitive dye Oregon Green 488 and laser scanning microscope LSM 510 (Zeiss, Germany) were used. Temporal series of images were registered for the intracellular calcium concentration assessment.

Results: Carried out experiments revealed no significant morphological differences between cultures obtained from APP and PSEN1 mutant and wild-type mice. Cell viability both types of cultures also did not significantly differ. However, changes in spontaneous calcium activity of investigated cultures were observed. The percentage of cells exhibiting calcium activity in cultures obtained from 5xFAD mice was significantly lower compared to control group during all observation period (10 DIV: "Control" 76±7.06%, "5xFAD" 41±8.63%; 14 DIV: "Control" 88±4.38% and "5xFAD" 60±10.12%, 21 DIV: "Control" 78±10.02%, "5xFAD" 44±7.02%). The frequency of calcium oscillations on these days was also significantly lower in the "5xFAD"group (10 DIV: "Control" 1.5±0.06, "5xFAD" 0.52±0.02 oscillation/min; 21 DIV: "Control" 0.95±0.05, "5xFAD" 0.59±0.02 oscillation/min). On 14 DIV this parameter did not differ between the groups. The duration of calcium oscillations in AD model was significantly increased on 10 and 21 DIV.

Conclusion: Cultures of wild and mutant types did not morphologically differ. However, the functional activity of primary hippocampal cultures obtained from 5xFAD mutant mice was significantly lower compared to cultures obtained from wild type mice.

The work was supported by the Russian Foundation for Basic research (grants No 18-015-00391A), Grant of the President of the Russian Federation MD-2634.2017.4 and also with the support of the state projects №№ 17.3335.2017/4.6 and 6.6379.2017/8.9.

Calcineurin Inhibition Suppresses the Neuronal Potassium-Chloride Cotransporter and Impairs the Neuronal Chloride Homeostasis

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Aims

Calcineurin Inhibitors (CnI) such as cyclosporine A (CsA) are instrumental for immunosuppression after organ transplantation but may cause serious neurologic side effects including seizures. Neuronal excitability depends on Cl- homeostasis maintained by cation-coupled chloride co-transporters (CCC), KCC2 and NKCC1. Since calcineurin is one of the phosphatases regulating CCC members, we tested the hypothesis that CsA impairs KCC2 or NKCC1 functions thereby causing neuronal hyperexcitability and neurotoxicity.

Methods

Effects of calcineurin inhibition on neuronal chloride homeostasis and KCC2 function were analyzed in CsA-treated rats, CsA-treated zebrafish larvae and calcineurin A beta (CnAb)-deficient mice (CnAb-/-) using immuno¬fluorescence, immunoblotting and co-immunoprecipitation, quantitative PCR, and electrophysiological measurements.

Results

In ex vivo experiments, rat pyramidal neocortical neurons responded to CsA (5 μM for 1h) with depolarized the GABAA reversal potential by 7.2 mV, increased the intracellular chloride [Cl-]I by 6.9 mM and prolonged chloride extrusion time by 3.9 s, suggesting reduced KCC2 activity. Binding- and co-localization studies in rodent brain tissue suggested interactions of CnAb with KCC2 and NKCC1. In vivo CsA administration to rats (5-25 mg/kg i.p.) enhanced levels of inhibitory tyrosine KCC2 phosphorylation at short term (+172%) and reduced levels of activating S940-KCC2 phosphorylation at long term (-61%). Phospho-S940-KCC2 levels were also reduced in CnAb-deficient mice (-78%). In contrast, NKCC1 and its activating kinase SPAK were stimulated upon calcineurin inhibition.

Conclusions

Our results provide several lines of evidence for calcineurin-dependent regulation of KCC2 and suggest that CnI attenuate KCC2 function but may stimulate NKCC1. These effects may alter neuronal excitability and contribute to CnI neurotoxicity.

Thalidomide Attenuates Development of Morphine Dependence in Mice by Inhibiting PI3K/Akt and Nitric Oxide Signaling Pathways

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Morphine dependence and the subsequent withdrawal syndrome restrict its clinical use in management of chronic pain. The precise mechanism for the development of dependence is still elusive. Thalidomide is a glutamic acid derivative, recently has been reconsidered for its clinical use due to elucidation of different clinical effects. Phosphoinositide 3-kinase (PI3K) is an intracellular transducer enzyme which activates Akt which in turns increases the level of nitric oxide. It is well established that elevated levels of nitric oxide has a pivotal role in the development of morphine dependence. In the present study, we aimed to explore the effect of thalidomide on the development of morphine dependence targeting PI3K/Akt (PKB) and nitric oxide (NO) pathways. Male NMRI mice and human glioblastoma T98G cell line were used to study the effect of thalidomide on morphine dependence. In both models the consequent effect of thalidomide on PI3K/Akt and/or NO signaling in morphine dependence was determined. Thalidomide alone or in combination with PI3K inhibitor, Akt inhibitor or nitric oxide synthase (NOS) inhibitors significantly reduced naloxone induced withdrawal signs in morphine dependent mice. Also, the levels of nitrite in hippocampus of morphine dependent mice were significantly reduced by thalidomide in compared to vehicle treated morphine dependent mice. In T98G human glioblastoma cells, thalidomide alone or in combination with PI3K and Akt inhibitors significantly reduced iNOS expression in comparison to the morphine treated cells. Also, morphine-induced p-Akt was suppressed when T98G cells were pretreated with thalidomide. Our results suggest that morphine induces Akt, which has a crucial role in the induction of NOS activity, leading to morphine dependence. Moreover, these data indicate that thalidomide attenuates the development of morphine dependence in vivo and in vitro by inhibition of PI3K/Akt and nitric oxide signaling pathways.

OM&P

Adeno-Associated Viral Expression of BDNF in Primary Neuronal Culture

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Brain-derived neurotrophic factor (BDNF) plays a major role in the regulation of neurogenesis, survival and formation of synaptic contacts between neurons in central nervous system. Neurotrophic molecules also play a role in maintaining neurons and their differentiated phenotypes. BDNF has strong neuroprotective properties that allows us to consider it as a promising therapeutic agent that can be used to correct ischemic and neurodegenerative processes in neural tissue.

Aims

The main goal of this research is to create adeno-associated viral vector for expressing of neurotrophic factor BDNF in neuronal cells.

Materials and Methods

In present study, we use the recombinant adeno-associated virus vector (AAV) for delivery of BDNF gene, which have emerged as potent and versatile tools of gene transfer without detected toxicity, low immune reaction, and long-term effect.
We used standard cloning techniques to create adeno-associated viral vector with fragment of mKate gene. AAV-Syn-BDNF-mKate viral vector represents a fusion construct consisted of BDNF gene fused to mKate far-red fluorescent protein (Ex/Em 594/635) which is necessary to visualize the expression. mKates far-red fluorescence allows us to separate the standard green fluorescent labels in dual-color high-throughput assays. The amplification of viral vector was performed in the culture of HEK 293T cells using DJ packaging system (Cell Biolabs). The viruses were purified and concentrated using Amicon Ultra columns centrifugal filter devices (Merck Millipore) to achieve necessary purity of the virus. To assess the efficiency of the construct we infected primary neuronal cultures received from the cortex of the C3H mouse line animals on embryonic day E18.5.

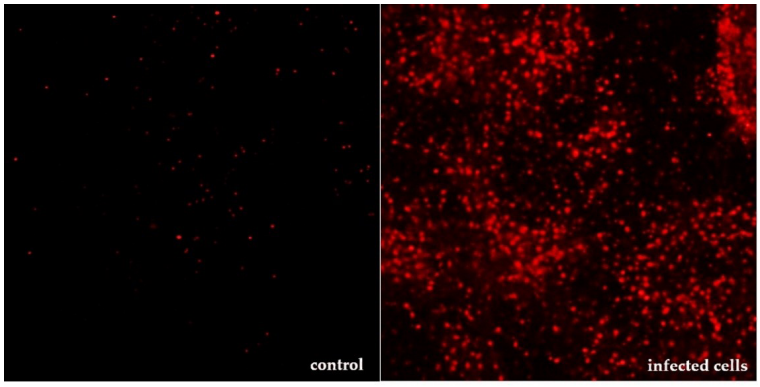

Fig. 1. *Immunocytochemistry of primary neuronal culture infected by AAV-Syn-BDNF-mKate virus. Images were obtained using a Zeiss LSM510 confocal laser-scanning microscope (Zeiss, Germany). Red channel – Alexa 647 (Ex/Em 650/665)*

Results

The cultures were infected on 10 DIV of culture development in vitro. Strong adeno-associated expression of neurotrophic factor BDNF was proved by immunocytochemistry on 6 day after transduction (Fig. 1).

Conclusions

To sum up it was created the adeno-associated viral vector contained the fragment of BDNF and mKate genes and it was tested on primary neuronal culture.

Investigation of the Oxygen Uptake by Brain Mitochondria during Chronic Prenatal Hypoxia in Mice

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Prenatal hypoxia as one of factor responsible for damage brain cells and neurodegenerative diseases development requires to additional study. Prenatal hypoxia modeling in mice leads in disruption of placental blood supply. This leads to disruption of central nervous system and respiration, particularly disruption of mitochondrial respiration. As a result, it changes the blood pH to acidic site, development of metabolic acidosis and decreased enzymes activity. Currently, understanding of the brain adaptation mechanisms during hypoxia in early ontogenesis investigated insufficiently. This causes problems in diagnosis and treatment the prenatal hypoxia effects. Thus, our research goal was to study the metabolic state of mitochondria during chronic prenatal hypoxia.

In this work was develop a technique modeling of chronic prenatal hypoxia. From 14th day old, pregnant female C57Bl/6 mice were place in a hypobaric pressure chamber (350-370 mm Hg) for 2 hours for modeling chronic prenatal hypoxia. For measuring the oxygen uptake rate by brain mitochondria, we used C57Bl/6 mice 1 days old. Measuring of the brain mitochondria respiration performed by estimating the oxygen uptake rate. The oxygen uptake rate by mitochondria recorded with a high-resolution respirometer Oxygraph-2k (Oroboros, Austria). It was shown that the basal rate oxygen uptake during oxidizing the substrates of glutamate and malate in the control group mice significantly increases by 45% and is 4053.875 ± 582.1 pmol / (s*ml), 1 mg of protein in comparison with the intact group. During the first complex of the respiratory circuts of ADP stimulating the parameters in the control group were 16016.375 ± 1196.9 pmol / (s*ml), which is 45% higher than in the intact group. Also was note activation of an alternative succinate-dependent respiratory tract. The parameters in the control group were 10,907.55 ± 884.6 pmol / (s*ml), 1 mg protein, which is 40% higher than in the intact group.

Thus, our modified method of chronic prenatal hypoxia leads to significant changes in the mitochondria metabolic activity. Potential neuroprotective effects has endogenous regulatory molecules, which can regulate the adaptation mechanisms of mitochondrial apparatus. This will be the subject of further research.

The study was supported by Russian Science Foundation (project No. 17-75-10149)

Brain Extracellular Matrix Failure Dependent Changes in Gene Expression in Mice Hippocampus in the Neonatal Period of Ontogenesis

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Aims

The extracellular matrix (ECM) plays an important role in regulating use-dependent synaptic plasticity. Distinct aggregates of ECM molecules surround cell bodies and proximal dendrites of some central neurons, forming so-called perineuronal nets (PNNs) [1, 2]. Preliminary data showed that the destruction of the extracellular matrix in the primary cell culture of mice hippocampus on the 17th day of development in vitro causes long-term changes in the bioelectric activity of cells characteristic of epileptic-like activity [3]. Using the methods of transcriptome analysis of gene expression and subsequent bioinformatics analysis, genes were found, the expression of which depended on the integrity of the molecular network of the extracellular matrix. However, the primary cell culture of the hippocampus is only a simplified model of neural networks of the brain, so for the correct interpretation of the data obtained from the primary culture, it is necessary to compare with in vivo samples that most fully reflect the properties of the

whole brain [4]. The purpose of this study is the transcriptome profile of C57BL/6 mice hippocampal tissue treated with hyaluronidase in early postnatal development (P17), as well as bioinformatic analysis and identification of signal pathways among a variety of molecular events in relation to mice behavior.

Methods

C57BL/6J mice were used to intra-hippocampal hyaluronidase injection. Hyaluronic acid, which is the basis of extracellular matrix, has been removed by 75U/ml hyaluronidase from Streptomyces hyaluroliticus (Sigma) by bilaterally injected into hippocampus (5 U/μl) using stereotaxic method. An equal amount of PBS was injected to control animals. Recognition memory was evaluated using the Object Recognition Test (ORT) in mice on next day after hyaluronidase injection and through 9 days. Memory was operationally defined by the discrimination ratio for the novel object (DIR), as the proportion of time the animals spent investigating the novel object minus the proportion spent investigating the familiar one during the testing period in Social Box (Panlab). «Open field» test, passive avoidance with using Shuttle-box (Panlab), and audiogenic seizures.

Transcriptomic analysis was performed by mouse full-genome 2-colour Agilent Microarrays. Normalization, processing and detection of differentially-expressed genes were performed by online free software – Babelomics 5.0 [2]. Functional annotation clustering was made by David bioinformatics resource [3].

Results

Based on transcriptome analysis, 1545 differentially expressed (DE) genes were detected. The data showed that hyaluronidase-dependent degradation of brain ECM resulted in significant changes of expression of TNF, ICAM, CREB1 и FGF2 against the background of other genes related to inflammatory nonspecific response of tissue cells to traumatic injury during injection into the hippocampus or lateral ventricle of the brain such as genes of the signal cascade of axonal growth; neuroinflammatory signal cascade; signal pathway associated with the efrin receptor; remodeling of epithelial adhesion contacts, etc.

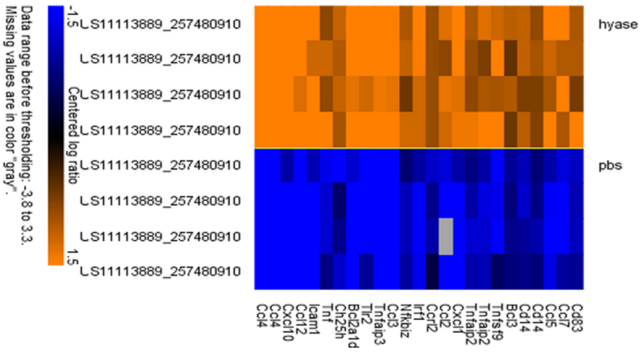

Fig. 1. *Heat map showing the degree of change in gene expression in the experiment (Hyase) and control (pbs).*

The hyaluronidase-dependent anxiety, reduction of the formation of short-term memory and decreased sociability were identified in behavioral tests, and 6% of animals had hyaluronidase-induced audiogenic seizures.

Conclusions

Hyaluronic acid digestion in mice hippocampus in the neonatal period of ontogenesis increases inflammatory reactivity of hippocampal tissue and caused changes in behavior due to the expression of important pro-inflammatory factors such as TNF, ect.

Acknowledgements

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Integrated Information in Coupled Genetic Repressilators

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Intercellular communication and its coordination allow to cells to exhibit multistable dynamics as a mechanism of adaptation. This conveys information processing from intracellular signaling networks enabling self-organizing between cells. How information is integrated in a functional manner and its relationship with the different cell fates is still unclear. In parallel, drawn originally from consciousness studies, integrated information, proposes an approach to quantify the balance between integration and differentiation in the causal dynamics among the elements in any interacting system. In this work, such an approach is considered to study the information flow in a genetic network of repressilators coupled by quorum sensing. Several attractors under different conditions are identified and related with integrated information to have an insight into the collective interaction and functional differentiation in cells. This particularly accounts to the open question about the coding and information transmission in living systems.

Activation of Heterodimeric Receptor to Erythropoietin with its Agonist CDEPO Regulates Brain Mitochondrial Bioenergetics after Local Acute Ischemia/Reperfusion in C57BL/6 Mice

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Ischemic lesions remain to be one of the main causes of physical disability and mortality worldwide. Furthermore, stroke is known to be followed by mitochondrial dysfunction and impaired cell respiration. Mounting evidence demonstrates that the cytokine hormone erythropoietin (EPO) is capable of activating signaling pathways that increase the brain's resistance to ischemia/reperfusion stress. After the discovery of EPO's heteroreceptor that promotes tissue protection [1], a number of attempts were made to develop non-hematopoietic EPO's derivatives, including CdEPO. However, the precise mechanisms implicated into protective CdEPO effect, notably on brain mitochondria, are still to be elucidated.

Aims

The purpose of current research is to elucidate the effect of non-hematopoietic derivative of erythropoietin (CdEPO) on brain mitochondria respiration rate on 4, 10 and 20 day after local acute ischemia/reperfusion in mice.

Methods

Male C57BL/6 mice (2 months old, weighing 18-23 g) were used in the study. Local acute ischemia in mice was induced with transient middle cerebral artery occlusion (tMCAO). Following 6 hours after ischemic exposure a fivefold intravenous CdEPO administration was carried out. In a control group the administration of sodium chloride was performed

in the same conditions. On 4, 10 and 20 day after reperfusion forebrains of animals were dissected to obtain isolated mitochondria. Bioenergetic studies were carried out using high-resolution respirometry (OROBOROS Oxygraph-2k, Oroboros Instruments, Austria). LEAK respiration or LEAK oxygen flux compensating for proton and electron leak, cation cycling, a dissipative component of mitochondrial respiration which is not available for performing biochemical work and therefore related to heat production, was measured in the presence of reducing substrates (glutamate and malate) [2]. OXPHOS capacity expressed in the mitochondrial respiration rate during oxidative phosphorylation (after ADP addition) was measured to study the activity of complex I. Significant difference (at least $p<0.05$) was tested by one-way ANOVA and Holm-Sidak post hoc.

Results

It was revealed that ischemia/reperfusion with tMCAO did not lead to significant alterations in LEAK respiration (Fig. 1 A). Intravenous CdEPO administration following 6 hours after ischemia/reperfusion did not exert any effect on LEAK respiration rate compared to control level. However, on day 20 after reperfusion brain mitochondrial OXPHOS respiration showed a significant decrease by 41% ($p=0.01$) in the control group compared to intact level (Fig. 1 B). Along with that on day 20 OXPHOS respiration rate was increased by 35% ($p=0.027$) in the CdEPO group in comparison with control level.

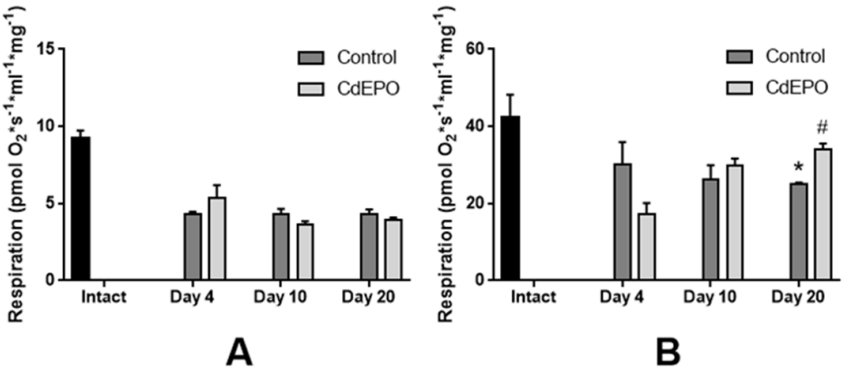

Fig. 1. *Effect of CdEPO on respiration of isolated forebrain mitochondria on 4, 10 and 20 day after local acute ischemia/reperfusion: A – LEAK respiration rate; B – OXPHOS respiration rate.*Significant difference (at least $p<0.05$) with intact level; #Significant difference (at least $p<0.05$) with control level*

Conclusion

The observed effect of CdEPO on forebrain mitochondrial bioenergetics might be implicated in the realization of protective mechanisms, which was induced by EPO's heteroreceptor activation, and resulted in postponed improvement of mitochondrial respiration after ischemia/reperfusion. Other effects of CdEPO on different parameters of mitochondrial bioenergetics require further investigation.

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WM-CLICK, a New Method for 3D Detection, Representation and Analysis of Dividing Cells in the whole Brain

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Recent years are marked by the active development of methods of three-dimensional optical visualization of the whole brain. Efficient imaging, quantification, and analysis carried out in 3D may reveal dynamics and hidden patterns of processes not recognized on conventional flat sections. This holds especially true for the study of neurogenesis both in the developing and adult nervous system where neural stem and progenitor cells divide in restricted regions and migrate along intricate trajectories to reach distant areas of the brain.

We have recently developed a new histological technique for 3D imaging of proliferating cells in the whole brain of developing and adult mice, based on labeling the dividing cells with 5-ethynyl-2'-deoxyuridine (EdU) and detecting them with fluorescent azide using whole-mount click-reaction (WM-CLICK). We also developed novel methods for automatic volume registration, counting and morphing of 3D images.

We applied these techniques, combined with scanning confocal microscopy, light-sheet microscopy and two-photon tomography for visualizing patterns of cell division in the mouse brain. We observed three proliferation/migration streams in the subventricular zone (SVZ) of adult mouse brain – dorsolateral, dorsomedial and ventral branches which merge together into a common rostral migration stream and then traced the dynamics of their formation. We also used WM-CLICK and our new mathematic algorithms to compare the 3D patterns of cell division in control and memantine-treated adult brain. Several areas with strong effects of memantine on cell division were revealed. The higher densities of EdU+cells were found in the CAs and DG regions of hippocampus, subcallosal zone, olfactory bulb, and piriform cortex. We also observed profound changes in the patterns of cell division at the perinatal age in two brain regions – SVZ, where streams are gradually formed during development, and cerebellum, where we observed heterochrony in proliferation intensity in the lateral and medial parts. The resulting data can be presented as pseudo time-lapse movie, thus allowing 4D representation of the dynamics of developmental changes in cell division and neurogenesis.

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Ubiquitin-Proteasome-Dependent Regulation of Cofilin Level in the Nerve Cells

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The ubiquitin-proteasome system is a major intracellular protein degradation system. Ubiquitylation is a post-translational modification of proteins that involves the covalent attachment of ubiquitin. Upon termination, ubiquitin is removed and recycled by deubiquitinases. Ubiquitylation regulates different catabolic and signaling pathways [1]. Cofilin (CFL) is a protein that binds to and forms clusters on actin filaments and it can also sever actin filaments. Actin and CFL were established to be involved in various physiological and pathological processes in the nerve system [2]. CFL can be modified by different types of polyubiquitin chains [3,4]. However, there are little data on the role of ubiquitylation in regulation of CFL level in the cells.

The aim of the present study was to analyze ubiquitin -dependent mechanisms in regulation of CFL level in the nerve cells.

Methods

Hippocampal cells were isolated from freshly dissected embryonic mouse brain of C57BL/6 mice. The cells were treated with 0.5 % DMSO (Sigma), 5 μM MG132 (Abcam) and 5 μM PR-619 (Abcam) for 2 hours. The method of immunocytochemistry was applied for the study of actin structure and cofilin localization in the nerve cells. To investigate CFL intracellular level we applied the methods of immunoprecipitation and western blot. Detection and quantification of band intensities was conducted using Gel Analyzer 2010a software.

Results

Cofilin was detected to be localized in the cytoplasm and nuclei of the nerve cells. In cytoplasm cofilin was co-localized with actin filaments. Western blot with anti-cofilin antibodies showed the presence of monomeric (19 kDa) and middle-weight molecular forms (proteoforms) of cofilin in nerve cells. To detect the type of polyubiquitin chains attached to CFL, cell lysates and CFL immunoprecipitates were probed with anti-K63-linked polyubiquitin chains antibodies. Cofilin proteoforms were revealed to be modified by K63-linked polyubiquitin chains. K63 chains are known to regulate proteasome-mediated degradation as well as proteasome-independent processes [5]. To study ubiquitin-proteasome-dependent regulation of cofilin level, the hippocampal cells were incubated with the inhibitor of proteasome MG132 and the inhibitor of deubiquitylating enzymes (DUBs) PR619. The treatment of the nerve cells with the inhibitors of the ubiquitin-proteasome system led to the changes in the intracellular level of middle-weight molecular forms of cofilin in comparison with intact hippocampal cells. At the same time, both MG132 and PR619 did not significantly influence the actin level in the nerve cells. However, proteasome inhibition led to the disruption in the structure of actin filaments and the decrease in the fluorescence intensity of cofilin. In contrast, the inhibition of deubiquitinases by PR619 did not significantly alter the structure of the actin filaments and the cofilin localization in the nerve cells.

Conclusions

Therefore, the effect of the inhibitors of proteasome and DUBs on CFL level and localization indicates the involvement of the ubiquitin-proteasome system in regulation of cofilin and actin dynamics in the nerve cells. Moreover, cofilin modification by Lys63-linked polyubiquitin chains suggest both catabolic and non-catabolic mechanisms of cofilin regulation. These ubiquitin-dependent mechanisms may play an important role in normal and pathological conditions.

Acknowledgements

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Behavioral Characterization of Two Mouse Lines Resulting from ENU-Induced Mutagenesis

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In the present study, two previously selected lines of mice susceptible to induced seizures by sound stimulation were investigated. The aim of the study was to characterize the basic behavioral features of mutant lines.

Mutant mice were generated during several stages of breeding, that included: injecting of C3H/HeN males (G0) with ENU, obtaining of males of the first generation (G1) from females C3H/HeN, mitigation G1 with C57Bl/6 females, backcrossing of G2 females with male G1, screening littermates G3 to sound susceptibility, selection of mice with sound susceptibility phenotype and crossing of these animals. The progeny G5 and G6 of males, called "S8-3" and "S5-1", demonstrating induced seizures by sound stimulation were selected. Susceptibility to sound-induced seizures was detected at P20-P25 by exposure to sound with a frequency of 110 dB for 60 sec or until the onset of seizures in the custom-made equipment. Spontaneous horizontal and vertical locomotor activities (Open field test), short-term memory (Passive avoidance test), startle response and prepulse inhibition was assessed at the age of 2 months. Tests were performed on the Panlab devices using standard software.

Our results confirm there are mice susceptible to induced seizures by sound stimulation in both lines. The S5-1 mice showed a high learning ability in the passive avoidance test, the intensity of the startle response, and low exploratory activity in the Open Field compared to the control group consisting of intact G3 hybrids. In the S8-3 line, on the contrary, a low learning ability compared to the control and a significantly higher motor and exploratory activity compared with both intact hybrids, and with mice from S5-1 was shown.

What Underlies the Evolutionary Increase in the Proliferative Potential of Cortical Progenitors?

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Evolutionary expansion of the neocortex in the mammalian lineage is thought to underlie the unparalleled cognitive abilities of humans. The size of the neocortex is mainly determined by the production of neurons during cortical development, which in turn is controlled by the proliferative potential of cortical progenitors. In particular, the proliferative capacity of basal progenitors is thought to play a key role for neocortex expansion. Although molecular differences in basal progenitors of various mammalian species have been extensively studied recently, the underlying mechanistic causes of the basal progenitor proliferative potential remain largely unknown.

Here, we propose the new concept that the basal progenitor morphology underlies the basal progenitor proliferative potential. We show that basal progenitors of species with an expanded neocortex, such as human and ferret, exhibit an increased number of processes compared to species with a small neocortex, such as mouse. To be able to genetically manipulate the morphology and subsequently the proliferative potential of basal progenitors, we first established a genome editing technique based on the CRISPR/Cas9 system. We applied this methodology to both in vivo analysis in mouse and ex vivo analysis in fetal human neocortical tissue. In this way, we functionally identified a molecular machinery which regulates the number of basal progenitor processes. Furthermore, we show that a targeted increase in the number of basal progenitor processes in embryonic mouse neocortex results in activation of cell receptors and the subsequent activation of several pro-proliferative signaling pathways, leading to an increased proliferative capacity of these cells.

In conclusion, our study provides a conceptually novel model in which an increased number of basal progenitor processes enables these cells to receive external signals, which in turn activate cell receptors and promote the pro-proliferative response. Thus, we provide evidence that cell morphology regulates proliferative capacity of cortical progenitors, contributing to the evolutionary expansion of the neocortex.

OM&P

Stem Cell Heterogeneity in the Adult Brain

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- Stem cells in the hippocampus of the adult brain produce neurones that have important functions in memory and mood control. Most adult hippocampal stem cells are quiescent while a small fraction proliferate and produce neurones in response to various physiological stimuli or to injury. How stem cells compute the diverse stimuli and downstream niche signals they receive to produce appropriate numbers of adult neurones remains an open question.

- We found that the transcription factor Ascl1 is essential for activation of hippocampal stem cell. We also obtained evidence that Ascl1 expression is controlled by different post-translational mechanisms at different stages in the hippocampal stem cell lineage. Ascl1 is transcribed in most quiescent stem cells but Ascl1 protein accumulation in these cells is suppressed by the transcriptional repressor Id4, via sequestration of Ascl1 dimerisation partner and degradation of monomeric Ascl1. Ascl1 protein is also actively degraded in proliferating hippocampal stem cells, by a different mechanisms involving the E3 ubiquitin ligase Huwe1. We are characterising the niche signals that controlling Ascl1 protein levels via regulation of Id4 and Huwe1.

- Further investigation of Huwe1 function in proliferating hippocampal stem cells has shown that active elimination of the pro-activation factor Ascl1 is essential for a fraction of these cells to return to quiescence. Moreover, examination of Huwe1 function in mice of different ages has revealed that stem cells that have previously proliferated and have returned to quiescence (which we call 'resting stem cells') have a unique role in maintaining homeostatic hippocampal neurogenesis. In contrast, stem cells that have not previously proliferated ('dormant stem cells') have a limited role in homeostatic neurogenesis, suggesting they may serve as a reserve stem cell population.

We are currently investigating whether resting and dormant stem cell populations are differentially activated by niche signals and by physiological neurogenic and injury stimuli.

NMDA Receptors Control Formation and Stability of Cortical Sensory Circuits

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Critical periods during circuit development are important in shaping the appearance of certain cortical circuits, particularly in sensory systems. How sensory stimuli regulate the formation of these cortical circuits and how such continued inputs affect the stability of these developmentally formed circuits is an area of great significance. We've been studying the role of NMDA receptors in the formation and stability of cortical circuits that are amenable to experimental manipulation. Glutamatergic neurotransmission underlies many forms of activity dependent plasticity, thus we reasoned that developmental functions of these receptors may be of major importance in providing activity dependent information to refine these circuits. In addition, in recent years it has become clear that there is a relatively common disorder caused by autoantibodies that interfere with NMDA receptor function that cause profound psychiatric and neurologic dysfunction in children and young adults. Our studies seek to provide a molecular understanding of how these receptors and this disorder may lead to defects in cortical circuit formation and integrity. We've found that knockout of NMDAR in cortical neurons leads to a non-cell autonomous defect in the formation of a prominent circuit connecting somatosensory cortex in the two hemispheres. We further show that this circuit can be disrupted by exposure to function blocking antibodies during the critical period of circuit formation and that this disruption is irreversible.

Targeting Olfaction

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Chemoreception in the mouse olfactory system occurs primarily at two chemosensory epithelia: the main olfactory epithelium and the vomeronasal epithelium. Their sensory neurons are olfactory sensory neurons and vomeronasal sensory neurons, respectively. In the main olfactory epithelium, the interaction with odorous ligands (smells) is mediated by the largest gene family in the mouse genome: 1100 odorant receptor genes. Each mature olfactory sensory neuron is thought to express just one odorant receptor gene. Axons of olfactory sensory neurons that express the same odorant receptor coalesce into the same structures in the olfactory bulb called glomeruli. We are interested in the molecular and cellular mechanisms that enable the expression of one odorant receptor per olfactory sensory neuron, and in the coalescence of axons into glomeruli.

Mechanisms Generating Cell-Type Diversity in Cerebral Cortex

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The concerted production of the correct number and diversity of neurons and glia is essential for intricate neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) are responsible for producing all neocortical neurons and certain glia lineages. We recently performed a quantitative clonal analysis by exploiting the unprecedented resolution of the genetic MADM (Mosaic Analysis with Double Markers) technology and discovered a high degree of non-stochasticity and thus deterministic mode of RGP behavior. However, the cellular and molecular mechanisms controlling the precise pre-programmed RGP lineage progression through proliferation, neurogenesis and gliogenesis remain unknown. To this end we use quantitative MADM-based experimental paradigms at single RGP resolution to define the sequential non-cell-autonomous and intrinsic cell-autonomous functions of candidate genes and signaling pathways controlling RGP-mediated cortical neuron and glia genesis and postnatal stem cell behavior.

Deciphering Species-Specific Properties of Human Corticogenesis

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The human cerebral cortex has undergone rapid expansion and increased complexity during recent hominid evolution.

One striking feature of human corticogenesis is that it is highly protracted in time, from early steps of expansion of progenitor pools and neurogenesis, to later stages of neuronal maturation and wiring.

This protracted timing is thought to contribute in an important fashion to several key features of the human brain, such as cortical size and complexity.

In vitro models and mouse-human neuronal xenotransplantation indicate that the species-specific timing of key steps of corticogenesis is largely intrinsic to cortical progenitors and neurons.

The underlying molecular mechanisms start to be uncovered, and include human-specific duplicated genes.

OM&P

Modes of Division and Differentiation of Neural Stem Cells

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Dynamic regulation of adult neurogenesis underlies cognitive function, neuronal plasticity, and response to therapies. Understanding the basic mechanisms of stem cell maintenance could guide the choice of strategies for stem cell therapies of neurodegenerative and neuropsychiatric disorders. Our results indicate that various modes of division and differentiation of neural stem and progenitor cells, while leading to seemingly identical outcomes, may be induced by different mechanisms and may have profoundly different long-term consequences, relevant to the prospects of brain rejuvenation.

Subcellular Resolution Neural Circuit Imaging in Translucent Mouse Brains: Implications for Assessing the Suitability of Neural Transplants for Restoring Neuronal Function

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In imaging there is usually a compromise between volume and resolution. In general, large volumes can only be imaged with low spatial resolution, while high spatial resolution is accomplished only over small volumes. This limitation is especially problematic in nervous tissue, where synaptically connected neurons can be spatially separated over very long distances and in three dimensions. However, critical details of neuronal connectivity occur at the subcellular level of neurites (axons and dendrites), structures that cannot be easily imaged in the context of extended neuronal networks in high resolution. We have realized large volume network imaging of nervous tissue in high resolution by combining virus-based transsynaptic tracing methods with novel tissue clearing protocols and high-resolution light sheet fluorescence microscopy imaging. Using this technique we succeeded in whole-brain 3D mapping of human neural transplant innervation. Our results indicate that transplant connectivity is largely dictated by the circuitry of pre-existing, endogenous fiber tracts and circuitries rather than the regional identity of the donor cells.
We expect our system to be helpful for benchmarking the synaptic integration potential of novel synthetic cell sources such as neural precursors and neurons generated by direct transcription factor-based cell fate conversion.

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Engineering Neurogenesis for the Postnatal Brain

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We explore the possibilities of generating new neurons where natural neurogenesis has ceased to occur, such as the postnatal or adult cerebral cortex. The approach we have taken is of lineage reprogramming glia or other brain-resident cells into induced neurons. Toward this end, we force the expression of developmentally relevant transcription factors such as Ascl2 and Neurog2 to induce a neurogenic program. Here, I will discuss our efforts (1) to induce neurogenesis from glial cells in the postnatal mouse cortex in vivo, and (2) to decipher the molecular and cellular trajectory of adult human brain pericytes undergoing conversion into neurons.

Developmental Impact of Auxiliary α2δ Subunits of Calcium Channels on Formation of Connectivity in Neuronal Networks

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Introduction

Voltage-gated calcium channels (VGCCs) are multi-unit membrane complexes that consist of pore-forming α1 subunit and auxiliary α2δ, β and γ subunits and mediate transient calcium influx upon membrane depolarization (1, 2). Calcium channels play pivotal role in multiple fundamental processes, such as triggering the neurotransmitter release and modulation of membrane excitability and plasticity. Earlier, auxiliary α2δ subunits were shown to modulate the trafficking and biophysical properties of the α1 subunit, as well as to promote formation of excitatory synapses in mouse cortical neurons and in Drosophila (3). Up- or downregulation of α2δ subunits was implicated in the pathogenesis of several syndromes and diseases including hyperalgesia, neuropathic pain, epilepsy and autism (2, 4).

Aims

Here, we evaluated the role of two α2δ isoforms, namely α2δ1 and α2δ3 that are expressed in the hippocampus, in the development of structural and functional connectivity in neuronal networks.

Methods

We used a combination of immunohistochemical, calcium imaging and electrophysiological techniques at single neuron and network levels.

Results

We found that overexpression of either α2δ1, or α2δ3 subunits led to significant increase in the number of excitatory synapses, whereas higher density of inhibitory synapses was evident only after upregulation of α2δ3 subunit. Furthermore, we observed a selective enhancement of presynaptic transmitter release in excitatory and inhibitory synapses by

α2δ1 and α2δ3 subunits, respectively. Additionally, analysis of axonal outgrowth revealed a significantly longer axons in GAD67-positive interneurons overexpressing α2δ3, but not α2δ1, subunit. The upregulation of either α2δ1, or α2δ3 subunit at different developmental stages in hippocampal cultures grown on microelectrode arrays led to enhancement of spontaneous network activity at two distinct temporal windows. The effect of α2δ3 overexpression was most prominent within first two developmental weeks, whereas upregulation of α2δ1 strongly enhanced the neuronal firing and functional network interaction during third and fourth week in vitro.

Conclusions

Our results demonstrate that α2δ1 and α2δ3 subunits play distinct but complementary roles in the early circuitry formation. Taken together, our findings demonstrate that α2δ subunits are intimately involved into development of synaptic and functional connectivity, and contribute to establishment of excitatory-to-inhibitory balance in neuronal networks.

Acknowledgements

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Molecular Mechanisms of Inhibitory Synapse Development

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Aim

In the mammalian brain, many types of interneurons make functionally diverse inhibitory synapses onto principal neurons (1, 2). While many molecules have been identified to function in inhibitory synapse development (3-5), it remains unknown whether there is a unifying mechanism for development of diverse inhibitory synapses. The aim of the current study is to determine the general molecular mechanisms underlying hippocampal GABAergic inhibitory synapse development

Methods

Primary hippocampal neuronal culture, mouse hippocampal slices, co-culture assay, whole-cell patch clamp, immunocytochemistry/immunohistochemistry, Western blot, immunoprecipitation, GST pulldown, protein purification, protein-protein binding affinity, molecular cloning and mutagenesis, and mouse genetics

Results

We have found that in early developing hippocampal neurons, the establishment of GABAergic inhibitory synapses depends on Neuroligin2 (NL2), an inhibitory synaptic cell adhesion molecule (CAM) (3-5). As the neuron matures, synapse development requires both NL2 and Slitrk3 (ST3) (3-5), another CAM. Importantly, NL2 and ST3 interact with na-

nomolar affinity through their extracellular domains to synergistically promote synapse development. Functionally, the NL2-ST3 interaction facilitates ST3 trafficking to the neuronal surface and augments their synaptogenic ability to induce presynaptic specializations. Selective perturbation of the NL2-ST3 interaction impairs inhibitory synapse development with consequent disruptions in hippocampal network activity and increased seizure susceptibility.

Conclusions

Our findings demonstrate how unique postsynaptic CAMs work in concert to control synaptogenesis and establish a general framework for GABAergic synapse development in hippocampus (Figure 1) (6).

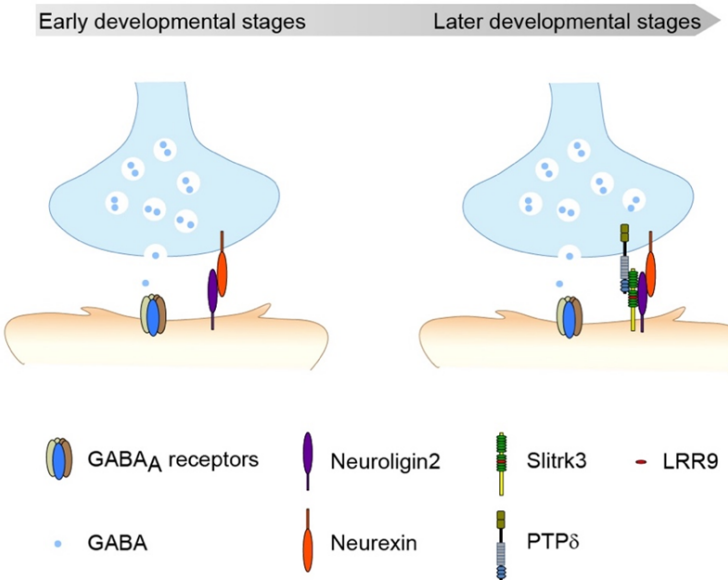

Fig. 1. *A model of the role of the NL2 and ST3 in GABAergic synapse development. In immature hippocampal neurons, NL2 is critical for GABAergic synapse development. In more mature neurons, both NL2 and ST3 are required for GABAergic synapse development. Importantly, at the later developmental stage, NL2 interacts with ST3 to facilitate ST3 trafficking to the neuronal surface and to synergistically promote GABAergic synapse differentiation.*

Acknowledgements

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Regulation of Memory Maintenance during Reconsolidation in Snails and Rats

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It is well known that reactivation of consolidated fear memory under boundary conditions of novelty and protein synthesis blockade results in an impairment of memory, suggesting that the reactivated memory is erased/destabilized and requires synthesis of new proteins for successful reconsolidation.
The role of nitric oxide (NO) in the development of memory was repeatedly described in a range of animal models. Recently in terrestrial snails it was shown that contextual fear memory was significantly impaired 24 hrs after memory reactivation under injection of a protein synthesis blocker (PSB) anisomycin (ANI), but similar reactivation of memory under a combined injection of ANI and a range of NO-synthase inhibitors (or the NO scavenger) demonstrated absence of impairment of the long-term contextual memory. Blockade of NO prevented erasure/destabilization of long-term memory. These results evidence that NO is involved in the destabilization (erasure) of a consolidated context memory in mollusks (Balaban et al., 2014). We tested the hypothesis of the NO involvement in memory destabilization during the reconsolidation process in rats using memory reactivation under different conditions. Administration of NO-synthase selective blockers 3-Br-7-NI or ARL in conditions of reactivation of memory under a PSB prevented destabilization of fear memory to conditioned stimulus. Obtained results support the role of NO signaling pathway in destabilization of existing fear memory triggered by reactivation, and demonstrate that disruption of this pathway may prevent memory reactivation-induced changes in long-term memory. Mechanism of NO effects is presumed to be activation of protein degradation in proteasomes in physiological conditions.

Acknowledgements

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Diffusion of Sodium Signals in Spiny Dendrites of the Mouse Brain

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Sodium ions play a fundamental role in neuronal physiology and excitatory synaptic transmission. While recent work has established that glutamatergic activity is accompanied by transient sodium signals in dendrites and spines, the spatial and temporal properties of such sodium signals are largely unknown. Moreover, basic parameters like diffusion of sodium along dendrites and its movement from and into dendritic spines have not been studied so far. In the present study, we have employed multi-photon sodium imaging combined with whole-cell patch-clamp in CA1 pyramidal neurons in mouse brain tissue slices to address these questions. We found that diffusion of sodium along spiny dendrites is significantly slower than that reported from non-spiny dendrites or axons. Furthermore, our data show that long spine necks serve as significant diffusional barriers for sodium, resulting in retention of sodium in spines that experienced direct influx and in their protection from dendritic sodium influx. Numerical simulations replicated these findings and also predicted active sodium extrusion from spine necks. The demonstrated restricted diffusion of sodium in spiny dendrites will influence the activity of sodium-dependent transporters and e. g. promote the proposed reverse operation of the sodium-calcium exchanger (NCX).
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Axon Growth and Navigation Monitoring Using Microfluidic and Electrophysiology Methods

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Dissociated neural cultures are often used in research of functional organization and development of the neural networks [1 - 4]. In particular, the neuronal culture is useful object to study axonal and dendritic navigation in the growing neural network. Over the past years a great progress has been made in identifying molecules guiding axonal navigation. It has been shown that several families of proteins of the receptor-receptor-type or receptor-secreted ligand type interact to guide axonal targeting in various regions of the brain, such as the spinal cord, dorsal root ganglia, cerebellum, etc. However, the mechanisms of axo-axonal interactions involved in the navigation of axon growth still are not uncovered [5 - 7]. To study the mechanisms of interaction between axons we developed the microfluidic chip that allows to observe growth and interaction of axons of different types of the neurons. In this work we investigate the navigation of individual axons by registration of the stimuli induced activity propagated through the axons.

Polydimethylsiloxane (PDMS) microfluidic chip consists of the four chambers and the microchannels. Two long microchannels connected chambers A and B, five short microchannels connected lateral chambers C and D with long channels. Microfluidic chip was mounted on the surface of a microelectrode array (MEA, Multichannel, Germany). We manually aligned the microfluidic chip with MEA so that 9 electrodes were placed in the A chamber. Cortical neuronal cells were dissociated from embryonic mice (E18) and plated in the C and D chambers of PDMS chip. Since 3 day in vitro (DIV) axons of neurons from lateral chambers reached the long channel and turned to the left or to the right.

On the 10 DIV electrodes placed in the short channels were stimulated by biphasic voltage pulses ±200 mV, 260 μs per phase, positive first, intervals between stimuli were 1 s. Series of 30 stimuli were applied through one electrode. The recording of the bioelectrical activity was performed using a multichannel USB-MEA120-Inv-2-BC-System (Multichannel systems, Germany). Evoked response propagated through the axons placed in the short channel. The signals registered on the electrodes in long channels were corresponded with the axons growth direction. The responses were average with respect to 30 stimulus and were visualize for each electrode. The local potential shift on the axonal membrane was registered as negative peak following 5 ms time window after stimuli.

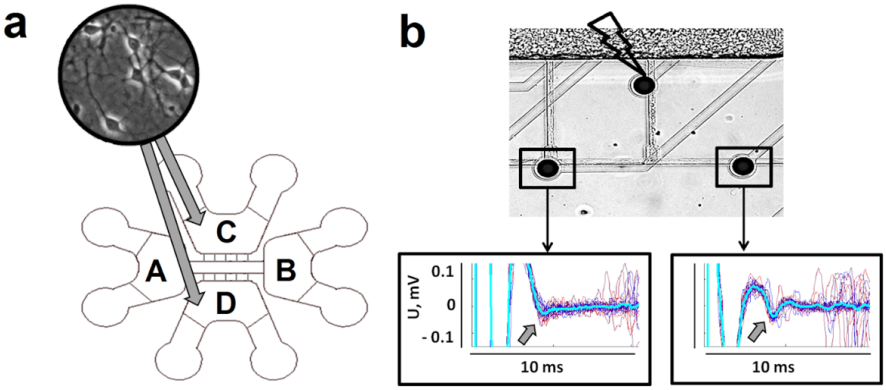

Fig.1. *Stimulus induced activity of axons a. Scheme of microfluidic chip consists of the four chambers and the microchannels. The arrows indicate the chambers of the chip into which the cells were planted. b. The signals registered on the electrodes in long channels after 60 stimuli.*

In this study we showed that axon growth and navigation can be monitored by precose electrophysiological methods.

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Microfluidic and Microprinting Methods to Study Axon Navigation and Axo-Axonal Interactions *in vitro*

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Neuronal circuits formation during neurogenesis and development is one of the key problems of neurobiology. There are areas of the brain composed of well-organized layers of neurons connected by unidirectional synaptic connections (e.g., cortex, hippocampus).

It is important to understand how different neurons in different layers and areas of the brain can form very precise networks and send their axons to remote target areas such as spinal cord.

The development of new tissue engineering methods allows to design neuronal cultures with a defined network architecture that addresses many fundamental questions such as intercellurar interactions [1,2] and guidance of axons to study brain development [3]. Several techniques were proposed for manipulation of axon growth in neuronal cultures, including micropatterning with microcontact printing of adhesive proteins [4, 5] and axon growth through microchannels using microfluidics [6-8]. In this study we investigated the growth of individual axons using both microfluidic techniques and microprinting of adhesive proteins. We compared two methods (microchannels and microprints) for isolation of axons growing in a grid structure.

Polydimethylsiloxane (PDMS) microfluidic chip consists of the four chambers and the microchannels. The opposite chambers (A-D and B-C) were connected by straight microchannels, forming a grid with perpendicular intersections. The surfaces of the prepared PDMS chips were mounted with cover glasses. Then PDMS-glass constructions were coated with the adhesion promoting molecule polyethyleneimine at a concentration of 1mg/mL (Sigma-Aldrich, P3143, USA).

Cortical cells of mice (E18) were plated in the Polydimethylsiloxane PDMS microfluidic chips. According to the first approach, the cells developed inside the microfluidic chip. Axons of cells sprouted into microchannels. According to the second approach, the microfluidic chip was removed on the first day after planting the cells. The axons of the cells grew on the microprints of the adhesive molecules left after removal of the chip.

The axons began to grow into the microchannels and onto microprints of adhesive molecules on 3 day in vitro (DIV). The axons of the cells dominantly grew on the microprints of the adhesive molecules. Nevertheless, a part of the axons grew outside the adhesive prints. More over glial cells could grew on a glass with no adhesive molecules. Glial cells migrating over the surface of the glass allowed the processes of neurons to grow outside the adhesive prints. The migration of glial cells and the growth of neuronal axons on a surface not covered by adhesive molecules reached 400-600 μm on 7 DIV.

On the other hand the microchannels reliably limited axon growth guiding them according a given structure throughout the culture time. Thus, microfluidics methods were more effective than microprints for isolation and navigation of individual axons.

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Creation of Viral Design, Carrying Gene Glynal Neurotrophic Factor (GDNF)

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Neurotrophic factors are polypeptides that regulate development, maintenance, functioning and plasticity of the vertebrate nervous system. The glial neurotrophic factor is one of the neuropeptides of this group. Mature GDNF realizes its biological functions in the form of a glycosylated disulfide-bound homodimer. GDNF includes a cascade of reactions through tyrosine kinase Ret receptors. Cascades of reactions lead to the activation of transcription factors, causing various responses. With this neurotrophic factor, the prospects of treating neurological disorders, Parkinson's disease, ischemia are associated, and GDNF has a neuroprotective effect.

The factor does not pass the blood-brain barrier, so it is necessary to find ways of introducing this gene, bypassing the meninges. Genotherapy is a convenient method for making changes in the genetic apparatus of human cells. Vector delivery is economical and safe, has directional action and stable gene expression.

In this regard, the aim of the study is to create a viral vector for expression in brain cells of the glial neurotrophic factor GDNF.

Considering various viral delivery systems, it was concluded that the viral construct based on adeno-asociated virus is the most successful system for this factor.

Adeno-associated vectors are non-pathogenic, provide an efficient and stable transduction of non-dividing cells, and in addition, allow the expression of viral proteins in infected cells to be excluded.

In the course of the work, RNA was isolated from the brain cells of an adult mouse. From it, the cDNA sequence was synthesized. Next, the GDNF sequence was amplified, which became an insert in the plasmid AVV-kid 2. During this phase of work, a great deal of work was done on the selection of the PCR amplification program, the selection of primers. After restriction and ligation, the plasmid AAV-GDNF-kid2 was obtained.

The cells of the constant HEK 239 cell line were transfected with plasmid. The plasmid showed persistent expression on day 3.

A viral vector is collected. Functionality of the construct was demonstrated by immunocytochemical analysis with antibodies Anti-GDNF (Abcam, ab18956), Goat Anti-Rabbit IgG H & L (Alexa Fluor® 555) (Abcam, ab150086) ex: 555 nm, em: 565 nm \ ex: 488 nm, em: 509 nm, visualized on a confocal microscope from Zeiss.

OM&P

Microfluidic Chip Design to Study Axo-Axonal Interactions and Navigation

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The technology of microfluidic systems in the culturing of the neuronal cells is a new and perspective approach to study the functions of axons. The method allows to isolate the processes from the cell bodies, direct them in the desired direction, and perform biochemical analysis of axons independently from the bodies of neurons and dendrites [1 - 3]. Isolation and navigation of the axons is used to study the development of the brain [4] and regeneration of injured nerves [5, 6].

We developed the microfluidic chip that allows to study the mechanisms of interaction between axons of different types neurons. Polydimethylsiloxane (PDMS) microfluidic chip consists of 4 chambers and microchannels (10 um width). Two long microchannels connected chambers A and B and five short microchannels connected lateral chambers C and D with long channels. The axons from neurons plated to chambers C and D interacted with guiding axons grow in long channel from chamber A in the channels intersections. In this work we investigate axon growth dynamics in the microchannel with different widths of intersections in order to find optimal design that allow to guiding axons lengthwise long microchannels without turning to lateral channels. We proposed two types of intersections: wide with 50 um width and tight with 20 um width. Axon growth dynamics were analyzed with commercial system for continuous monitoring of living cells in culture and image analysis (Cell IQ, ChipMan Technologies, Finland).

Cortical neuronal mice cells (E18) were plated in the chamber A of PDMS microfluidic chip. We started to monitor axon growth on 2 DIV when the axons began to grow in long microchannels. Every 20 min the CellIQ system acquired phase contrast images of each channels intersections. On the 7 day in vitro (DIV) the axons had to grow into an opposite empty chamber B lengthwise long microchannels. However in some cases the axons turn in the lateral channels to chambers C and D.

We estimated the cases of axons turning for 20 um and 50 um width intersections on 7 DIV. We found that the percentage of the turned axons in the chambers with wide intersections compose 44% (n = 5 chips, 29 intersections). Whereas the percentage of the turned axons in the chambers with tight intersections compose 22% (n = 4 chips, 22 intersections). Therefore the 20 um channels intersections more optimal for guiding axon direct growth.

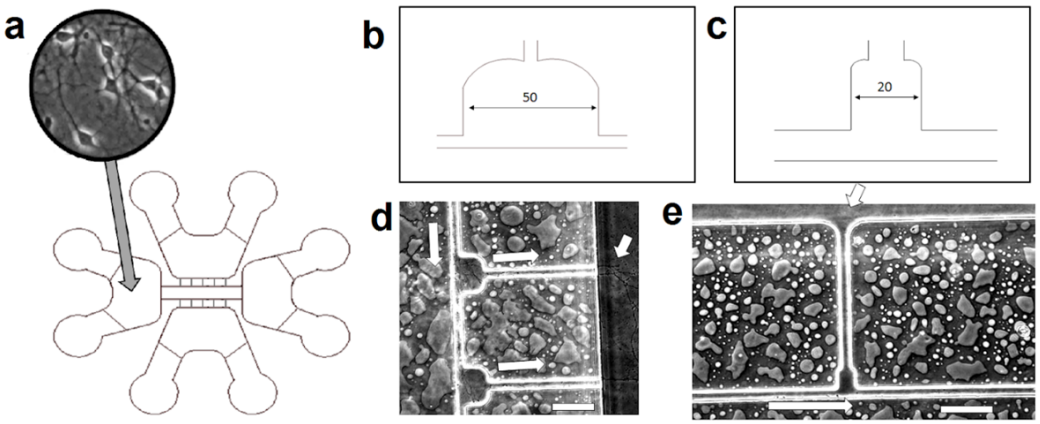

Fig.1. *Experimental model for the study of navigation of individual neocortex axons in microchannels. a. Scheme of microfluidic chip consists of the four chambers and the microchannels. The arrow indicates the chamber into which the cells were planted. b. The wide intersection with 50 um width. c. The tight intersection with 20 um width. d. The wide intersection: axons turned in the lateral chambers. e. Example of direct growth of axons in microchannels with the tight intersection.*

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Monitoring of Calcium Activity in Presynaptic Neurons of Mice Experiencing Hippocampal Slices

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For many decades synaptic circuits have been associated solely with cell-cell neuronal connections represented by the presynaptic terminal, which releases a neurotransmitter, and the postsynaptic neuronal specialization, a site where the neurotransmitter can activate synaptic receptors. However, due to technical limitations these studies usually were linked only to the postsynaptic site. For a long while, the widespread techniques that rapidly advanced neurophysiology have been little used in understanding the way how Ca^{2+}-dependent release of the excitatory neurotransmitter glutamate from neuronal axons can be measured directly. Only with the advance of live cell imaging, it became possible to detect internal Ca^{2+} dynamics in presynaptic boutons with the high temporal resolution.

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