CORTICAL CIRCUITS IN HEALTH AND DISEASE

The Imbalance of Homocysteine Induces Seizure-Likes Events in Rat Hippocampus

Yakovlev A., Gerasimova E., Gataulina E., Shakhmatova V., Sitdikova G.*

Kazan Federal University, Kazan, Russia.
* E-mail: alv.yakovlev@gmail.com

Homocysteine, a thiol-containing amino acid derived from dietary methionine through demethylation. The breakage of metabolism due to genetic alteration in metabolic enzymes or deficiency in cofactors may lead to hyperhomocysteinemia. Abnormal accumulation of homocysteine during pregnancy induces learning deficits in offspring at early postnatal development. It was shown, that HHCy can contribute to seizures in patients with Down syndrome, depression and in alcohol withdrawal. The goal of this study was to estimate the sensitivity of hippocampal slices of newborn rats with prenatal hyperhomocysteinemia (pHHCy) to 4-aminopyridine–induced seizure-likes events (SLE) and the effects of homocysteine derivatives - homocysteine-thiolactone on the neuronal activity in rat hippocampus *in vivo*. Experiments were performed on slices of Wistar rats during second and third postnatal weeks (P9-19, P0-day of birth) using extracellular field electrodes in the CA3 pyramidal cell layer of hippocampus. To determinate the threshold of 4-aminopiridine for generation of SLE the convulsant was added by increasing doses. Pups with pHHCy were born from females received daily methionine with food. *In vivo* experiments Wistar rats of three age groups P5-7, P10-15 and P35–60 were used. Extracellular neuronal activities were recorded from hippocampus using 16-site linear silicon probes. D,L-homocysteine-thiolactone was administrated by intrahippocampal injection using glass pipette. Multiunit activity (MUA), local field potential (LFP) were detected and analyzed using MATLAB environment. Differences were considered as statistically significant at $p < 0.05$ in at least four independent experiments.

In control group application of 15-35 μM 4-aminopyridine induced a gradual increase of the frequency of multiunit activity of hippocampus neurons. In concentration of 50-75 μM 4-aminopyridine induced SLE in 52% cases (n=35) after 7.6±1.1 min of perfusion. In slices prepared from the hippocampus of rats with pHHCy the application of 15-35 μM 4-aminopyridine induced SLE in 40% of the cases with 7.1±1.2 min of seizure onset (n=31). Our findings indicate that pHHCy significantly lowers the threshold of 4-aminopyridine–induced SLE. It is known that homocysteine and its metabolites are potent agonists of NMDA-receptor, which are linked with epileptogenesis. The lowest dose of homocysteine-thiolactone used (0.03mg/2μl) produced seizures in 75% of immature rats. The spectral power of LFP increased up to 1331±662%, frequency of MUA - up to 1155±598% compared with basal neuronal activity in CA1 region of hippocampus. In P10 and P35 animals that 0.03mg/2μl of homocysteine-thiolactone increased only MUA frequency and SLE appeared only at 0.06mg/2μl (in 100% of animals). The spectral power of LFP increased up to 668±79% and 254±32%, frequency of MUA up to 358±45% and 396±126% compared to control in P10 and P35 animals correspondingly. The spectral analysis of LFP indicated that homocysteine-thiolactone increased the power of SLE in theta and alfa band frequency. Our data suggest that hippocampal neurons of immature rats have higher sensitivity to homocysteine-thiolactone which may underlie a high risk of seizure appearance in postnatal life in case of maternal hHCY. It is possible that pHHCy can induce the hyperexci\tability of neuronal network of immature hippocampus by stimulating NMDA-receptors and changing the electrophysiology property of neurons. This work was supported by RFBR № 18-015-00423

Consequences of Hippocampal Cholinergic Deficit Induced by 192igG-Saporin

Bolshakov A.P., Dobryakova Y.V., Zaichenko M.I., Manolova A.O., Stepanichev M.Y., Markevich V.A.*

Institute of Higher Nervous Activity and Neurophysiology, Moscow, Russia.
* E-mail: al230679@yandex.ru

Alzheimer's disease (AD) is associated with degeneration of cholinergic neurons in the basal forebrain. Intracerebroventricular administration of the immunotoxin 192IgG-saporin to rats, an animal model of AD, leads to degeneration of cholinergic neurons in the medial septal area. 1.5 months after injection, we studied effects of the immunotoxin on the behavior of rats, gene expression in the dorsal and ventral hippocampus using RNA-seq approach, responses of microglia and astrocytes using immunohistochemistry, and histopathology of the hippocampus. 192IgG-saporin-induced degeneration of cholinergic septal neurons impaired memory retention in Morris water maze. RNA-seq analysis showed that 192IgG-saporin strongly upregulated expression of microglia-specific genes only in the dorsal hippocampus. The following immunohistochemical examination showed that the number of microglial cells increased in the dentate gyrus of the dorsal hippocampus. In addition, treatment with 192IgG-saporin resulted in neuronal loss in the CA3 field of the hippocampus. Taken together, our data suggest that cholinergic degeneration in the medial septal area induced by intracerebroventricular administration of 192IgG-saporin results in an increase in the number of microglial cells and neuronal degeneration in the dorsal hippocampus.

Computing Hubs in the Hippocampus and Cortex

Wesley Clawson, Ana F. Vicente, Christophe Bernard, Demian Battaglia, Pascale P Quilichini*

Aix Marseille Univ, Inserm, INS, Institut de Neurosciences des Systèmes, Marseille, France.
* E-mail: christophe.bernard@inserm.fr

Neural computation, which relies on the active storage and sharing of information, occurs within large neuron networks in the highly dynamic context of varying brain states. Whether such functions are performed by specific subsets of neurons and whether they occur in specific dynamical regimes remain poorly understood. Using high density recordings in the hippocampus, medial entorhinal and medial prefrontal cortex of the rat, we identify computing microstates, or discreet epochs, in which specific computing hub neurons perform well defined storage and sharing operations in a brain state-dependent manner. We retrieve a multiplicity of distinct computing microstates within each global brain state, such as REM and nonREM sleep. Half of recorded neurons act as computing hubs in at least one microstate, suggesting that functional roles are not firmly hardwired but dynamically reassigned at the second timescale. We identify sequences of microstates whose temporal organization is dynamic and stands between order and disorder. We propose that global brain states constrain the language of neuronal computations by regulating the syntactic complexity of these microstate sequences.

Intracellular Dynamics of CA3 Pyramidal Cells *In Vivo* Across Brain States

*Meryl Malezieux, Ashley L. Kees and Christophe Mulle**

Interdisciplinary Institute for Neuroscience, CNRS UMR 5297, University of Bordeaux, F-33000 Bordeaux, France.
* E-mail: christophe.mulle@u-bordeaux.fr

During quiet wakefulness, the hippocampal local field potential (LFP) displays large irregular activity (LIA) punctuated by sharp-wave ripples, which play a role in memory consolidation, whereas during exploratory behaviors, hippocampal LFP oscillates at both theta and gamma frequencies. Monitoring the intracellular dynamics of individual neurons *in vivo* such as membrane potential, intrinsic excitability, and synaptic efficacy is essential to understand how single-cell properties contribute to network processing across these different brain states. We have explored the intracellular dynamics of pyramidal cells (PCs) in the area CA3 of the hippocampus in awake mice. CA3 is important for rapid encoding of memory and integrates multimodal information from the entorhinal cortex, dentate gyrus, and CA3 itself before output to CA1. We have used whole-cell patch-clamp recordings from CA3 PCs in awake head-fixed mice combined with measurements of pupil diameter, treadmill running speed and LFP recordings of oscillatory activity. Our findings show that a large proportion of CA3 PCs are prone to intracellular modulation during changes in brain states. During theta states, most CA3 PCs hyperpolarized, reduced their firing, and had lower Vm variance as compared to LIA states. These results demonstrate a specific role for CA3 PCs during theta as compared to CA1 and DG cells which do not show any intracellular modulation. During periods of hyper-arousal such as theta states, a small subpopulation of CA3 PCs is however selectively active while most CA3 PCs are silenced. This modulation at the single-cell level in CA3 could play a role in the emergence of oscillations, and underlie the ability of CA3 to perform different memory functions during different brain states.

Acute Changes in Cortical Network Functions During Endothelin-1 Induced Local Brain Ischemia

Daria Vinokurova, Andrey Zakharov, Nailya Lotfullina, Kseniya Chernova, Gulshat Burkhanova, Julia Lebedeva, Roustem Khazipov*

Kazan Federal University, Kazan, Russian Federation.
* E-mail: dariavinokurova.kfu@gmail.com

Subarachnoid hemorrhage (SAH) is often complicated by cerebral vasospasm and delayed ischemic damage. Key player in this process is a potent vasoconstrictor endothelin-1 (ET-1). In the present study, we explored the effects of epipial application of ET-1 on spontaneous and sensory-evoked activity at different depths of the cortical column of rat barrel cortex (BCx - whiskers representation area) using linear 16-channel silicon probes. ET-1 was delivered epipially for 1 hour and then washed by ACSF during 3 hours. ET-1 application first caused several minutes long suppression of both spontaneous and evoked activity in all cortical layers, followed by a short burst of multiunit activity organized in gamma oscillation in layers 5 and 6 and then by cortical spreading depression (CSD). In most cases spontaneous and sensory-evoked activity was fully blocked after CSD episode and remained severely depressed during ET-1 application. In some cases, activity partially recovered in deep layers after the first CSD and several recurrent CSDs restricted to the deep layers were observed. In all cases slow large negative shift of the extracellular potential developed through the time course of ET-1 application attaining maximal values of up -80mV in deep layers. Washing of ET-1 resulted in recovery of the DC potential, but only weak recovery of spontaneous and evoked activity was observed. Histological examination of brain sections revealed focal ischemic damage in the barrel cortex at the site of ET-1 application. Thus, ET-1 induced focal ischemia induces sequential cortical layer specific changes in spontaneous and sensory-evoked activity in cortex that could be useful for the rapid detection of the onset of cerebral vasospasm and ischemia and for the prevention of the delayed ischemic damage in patients with subarachnoid hemorrhage.

This work was supported by RSF (17-15-01271) and performed in the framework of the Program of Competitive Growth of Kazan Federal University.

OM&P

Gene Therapy for Refractory Epilepsy

*Dimitri Kullmann**

UCL, Institute of Neurology, London,United Kingdom.
* E-mail: d.kullmann@ucl.ac.uk

Epilepsy is common and 25-30% of affected individuals continue to experience seizures despite optimal medication. Conventional anti-seizure drugs mainly work by reducing excitation or promoting inhibition, but have a limited therapeutic index. First, the entire brain is exposed, and not only the epileptogenic zone. Second, drugs generally act indiscriminately on excitatory and inhibitory neurons. Gene therapy offers the opportunity for treatment to be targeted to a specific region of the brain, and, by use of cell-type specific promoters, to bias expression of therapeutic transgenes to specific cell types. I shall summarise work showing successful suppression of seizures in rodent models of epilepsy by overexpressing the potassium channel Kv1.1 in excitatory neurons, and by using optogenetics and chemogenetics. I shall also describe an autoregulatory strategy that uses a glutamate-gated chloride channel to suppress seizures in response to pathological elevations of glutamate. Some of these strategies are ready for clinical translation.

Mathematical Modeling of Brain Logistics: The Meeting Point

*Dmitry Postnov**

Saratov State University, Russia.
* E-mail: postnovdmitry@googlemail.com

Recent advances in the understanding of complex interactions between the processes that maintain and control the physiological state of brain parenchyma provide new challenges for modeling studies on the topic. Indeed, any considerable alteration of neural activity is accompanied by measurable changes in physiological parameters. Intercellular interactions within the so-called "neuro-glial-vascular unit" not only coordinate local responses to changes in neural activity, and also lead to the formation of spatially extended patterns within functional subsystems of brain "logistics", such as the activity of astrocyte networks, transfer of substances in the intercellular space, or the propagated vascular responses. Being usually subtle, these physiological mechanisms become dominant during the extreme states of the cerebral cortex, such as spreading cortical depression, migraine with aura, and also the propagation of depolarization waves during stroke or as a result of brain injury.

New impuls to research in this area has been given by discussion of the mechanisms that provide drainage of the brain parenchyma. The brain is the only organ in whose parenchyma no lymphatic system has been found to date. In 2012, research group leaded by M. Nedergaard proposed a hypothesis about the so-called "glymphatic" drainage system, in which the key role is played by the flow of water through the pores of the astrocyte membrane and the process of "pumping" the cerebrospinal fluid into the parenchyma due to pulsations of arterial walls. The latter has caused a heated debate, continuing at the present time. Note that a reliable understanding of these mechanisms is extremely important for a wide range of medical problems, such as Alzheimer's disease, or the delivery of drugs through the blood-brain barrier. Currently, there are a number of attempts to simulate the process of "pumping" the cerebrospinal fluid into the intercellular space, mainly related to the criticism of the propulsive mechanism.

Thus, a whole range of actual problems in the physiology of the brain urgently requires an understanding of how the cellular "machine" of the brain parenchyma (neurons, astrocytes, vascular smooth muscle cells, and endothelial cells) functions as a whole, including coordinating changes in cell activity with modulation of the volume space and intensity of transport of substances in it. This large-scale task is simultaneously a promising prospect and a serious challenge for model-theoretical research in this area. One may say, in this focus point different physiological and medical problems meet in demand the adequate and self-consistent mathematical modeling.

Dynamical Modeling of Absence Seizures by a Complex Network of Neuronal Oscillators

T. M. Medvedeva[1], M. V. Sysoeva[2], A. Lüttjohann[3], G. van Luijtelaar[4] and I. V. Sysoev[5,6]*

[1] Institute of Higher Nervous Activity and Neurophysiology of RAS, Moscow, Russia;
[2] Yuri Gagarin State Technical University of Saratov, Saratov, Russia;
[3] Institute of Physiology I, Westfalische Wilhelms University, Munster, Germany;
[4] Donders Centre for Cognition, Radboud University Nijmegen, Nijmegen, the Netherlands;
[5] Saratov State University, Saratov, Russia;
[6] Saratov Branch of the Institute of Radioengineering and Electronics of Russian Academy of Sciences.
* E-mail: golova93tanya@gmail.com

Absence epilepsy is a generalized form of epilepsy characterized by short-term loss of consciousness without convulsive manifestations. This form of epilepsy is usually observed in children and adolescents [1]; it may either remit [2] or turn into other convulsive forms [3] with time. Electroencephalography is the main method to register manifestations of absence epilepsy, since EEG recordings show the typical spike-wave discharges (SWDs) during absence seizures.

To understand the mechanisms of absence epilepsy occurrence it is important to build computational models that would reproduce main features of SWDs. Such models are known at various levels of organization: from models of an individual neuron excitation due to pathological changes in the concentration of neurotransmitters [4], to a large phenomenological model in the form of an ensemble of Kuramoto phase oscillators [5].

The importance of the neural network structure for the occurrence of spike-wave discharges remains an open question. Therefore, the aim of this work is to create such a model, which will take into account the hierarchy of the functional units organization of neural connections that are involved in the initiation and maintenance of absence discharges.

We used a two level mesoscale model. The first level consists of four structures: the nervus

trigeminus serving as an input, the the ventro-posterior medial nucleus of the thalamus (VPM), reticular nucleus of the thalamus (RTN) and the somatosensory cortex; the second level is represented by nearby situated neurons belonging to one of four modeled structures. The dynamics of an individual element was presented by FitzHugh–Nagumo equations [6]. The model reproduces the main features of the transition from normal to epileptiformic activity (an increase in the oscillation amplitude, the emergence of the main frequency and its higher harmonics) and its spontaneous abortion.

The work was supported by Russian Foundation for Basic Research, Grant No. 18-015-00418.

References

1. Panayiotopoulos CP. Treatment of typical absence seizures and related epileptic syndromes. Paediatric Drugs. 2001; Vol. 3. P. 379-403
2. Sadleir L. G., Farrell K., Smith S. et al. Electroclinical features of absence seizures in childhood absence epilepsy. Neurology. 2006; Vol. 67, № 3. P. 413–418.
3. Berg, A., Levy, S., Testa, M., & Blumenfeld, H. Long-term seizure remission in childhood absence epilepsy: might initial treatment matter. Epilepsia. 2014; Vol. 55 № 4. P. 551–557
4. Destexhe A, McCormick DA, Sejnowski TJ. A model for 8–10 Hz spindling in interconnected thalamic relay and reticularis neurons. BiophysJ. 1993; Vol. 65. P. 2473–7.
5. Schmidt H, Petkov G, Richardson MP, Terry JR. Dynamics on networks: the role of local dynamics and global networks on the emergence of hypersynchronous neural activity. PLoS Comput Biol. 2014; 10:e1003947.
6. FitzHugh, R. Mathematical models of threshold phenomena in the nerve membrane. Bulletin of Mathematical Biophysics. 1955; Vol. 17. P. 257–269