

THE IMPACT OF ASTROCYTES MORPHOLOGY ON THEIR Ca^{2+} CHARACTERISTICS

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Astrocytes, an abundant type of glial cells in mammalian brain and spinal cord, play an important role in regulation of neuronal network functions. During the last decade it has become evident that astrocytes can also be directly involved in modulation of synaptic signalling and synaptic plasticity, and that these astrocyte functions are related to the intracellular Ca^{2+} dynamics. Ca^{2+} signals in astrocytes can occur spontaneously and mainly rely on Ca^{2+} release from intracellular stores. More importantly, Ca^{2+} diffusion in astrocytes depends on cell morphology. Because of their unique morphology, astrocytes can modulate the functional properties of thousands of synapses over defined anatomical regions. However, the mechanisms involved in functional interplay between astrocyte morphology and Ca^{2+} signalling in astrocytes remain poorly understood.

We aim to elucidate the role of astrocyte morphology and its dynamical changes in shaping Ca^{2+} characteristics in cultured hippocampal astrocytes. We show how to uncover Ca^{2+} transients in astrocytes from GCaMP6s fluorescence signals and further quantify and characterize astrocyte Ca^{2+} signalling. By corresponding analysis of astrocyte morphology, we correlate astrocytes Ca^{2+} characteristic to their geometry. Furthermore, by actively modulating astrocytes morphology by up- or downregulating the activity of small GTPases e.g. via the serotonergic system, we show that astrocytes Ca^{2+} characteristics are directly coupled to their morphology.

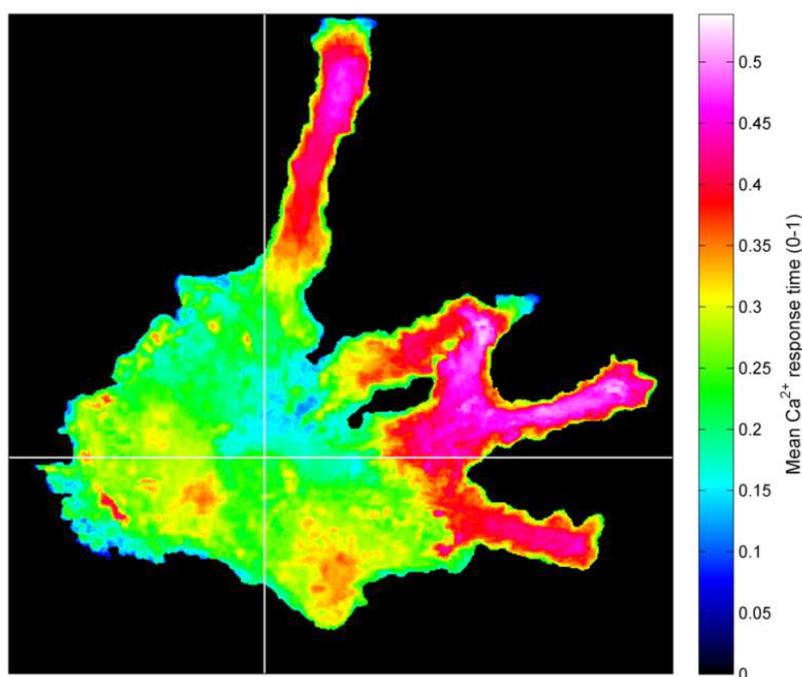


Fig. 1. Color-coded Ca^{2+} response time of a mouse hippocampal astrocyte expressing GCaMP reveals distinct areas of main activity.

CELLULAR SODIUM LOADING IN METABOLICALLY COMPROMISED CORTEX *IN SITU* AND *IN VIVO*

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The brain is strictly dependent on a steady supply with oxygen and glucose. Most energy is needed by the Na⁺/K⁺-AT-Pase (NKA), which maintains a steep inwardly directed sodium gradient. NKA thereby mediates a direct, imperative link between sodium regulation and metabolism in the brain. In ischemic stroke, the disruption of blood flow leads to a breakdown of energy homeostasis and, as a consequence, a decrease in intracellular ATP and NKA activity. This causes a decrease or even failure in sodium export, mandatory to recover from sodium influx e. g. following opening of voltage- and ligand-gated channels. Despite its fundamental role and direct link to cellular energy metabolism, there is a surprising lack of experimental data on the effects of metabolic failure on intracellular sodium of neurons and astrocytes in the intact tissue and, up to date, no *in vivo* measurements exist. Moreover, the consequences of changes in intracellular sodium concentrations are largely unclear.

In the present study, we analyzed the effects of energy deprivation on sodium concentrations in astrocytes and neurons of the somatosensory cortex *in vivo* and in acute cortical tissue slices. To this end, we performed a permanent middle cerebral artery occlusion (pMCAO) for stroke induction *in vivo* or classical chemical ischemia (induced by sodium azide and 2-deoxyglucose) to mimic stroke-like conditions *in situ*. Intracellular ion changes were monitored using two-photon or wide-field fluorescence microscopy. Our data reveal that acute pMCAO induces massive transient increases in the intracellular sodium concentration of neurons and astrocytes in the somatosensory cortex that spread to neighboring cells in a wave-like manner, indicating a peri-infarct depolarization wave. Sodium increases reached 20-30 mM in both cell types and lasted over minutes, before slowly recovering to baseline. In acute cortical brain slices, chemical ischemia caused similar wave-like sodium elevations, albeit of smaller amplitudes. In addition, prominent transient elevations in intracellular calcium were observed. These calcium signals were strongly dampened during perfusion with KB-R7943, a blocker of the sodium/calcium exchanger (NCX).

Taken together, our study provides the first experimental data on sodium changes *in vivo* in response to pMCAO. In addition, we found evidence for a significant contribution of NCX reversal to calcium signalling during energy deprivation. This suggests that changes in intracellular sodium in neurons and glia are among the very first consequences of ATP shortage following ischemic stroke, triggering secondary calcium influx and cellular damage.

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A NOVEL ROLE FOR ASTROCYTES IN BRAIN HEMORRHAGIC STROKE

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The G-protein coupled, protease-activated receptor 1 (PAR1) is a membrane protein expressed in astrocytes. Fine astrocytic processes are in tight contact with neurons and blood vessels and shape excitatory synaptic transmission due to their abundant expression of glutamate transporters. PAR1 is proteolytically-activated by bloodstream serine proteases also involved in the formation of blood clots, suggested to play a key role in pathological states like thrombosis, hemostasis and inflammation. What remains unclear is whether PAR1 activation also regulates glutamate uptake in astrocytes and how this shapes excitatory synaptic transmission among neurons. Here we show that, in the mouse hippocampus, PAR1 activation induces a rapid structural re-organization of the neuropil surrounding glutamatergic synapses, which is associated with faster clearance of synaptically-released glutamate from the extracellular space. This effect can be recapitulated using realistic 3D Monte Carlo reaction-diffusion simulations, based on axial scanning transmission electron microscopy (STEM) tomography reconstructions of excitatory synapses. The faster glutamate clearance induced by PAR1 activation leads to short- and long-term changes in excitatory synaptic transmission. Together, these findings identify PAR1 as an important regulator of glutamatergic signaling in the hippocampus and a possible target molecule to limit brain damage during hemorrhagic stroke.

MODELING CALCIUM DYNAMICS IN ASTROCYTE NETWORKS WITH REALISTIC MORPHOLOGY

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We use a data-driven approach to construct realistic astrocyte network patterns suitable for simulation. Interplay between spatial structure and geometry-dependent mechanisms of calcium dynamics leads to rich and complex spatiotemporal patterns of noise-driven activity.

Astrocytes are morphologically complex cells and are now often regarded as key moderators of multiple aspects of the central nervous system physiology, from uptake and turnover of glutamate and other neurotransmitters to synaptic plasticity to modulation of neuronal excitability to control over cerebral blood flow. Intracellular and collective calcium signaling in astrocytes has important implications in these functions. The local neuronal and synaptic activity is assumed to be the primary drive of the astrocytic calcium signals.

Interestingly, calcium dynamics is the richest and most complex in the extremely thin processes, while somatic calcium signals are more sparse. Moreover, the dynamical mechanisms bringing about these calcium transients are different at the soma and thick branches, where cytoplasmic calcium level is dominated by IP₃-dependent exchange with the endoplasmic reticulum and in the thin processes and lamellae, where it is the influx through the plasma membrane that sets the pitch.

This motivated us to investigate how the interplay between structural diversity and intracellular mechanism variations shapes the calcium activity from the single-cell level to astrocytic networks in a spatially extended modeling study. To this end, we committed to creating as realistic spatial patterns as possible and developed a way of how to convert these patterns into markup for mathematical modeling and parallel computer simulations on GPU. Spatial patterns in our approach are based on tiling the simulation space with transformed microscopic images of astrocytes, which are later segmented into areas with somatic dynamical mechanisms and areas with dynamics specific for thin branches. We also stochastically model local synaptic activity as spatially independent Poisson streams of glutamate pulses which causes calcium entry in the thin processes and IP₃ production. Low or high synaptic drive can thus be set as different rates of the Poisson spike streams, which allows us to model a rich repertoire of calcium activity, ranging from local random calcium sparks to complex spatiotemporal patterns and spreading calcium waves. To describe and understand the latter, one needs to apply modern methods of data analysis and dimensionality reduction, which also bridges the gap between the modeled activity and the experimentally observed one.

Using three independent super-resolution imaging methods, on both genetically tagged and endogenous receptors, we have demonstrated that, in live hippocampal neurons, AMPAR are highly concentrated inside synapses into a few clusters of around seventy nanometers. AMPAR are stabilized reversibly in these domains and diffuse freely outside them. Nanodomains are themselves dynamic in their shape and position within synapses as they can form and disappear within minutes, although they are for the most part stable for at least up to an hour. These results open the new possibility that glutamatergic synaptic transmission is controlled by the regulation at the nanometer scale of the position and composition of these highly concentrated nanodomains. In support of this hypothesis, we recently demonstrated that AMPAR conformation strongly impacts their mobility, indicating that desensitized AMPAR can escape synapses. This finding provides a functional support to our hypothesis that fast AMPAR surface diffusion can tune short term plasticity by allowing fast replacement of desensitized AMPAR by naïve ones during high frequency stimulation.

NEW TRENDS IN THE ANALYSIS OF FUNCTION AND MODULATION OF THE CELLS OF NERVOUS SYSTEM

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Several new areas for analysis and functional regulation of nervous system function have been developed in recent years. Among them connectomics, clarity, optogenetics, optosensorics and optopharmacology provide an excellent tools for detailed investigation of the nervous system organization, on clarifying of neuronal networks functioning and control of some biological organisms behaviour.

A crucial factor for the establishment of optogenetics become the discovery of algal light-gated ion channels (channelrhodopsins) and pumps (halorhodopsins), which can be easily incorporated in different cell types. This provided unprecedented tolls for the control of neural activity with light. Consequently, a number of other light-sensitive modules were discovered and engineered to generate new photoswitches to control protein activity, protein localization, and gene expression. With the help of light one can investigate the function of cells controlling their activity, to measure the concentration of ions, ATP and other cellular components, to control the behaviour of organisms, as well as to seek for novel ways to treat certain diseases. Optogenetic approaches have been used in many models with medical orientation, including the study of stress, schizophrenia, memory disorders, drug addiction, psychiatry and motor functions, vision, pain, functional recovery after stroke and epilepsy.

Photopharmacology is a direction based on the creation of chemical compounds capable of controlling the functions of biological molecules possessing photosensitive switches. Photochromic compounds that activate or inhibit the activity of key cellular proteins, especially ion channels, represent a powerful tools for non-invasive control of neuronal network activity and, consequently, functional control of organisms and behavior. Photochromic switches can be divided into two main classes: (i) soluble photochromic ligands and (ii) compounds covalently bounding to target proteins. On this basis, potassium channel blockers, modulators of glutamate and GABA receptors, as well as cationic TRP channels were created. Photopharmacology offers great opportunities in the regulation of pain, restoration of the functions of the retina and other physiological functions.

Optosensorics uses genetically encoded biosensors for non-invasive imaging of concentrations of ions, the activity of enzymes, distribution of small molecules, proteins and organelles, and the protein interactions in living cells. These fluorescent molecules are used either at the transient expression in cultured cells or organisms or at stable expression producing transgenic animals possessing heritable and functional biosensors. Using the mouse Thy1 mini-promoter, we generated two lines of transgenic mice for the monitoring of intracellular chloride (Cl^-) and for the simultaneous measurements of intracellular and pH. To reveal the ClopHensor expression pattern across the brain of transgenic mice we obtained transparent brain samples using CLARITY method and imaged them with confocal and light-sheet microscopy. This analysis provides the map of the brain areas available for non-invasive monitoring of intracellular Cl^-/pH in normal and pathological conditions.

These and other directions will be presented in the talk.

SPATIOTEMPORAL CHARACTERISTICS OF ASTROCYTE CALCIUM DYNAMICS IN CHRONIC EPILEPSY

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Epilepsy is diagnosed annually in 2.4 million people (WHO 2017). Epileptic status (ES) is the main symptom and is the most severe manifestation of generalized epilepsy. It is a series of seizures with tonic and clonic phases, leading to neurodegeneration and astrogliosis. The influence of these processes on the neuron-astrocytic interaction, in particular on the calcium events in the astrocytic network, remains poorly studied. The study of astrocytic activity can give new knowledge about the mechanisms of epilepsy and determine the direction of creating new methods of treatment.

As an experimental model in this project, a lithium-pilocarpine model of epileptogenesis was chosen. It reflects the various phenomenological features characteristic of temporal epilepsy in humans.

Sprague-Dawley rats (age 18-25 days) were used in the experiments. 2-4 weeks after ES, the changes in calcium dynamics in the neuron-astrocytic network stratum radiatum of the CA1 field on the hippocampal slices were stud-

ied. The thickness of the sections was 350 μm , the staining was performed with a calcium indicator Oregon Green 488 BAPTA-1 AM and an astrocytic marker sulforodamine 101. A confocal fluorescence microscope LSM Zeiss 510 DuoScan was used to visualize the calcium events. To analyze the space-time characteristics of calcium dynamics in the network of astrocytes, software was developed on the basis of Matlab, which allowed identifying the space-time characteristics of individual events. The following parameters were investigated: duration of events, maximum projection of events and frequency.

The distribution in accordance with the power law was observed both for the sizes and for the maximum projection of the calcium events. In particular, with ES, a statistically significant increase in the maximum projection was observed, indicating a decrease in the number of large events in comparison with the control. The change in the frequency of events is not statistically significant. This change is pathological in terms of the functioning of the calcium signaling of the astrocytic network.

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K⁺ MEDIATED SIGNALING WITHIN TRIPARTITE SYNAPSE

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Living cells had evolved with high intracellular concentration of K⁺ and with low concentration of Na⁺. Transmembrane gradients of these ions drive cellular excitability. Therefore, general belief is that K⁺ efflux is mostly needed for membrane repolarization during action potential. In fact, significant amount of K⁺ is also released via postsynaptic glutamate receptors during excitatory synaptic transmission in the CNS. This reduces postsynaptic depolarization making synaptic transmission less efficient and more energy costly. Why such K⁺ permeability is preserved in these receptors? Here, we report that NMDA receptor-dependent K⁺ efflux can provide a retrograde signal in the synapse. In hippocampal CA3-CA1 synapses, the bulk of astrocytic K⁺ current triggered by synaptic activity reflects K⁺ efflux through local postsynaptic NMDA receptors. The local extracellular K⁺ rise produced by activation of postsynaptic NMDA receptors boosts action potential evoked presynaptic Ca²⁺ transients and neurotransmitter release from Schaffer collaterals. Perisynaptic K⁺ accumulation during synaptic transmission also affects astrocytic transporter currents, making them slower. This suggests activity dependent enhancement of glutamate spillover also depends on postsynaptic cell. Our findings indicate that postsynaptic NMDA receptor-mediated K⁺ efflux contributes to use-dependent synaptic facilitation and increased glutamate dwell time, thus revealing a fundamental form of ionic signaling within tri-partite synapse.

RAPID ASTROCYTE MORPHOLOGY CHANGES SUPPORT EPILEPTIC ACTIVITY

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Astrocytes actively contribute to neuronal network function. The close contact of individual astrocytes to thousands of neurons enables them to maintain and modulate neuronal function effectively by, for example, buffering potassium and glutamate clearance. A disruption of this spatial relationship could be of pathophysiological significance. Indeed, astrocyte dysfunction and long-term morphology changes have been implicated in numerous diseases including epilepsy. How rapid astrocyte morphology is altered by the onset of epileptiform activity and to what degree it contributes to aberrant network behavior is largely unknown. Combining established protocols of hippocampal epileptogenesis, electrophysiology and two-photon excitation fluorescence microscopy allowed us to monitor astrocyte morphology changes during the induction of epileptiform activity in acute hippocampal slices. Analysis revealed that small and medium-sized astrocyte processes shrink acutely within minutes after epileptiform discharges appeared in the CA1 region. Importantly, similar astrocyte morphology changes were also detected shortly after induction of status epilepticus in vivo by intracerebral kainate injection. In vitro, these astrocyte morphology changes outlasted the induction of epileptiform activity, persisted after pharmacological termination of epileptic activity by TTX and were sensitive to inhibition

of Rho-associated protein kinase (ROCK, Y-27632). Importantly, ROCK inhibition also reduced epileptiform activity, indicating that rapid astrocyte morphology changes support epileptic activity. A modification of glutamatergic or GABAergic synaptic transmission did not underlie the proconvulsive effect of astrocyte morphology changes. Instead, we observed that intracellular diffusion in astrocytes and diffusion between astrocytes via gap junctions were significantly decreased in parallel to morphology changes. The reduced astrocyte gap junction coupling is likely a consequence of reduced intracellular diffusion because no changes of connexin 43 and 30 expression and phosphorylation were observed. Thus, astrocytes respond to epileptic activity with morphology changes on a time scale of minutes, which reduces intra- and intercellular diffusion in the astrocyte network and supports further epileptic activity. A faster glutamate accumulation, which we detected using the glutamate sensor iGluSnFR, after induction of epileptiform activity may link astrocyte remodeling and maintenance of epileptiform activity.

SYNCHRONIZATION IN MULTIPLEX GLIAL-NEURAL NETWORKS

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In work we investigate impact of the glial cells activities on synchronizability of neural cells in multiplex networks framework. Connections among the «glial» cells form a regular star like periodical structure in which each cell is connected to the four other neighbour cells whereas connections, among «neural» cells are represented by an Erdős–Rényi random network with average quantity connections is equal by four.

A multiplex network in which one layer represents interactions among the glial cells and the other layer represents those of neural cells is taken. Connections among the glial cells form a regular star like periodical structure in which each cell is connected to the four other neighbour cells whereas connections, among neural cells are represented by an Erdős–Rényi random network with average quantity connections is equal by four. Inter-layer links are such that each node in the neural layer is connected to its mirror in glial layer and all the four neighbours of the mirror node. The dynamical evolution of the oscillator nodes in this multiplex network is given by the coupled Kuramoto model.

At first case we focus on the case when neural and glial layers are not coupled. Our aim is twofold: we want to capture the effect of network topology on synchronization and study size dependence.

The main results in case of uncoupled layers are here:

- Kuramoto order parameter r in neural layer does not depend from layer size and has classical Kuramoto like behaviour (“all-to-all” links).
- In glial layer Kuramoto order parameter strongly depends from layer size: r_{glial} decrease due to layer size increasing.
- In limit $N \rightarrow \text{Inf}$ parameter $r_{\text{glial}} \rightarrow 0$, that correspond to 1-D nodes chains (they has no mean field).

At second case neural and glial layers are coupled.

We can conclude several points about synchronization case:

- Mean field in glial layer is born with the interaction of neural layer.
- There is partial desynchronization in glial and neuron layers.
- There is abrupt transition to synchronization.

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SOFTWARE DEVELOPMENT FOR CUSTOM-MADE TWO-PHOTON SYSTEM FOR IMPLICATIONS IN NEUROSCIENCE

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Two-photon microscopy is one of the main and quickly developing imaging techniques in neuroscience. Therefore, scientists need to regularly modify their systems according to changing in experimental needs. This task is not always possible with commercially available microscopes. Earlier we have reported how to build a custom-made laser scanning microscope (LSM) that can be easily modified by the user for a specific task in *in vivo* and *in vitro*. Here we describe our custom-made software - AMAScan (MATLAB) to control LSMs in a flexible and user-friendly manner.

Modern imaging techniques with high spatial and temporal resolution provide key tools for studying the brain functioning significantly advance neuroscience field. Developing new tools and upgrading available ones is crucial for further progress [1]. Thus, we developed custom-made two-photon microscope that took into account specific needs of various types of experimental approaches *in vitro* and *in vivo*. This system allowed us to significantly reduce the cost and increase the quality of obtained images by implementing the BM3D filtering algorithm for the noise reduction [1, 2]. We optimized the optical path in order to minimize the excitation lose and tissue damage in combination with increased efficiency of the signal detection.

Notably, this system can be easily modified for cultures, brain slices and *in vivo* recordings from behaving animals. For the first two types of samples, we minimized the distance between the objective and the condenser in order to optimize the quality of images for morphometry and line-scan imaging. In contrast, for *in vivo* procedures on behaving animals, the condenser can be removed and distances can be easily adjusted to fit in a platform for virtual reality and a floating sphere where an animal can run.

Specifically we focused on the software development realizing its crucial role for different research paradigms. In order to improve the quality of obtained images we optimized not only the technical (physical) characteristics of the microscope [3–5], but also the software. The system is fully controlled via three types of connections: 1) digital-to-analog converter (DAC), 2) analog-to-digital (ADC) converter (National Instruments boards), 3) the USB, RS232 connections to control the microscope components (e.g.). Importantly, we implemented existing well-optimized mathematical algorithms to control the scanning mirrors and the detection process [6]. Despite that, numerous types of the software are available on the market (software (ScanImage, Micro-Manager, YouScope, etc.)), in this system we developed a custom software (Matlab, LabView, Python), so we could adjust to the hardware in task-specific manner. Here we created a few sub-programs for each element to be controlled by the software (e.g. manipulators, objective, lasers, shutters, scanners, acousto-optic modulator (AOM), detector, etc.). After maintaining the control over each element we combined and optimized those sub-programs to the single program that controls and synchronize their work. Currently we are optimizing the interface in a quickly adjustable and user-friendly way. Particularly, we developed a graphical interface that is controlling the system and collects images in various scan modes (2D, 3D, line scan mode). For the maintenance purposes, the software interface can be also accessed as a command line tool.

In order to simplify the routine laser alignment process in the software we implemented the option to identify the laser positions where you can monitor it and perform the alignment in relative coordinates where (0; 0) represents the center and the optimal laser position. In this position, the laser beam should pass through the centers of all optical elements. The second procedure to optimize the scanning before the experiments can be also easily performed in the software. It is implemented as a circular scanning that allows to measure aberrations under the objective at the focal plane. These manipulations are implemented in the “Beam Calibration Setup” service mode and significantly simplify the routine adjustment procedure.

To summarize, here we described the development of the software to control and improve custom-made two-photon systems. This approach significantly reduces the costs, increases efficiency, flexibility and improves the quality of obtained data from various types of samples including cultures, brain slices and *in vivo* recordings on behaving animals.

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NETWORK Ca^{2+} -CELL ACTIVITY FIELD CA3 HIPPOCAMPAL SLICES OF RAT EARLY AND LATE POSTNATAL DEVELOPMENT

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Information processing in the brain - is the result of the constant interaction between two cellular networks: the neuronal and the glial. Hippocampus - the structure of the central nervous system, which is involved in the mechanisms of emotion and memory consolidation. The hippocampus has a certain topology distribution of cellular elements, which provides the many cellular networks. One of them is the network of neurons in the CA3 field. This network receives inputs from cells of the entorhinal cortex and the dentate gyrus, in addition CA3 pyramidal neurons form the connection between themselves and interneurons, forming a closed network that operates in conditions of acute slice and generates spontaneous Ca^{2+} activity. Neuronal network interacts with the glial network, the main manifestation of activity which are Ca^{2+} oscillations. Therefore, to estimate the age dependence of Ca^{2+} activity in the cells were investigated Ca^{2+} oscillations in neuronal and glial networks and the interactions between them.

In this work, we investigated changes in the characteristics of Ca^{2+} oscillations cells of rat hippocampal CA3 field in early (P5-8, P14-16) and late (P21-25), postnatal development. Also shown the effect of temperature of perfusion solution on cells Ca^{2+} activity of CA3 field hippocampal slices of rats in different postnatal periods. Besides in the study was valued role of network activity in the formation of spontaneous Ca^{2+} oscillations cells of rat hippocampal CA3 field in early and late stages of postnatal development. Experiments were carried out on acute hippocampal slices from rats. Was used laser scanning confocal microscope Carl Zeiss LSM 510 DuoScan (Germany). Recording fluorescence kinetics were carried out in full frame (field of view of 400x400 mm), with a resolution of 512x512 pixels digital and scanning frequency of 1 Hz. Fluorescence indicators recorded in the range 500-530 nm (Oregon Green 488 BAPTA-1 AM) and 650-710 nm (Sulforhodamine 101). The fluorescence intensity (s.u.) shows the dependence of the concentration of $[Ca^{2+}]_i$ in time, indicating the metabolic activity of cells. Method of cross - correlation analysis was used to evaluate synchrony of Ca^{2+} oscillations cells of CA3 field of rat hippocampus. We chose the time interval size in 3 seconds and within this interval were found synchronous Ca^{2+} oscillations in all possible pairs of cells. Further, the number of synchronously occurring Ca^{2+} oscillations were normalized to the minimum number of Ca^{2+} oscillations in one of the cells analyzed pairs.

The studies have shown that the parameters of cell Ca^{2+} oscillations field CA3 of hippocampal slices vary depending on the period of postnatal rats. Reducing the amount of Ca^{2+} oscillations with age due to the formation and complexity of synaptically connected neural networks, the transition of electrical synapses in the chemical. Transitional period is 14-16 days of postnatal development, and for 21 days - there is a fully formed neural network. Electrically connected network is weakly controlled, excitement is freely distributed over the network, involving work of all cells, resulting in a high Ca^{2+} activity in rat hippocampal cells of younger age group. In mature hippocampal brain slices spontaneous Ca^{2+} activity with low due to lack of active neural network. In this case, the spontaneous Ca^{2+} oscillations are due mainly metabolic activity of cells has been shown in our experiments. This study showed that changes in Ca^{2+} activity in the cells of rat hippocampal CA3 fields occurring during postnatal development directly related to the functioning of the neural networks, and the metabolic state of the cells. Ca^{2+} signaling in mature brain - is a complex multicomponent process involving various receptor systems capable of mutual substitution in violation of the normal functioning of one or more of them.

VESICULAR GLUTAMATE RELEASE FROM ASTROCYTES AT THE INTERFACE OF SIGNALING AND METABOLISM

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Astrocytes synthesize glutamate *de novo* owing to pyruvate entry to the citric acid cycle via pyruvate carboxylase. Pyruvate is sourced from the utilization of two metabolic fuels, glucose and lactate. Glucose can be polymerized to glycogen and stored as fuel within astrocytes and/or lysed to pyruvate, while lactate can be converted to pyruvate. To that end, we investigated the role of the above energy sources, glycogen, glucose and lactate, in exocytotic glutamate release from astrocytes. We used purified primary astrocyte cultures acutely incubated (1 hour) in glucose and/or lactate-containing media. We used mechanical stimulation, known to increase intracellular calcium levels and cause exocytotic glutamate release. Using single cell fluorescence microscopy, we monitored stimulus-induced intracellular calcium responses as well as glutamate release to the extracellular space. Our data indicate that glucose, either taken-up from media or mobilized from the glycogen storage, sustained glutamate release, while the availability of lactate significantly reduced the release of glutamate from astrocytes. Based on further pharmacological manipulation, it appears that lactate caused metabolic changes consistent with an increased synthesis of fatty acids. The above metabolic and functional changes were corroborated by tandem mass spectrometry proteomics analysis which confirmed appropriate altered protein expression. These findings support the notion that the availability of energy sources and metabolic milieu play a role in glial-neuronal interactions and modulation of synaptic activity in health and disease.

QUANTIFICATION OF DEVELOPMENTAL AND PATHOLOGY-RELATED CHANGES IN PERINEURONAL NETS

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Perineuronal net (PNN) is a highly specialized portion of the extracellular matrix (ECM) in the brain and spinal cord. PNN surround synaptic boutons on somata and proximal dendrites of multiple neuronal populations including interneurons of the brain cortex and spinal cord. While molecular composition and physiological roles of PNN have been studied in detail over the past decades, the PNN microstructure remains largely unstudied.

Here we propose a method for semiautomatic quantification of the PNN structural parameters in fluorescent microscopy images of murine brain and spinal cord. We use *Wisteria floribunda* agglutinin (WFA) staining and epifluorescent microscopy to visualize chondroitin sulfate (CS) chains within the chondroitin sulfate proteoglycan component of PNN. The resulting microscopy data are used for quantification of the single cell PNN area and intensity of the WFA staining. The density of the PNN-bearing cells is also quantified. The image analysis approach utilizes autothresholding algorithms of the FIJI software. As a proof of principle we analyse single cell PNN size and CS enrichment in developing brain and posttraumatic spinal cord. Image stitching allows acquisition of quantitative parameters from all cells of a particular brain area in a tissue section. That feature is further used for comparison between brain areas. We demonstrate combination of the method with high content imaging using motorized and programmable microscope stage. The method allows retrieval of the PNN quantitative parameters for the studies on the CNS development and pathology mechanisms.

INTERACTIVE REALISTIC MODEL OF PROTOPLASMIC ASTROCYTE

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Electrically non-excitabile astrocytes appear able in transducing, integrating and propagating physiological intracellular diffusion signals. Decrypting this type of signalling, however, poses a conceptual difficulty because it requires an understanding of molecular interactions in the massive morphological structure of nanoscale-thin leaf-like processes which constitute the bulk of astrocyte geometry. How a particular cell signalling engages a precise type of geometry remains therefore poorly understood.

There have been no attempts to develop an astrocyte model with such a spongy morphology even though this could provide the key to mechanistic insights into astrocytic physiology and Ca²⁺ signalling.

To understand the role of complex pattern in cell function we have adapted the NEURON modelling environment to build a simulation tool to produce different astrocyte models with the detailed morphology, membrane properties and known molecular signalling mechanisms. The tool enables to design a distributed Ca²⁺ homeostasis mechanisms including diffusion, wave propagation, gap-junction escape or channel currents whereas the simulation environment also has the capability to mimic uncaging, membrane physiology, volume current injections or fluorescence recovery after photobleaching (FRAP) experiments in the 3D tissue volume containing the astrocyte.

In our illustration study, the tool generates astrocyte which has adapted the features of hippocampal protoplasmic astrocytes (area CA1) documented through a combination of experiments involving electrophysiology, two-photon excitation imaging, a FRAP super-resolution technique and quantitative electron microscopy. We demonstrate how simulations with the model could help to unveil some fundamental features of astrocytic morphology and Ca²⁺ signalling that are not accessible to direct experimental probing.

To our knowledge, this is the first attempt to have a full-scale tool for astroglia simulations, which we believe will attract significant interest among a broad audience of cell biologists and neuroscientists.

MODELING OF NEURAL NETWORKS WITH TETRAPARTITE SYNAPSES

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Uncovering the key role of different brain cells and structures in information processing and health and disease is the main task of modern neuroscience. Many years neurons were considered as the key players in information processing. Recent experimental study has uncovered crucial role in information processing of extracellular matrix molecules and astrocytic cells activity [1-4]. The neuron-glia interaction is mediated by spillover (diffusion) of neurotransmitters in the extracellular space and their binding to astrocytic membrane receptors. For example, released D-serine and ATP from astrocyte after binding of neurotransmitter to astrocytic membrane receptors might induce currents through ionotropic receptors on the postsynaptic side as well as lead to long-term changes through activation of metabotropic receptors. Activation of kainite receptors by astrocytic glutamate induces axonal depolarization and reduction of AP generation threshold, and hence changes the properties of spontaneous IPSCs, namely increases their frequency and amplitude [3]. In a series of experimental investigation, the role of extracellular matrix (ECM) molecules in regulation of synaptic transmission and neuronal excitability was highlighted. It is assumed that ECM-mediated regulation mechanisms are involved in homeostatic modulation of neuronal activity on extended time scales [1,2]. ECM-induced homeostatic plasticity helps prevent the pathological hypo- and hyper-excitation of neurons which may cause dysfunction and cell death. For instance, an experimentally observed effect referred to as synaptic scaling helps neurons maintain the extent of their activity within a certain range under different inputs [5,6]. Besides the neuron-glia and ECM-neuron interaction pathways there also exists ECM-astrocyte interaction which format the structure in the brain - tetrapartite synapse. Activation of glial cells is not only elicited by the diffusing neurotransmitter, but also by ECM

molecule production [1]. According to the experimental data the influence of ECM molecules on astrocytes is associated with the change in the number and properties of glial cells (specifically, changes in cellular morphology and intracellular pH [7]), which would in turn modulate the efficiency of neuron-glia interaction.

We present a computational model of neuronal network with tetrapartite synapse. The model describes the dynamics of excitatory and inhibitory neuron populations in the presence of glial and ECM regulations and based on mean-field approach. Neuron dynamics we modeled by Wilson-Cowan mean – field model [8]. The astrocyte is described by gliatransmitter concentrations depending on excitatory neuron population. We found that interaction between ECM, astrocytes and neuronal populations lead to spontaneous activity oscillations on extended timescales. The interaction parameters determine the oscillation period (hours to days) and their existence and switching to bistable regimes.

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ROLE OF EXTRACELLULAR SIGNALING IN THE NEOCORTICAL DEVELOPMENT

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In the developing cerebral cortex different types of neuron and glial cells are born in a precise sequential manner from specialized radial glia progenitors. After leaving mitotic cycle, they migrate out of the germinative zone and initiate differentiation program. We identified a novel mechanism that controls cell identity in the developing cerebral cortex via “feedback” of secreted factors to neuronal progenitors. A defined set of secreted factors including neurotrophin-3, Sfrp1 and Fgf9 were found to induce premature and excessive production of upper layer neurons at the expense of deep layer neurons on one hand and precocious generation of glial precursors on the other hand. This “feedback” is likely to act at the distal part of a radial glia process located in the area of young differentiating neurons. We suggest that various concentrations of secreted factors can be sensed by the radial glia process inducing cell fate switch. We identified non-canonical TrkC receptor as a major mediator of this extracellular signaling that controls cell fate switch in the developing neocortex.

ASTROCYTIC GLUTAMATE UPTAKE IS REGULATED BY K⁺ ACCUMULATION IN THE SYNAPTIC CLEFT

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K⁺ accumulates in the synaptic cleft during synaptic transmission, depolarizes presynaptic terminal and increases glutamate release probability (Shih et al., 2013). Here we investigated if such K⁺ accumulation can also affect electrogenic glutamate uptake in astrocytes. Glutamate transporter currents were recorded in CA1 *str. radiatum* passive astrocytes of hippocampal slices from C57BL/6J mice (P28-P35) in response to local glutamate uncaging or electrical stimulation of Schaffer collaterals. Increases in the extracellular K⁺ from 2.5 mM (control) to 7.5 mM or 20 mM depolarized the astrocytes and significantly reduced uncaging induced transporter currents. Equivalent depolarization of the cell through patch pipette reduced the transporter current to the same extent, suggesting voltage dependent mechanism of K⁺ action. Repetitive synaptic stimulation (5 stimuli at 50 Hz) induced progressive increase in the decay time of the transporter currents, which was abolished by D-APV, NMDA receptor antagonist. This is consistent with previous finding that NMDA receptors is a major source of K⁺ in the synaptic cleft. A detailed biophysical model also complemented experimental observations. Thus, NMDA receptor dependent accumulation of K⁺ during repetitive synaptic activity can inhibit local glutamate uptake, potentially extending glutamate well-time in the synaptic cleft.

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EXCITATORY AMINO ACID TRANSPORTERS (EAATS) AS POTENTIAL TARGETS FOR THE TREATMENT OF NEUROLOGICAL DISORDERS

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Glutamate is the main excitatory neurotransmitter in the central nervous system and plays a key role in brain development, neuroplasticity, and neurological disorders. The mechanism and importance of the glutamate removing from synaptic clefts were unknown until the 1970s. Since then, a family of five glutamate transporters has been described that are responsible for regulating glutamate concentrations in both synaptic and extrasynaptic sites. The glutamate transporter 1 (GLT1 or EAAT2) is responsible for more than 90% of the transport of glutamate from the synaptic cleft. To date, very complex regulation of EAAT2 has been identified both at the level of expression, including alternative splicing and at the protein level. EAAT2 dysregulation is shown in many mental and neurological disorders, including amyotrophic lateral sclerosis, schizophrenia, mood disorders, Alzheimer's disease, epilepsy, strokes, Huntington's disease, brain trauma, glaucoma, dementia associated with HIV, and drug addiction. Animal studies show that increased expression of EAAT2 provides neuroprotection in many models of neurodegenerative diseases. Expression activators and EAAT2 modulators can act as promising therapeutic agents that prevent excitotoxicity and neuronal death. The lecture summarizes current information on this issue and discusses prospects for the use of glutamate transport activators.

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ASTROGLIAL VESICULAR NETWORK: EVOLUTION AND FUNCTION IN HEALTH AND DISEASE

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Astrocytes, the most heterogeneous glial cell type in the brain, have been scientifically neglected for almost a century. By being merely “nervenkitt”, as proposed by Virchow in 1858, they were considered to play only subservient roles to neurons. However, in the last two decades a renewed interest into these cells emerged. Astrocytes, get excited when neurotransmitters bind to their membrane receptors and signal back to neurons by releasing their own transmitters. As in neurons this involves vesicles, which store chemicals termed gliotransmitters or more generally gliosignaling molecules. While the vesicle-based chemical signal release is similar to that in neurons, however it is much slower vs. that in neurons. The slow kinetics of this signaling makes them integrators that provide energy (astrocytes contain glycogen) to neurons in a similar time domain. Vesicle dynamics greatly depends on intermediate filaments, which get overexpressed in pathological conditions. Therefore, altered vesicle dynamics may be associated with the diseases such as amyotrophic lateral sclerosis, multiple sclerosis, autistic disorders, Alzheimer’s disease, trauma, edema, and states in which astrocytes contribute to neuroinflammation. In multiple sclerosis, for example, fingolimod, a recently introduced drug, apparently also affects vesicle traffic and gliosignaling molecule release from astrocytes, indicating that this process may well be used as a new physiologic target for the development of new therapies.

CONNECTOR DEVICE FOR LONG-TERM STIMULATION OF NEURONAL CULTURES GROWING ON MICROELECTRODE ARRAYS

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Neuronal cultures in vitro can be used to study memory, learning and information processing in the brain. Bioelectrical activity of the cultured networks in vitro differs from the normal physiological activity of the brain possibly because of absence of an afferent signal. One of the ways to simulate sensory inputs can be chronic electrical stimulation. In this study we present the connector device for long term stimulation of the neuronal network cultured on microelectrode arrays.

Neuronal cultures in vitro can be used to study memory, learning and transfer of information signals in the brain. The monolayer of neuronal cells is organized in a synaptically connected two-dimensional neural network. The formation and modification of the synaptic connections in the network can be investigated with electrophysiological methods [4].

Spontaneous bioelectrical activity registered in neural networks in vitro differs from normal physiological activity in the living brain [2]. This phenomenon can be explained by absence of an afferent signal [1, 3]. Functional development of neural networks in the brain is significantly modulated by sensory inputs and associated with an increase of the number of cells, its density and synaptic connectivity change.

However, most of the studies of the cultures are made without external stimulation that simulates sensory inputs. Some attempts of long-term stimulation were made, which consisted of single stimulation trial in three-four days a week and lasted no more than 40 minutes.

Here we present a device that allows long-term continuous stimulation of the neuronal cultures. We suggest that the morphological and functional organization of the culture and its activity which developed under such conditions will reproduce in vivo features.

Materials and methods

The presented device (connector) for chronic stimulation is intended for keep cell cultures grown on microelectrode arrays (MEA). MEAs consists of microelectrodes on a glass substrate and has conductive pads on perimeter. The

signal from the stimulus generator transmitted to the conductive pads by a contact board of the connector and then applied to the neuronal culture.

The connector consisted of a plastic box and a PCB board, which transmit stimuli for MEA. The box was designed in the Tinkercad software and printed on a 3D printer Ultimaker 2 plus (Ultimaker B.V., Netherlands) from PLA plastic with 2.65 mm diameter. The PCB board contained 4 gold pressure pins (Preci-Dip, Switzerland) which were connected by conductive paths with a connector to the stimulator.

Results and discussion

Developed connector consisted of the contact plate with pressure contacts and the plastic box for MEA installation. The MEA was installed in the cavity of the box base, the contact plate was fixed upon it, so the pressure electrodes touch the contact pads of the MEA. The special hole in the box base covered with a cover glass allowed to observe the neural network morphological development on an inverted microscope in sterile conditions inside the box. The gold pressure electrodes of the contact plate was connected by conductive paths with a connector and then with stimulus generator. A cylindrical protrusion was on the cover for a filter film, which transmits the CO₂ that is necessary for the cells metabolism. The connector was placed in the incubator during chronic experiment where the neuronal culture was in optimal conditions for the development.

To test the survival of the neurons developed under conditions of chronic stimulation in the connector, we plated differentiated hippocampal neurons of mouse embryos (E18) on the microelectrode arrays. On the 2nd day after planting the MEA was installed into the connector for stimulation. The chronic stimulation consisted of loop of trains of 5 bipolar pulses with an amplitude of 600 μ V, 20 ms interpulse interval and an interval between trains of 120 ms. We found that such chronic stimulation did not affect of the culture negatively and the cells showed good viability. The developed connector can be used to study the neuronal cultures in vitro under conditions of chronic stimulation that simulates afferentation in brain. We suggest that such approach will induce activity similar to that observed in vivo conditions.

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SOFT MULTIMODAL NEURONAL INTERFACE TO RESTORE MOTOR FUNCTION AFTER SPINAL CORD INJURY

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The mechanical mismatch between soft neural tissues and stiff neural implants hinders the long-term performance of implantable neuroprostheses. We designed and fabricated soft neural implants with the shape and elasticity of dura mater, the protective membrane of the brain and spinal cord. The electronic dura mater, which we call e-dura, embeds interconnects, electrodes, and chemotrodes that sustain millions of mechanical stretch cycles, electrical stimulation pulses, and chemical injections. These integrated modalities enable multiple neuroprosthetic applications. The soft implants extracted cortical states in freely behaving animals for brain-machine interface and delivered electrochemical spinal neuromodulation that restored locomotion after paralyzing spinal cord injury.

DIFFERENTIATION FATE OF NEURAL STEM CELLS IN THE HIPPOCAMPUS OF EPILEPTIC RATS

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Epilepsy is the 4th most common neurological disease after migraine, stroke and Alzheimer's disease. Along with well-known neurotransmission imbalance epilepsy also leads to pathological defects in neuronal architectonics, particularly in the hippocampus. Morphological alterations, such as astrogliosis and dispersion of granular and pyramidal layers were shown in both human epileptic hippocampus and in various rodent models of epilepsy. Moreover it was shown that epileptogenesis is accompanied with increased neural stem cell (NSC) proliferation in the granular layer of the dentate gyrus, which leads to formation of ectopic hilar granule cells and probably contribute to astroglial proliferation. Now it is becoming clear that glia cells could play a role in seizure susceptibility and epilepsy development. It was demonstrated that neuronal hyperexcitability could be mediated by astrocytic glutamate receptor activation or by changed expression of astrocytic enzymes.

We chose Krushinsky-Molodkina (KM) rats, genetically prone to audiogenic seizure (AGS), as a model in our study. AGS in KM rats expresses in age-dependent manner and acquires during maturation. These rats were selected from Wistar, which we used as a control. We hypothesized that astrogliosis and ectopic cells appearance caused by abnormal neurogenesis could be genetically determined in KM rats. Comparative analysis revealed significant upregulated ERK1/2 activity in the hippocampus of immature young KM rats. It is known that ERK1/2 kinase is a key player in the regulation of cell proliferation and differentiation. Thus increased ERK1/2 activity could stimulate neurogenesis in these rats. Indeed in the hippocampus of these rats we observed increased proliferation level of NSCs. *In vitro* analysis of differentiation fate demonstrated that NSCs of KM differentiate mainly in astrocytes and glutamatergic cells and the maturation of these cells completed faster than in culture of Wistar NSC. *In vivo* analysis of the hippocampus also showed increased number of astrocytes in KM rats. Moreover we revealed a significant upregulation of glutamatergic activity in the hippocampus of KM rats that correlates with published data demonstrated a contribution of reactive astrocytes to the abnormal glutamatergic transmission.

Thus we demonstrated predisposition the NSC of KM rats to differentiation into the glial cells that could determine revealed alteration in glutamate transmission and mediates seizure susceptibility.