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NEURON-GLIA INTERACTIONS

Astroglial Control of The Respiratory Rhythm-Generating Circuits

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Astrocytes are implicated in modulation of neuronal excitability and synaptic function, but it remains largely unknown if these glial cells can directly control activities of motor circuits to influence complex behaviours *in vivo*. Our studies conducted in collaboration with Prof Sergey Kasparov (University of Bristol) and Dr Jeffrey C. Smith (National Institutes of Health) focused on the vital respiratory rhythm-generating circuits of the preBötzinger complex (preBötC) located in the lower brainstem and determined how compromised function of the preBotC astrocytes affects breathing in conscious experimental animals (rats). It was found that blockade of vesicular release in preBötC astrocytes reduces the resting breathing rate and frequency of periodic sighs, decreases rhythm variability, impairs respiratory responses to hypoxia and hypercapnia, and dramatically reduces the exercise capacity. These findings indicate that astrocytes modulate the activity of CNS circuits generating the respiratory rhythm, critically contribute to adaptive respiratory responses in conditions of increased metabolic demand and determine the exercise capacity.

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Changes of Theta-Rhythm Epochs are Evaluated by Activity-Dependent Increase in Tonic GABAA Conductance

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Theta-rhythm appears is epochs that contribute to encoding of spatial and behavioral Information in the hippocampus. Here we sought to study a mechanism responsible for occurrence of these second-order oscillations. We recorded hippocampal theta rhythms with extracellular electrode through a wireless system in freely moving mice. Rhythmic modulation of hippocampal theta power was studied using wavelet analysis. 1 μM picrotoxin was injected through cannula into the mice hippocampus to selectively block tonic GABAA conductance. The concentration significantly increased the length of theta rhythm epochs and decreased inter-epoch interval. Larger concentrations of picrotoxin (10 μM and 100 μM), which are known to block both tonic GABAA currents and IPSCs, made theta rhythm epochs shorter and inter-epoch intervals longer. This finding is consistent with important role of synaptic GABAergic connections in rhythm generation. Then we facilitated activity dependent tonic GABAA conductance by blocking GABA uptake. Both the GAT-3 inhibitor SNAP-5114 (100 μM) and GAT-1 inhibitor NNC-711 (10 μM) significantly shortened the epochs and increased inter-epoch intervals. 100 nM allopregnanolone shortened the epochs and increased inter-epoch intervals in the same manner.

We suggest that accumulation of extracellular GABA during synchronized neuronal activity inhibits neurons and stop their firing, hence rhythmic activity. When neuronal activity is reduced, ambient GABA concentration also decreases. Such fluctuations in ambient GABA are responsible for rhythmic modulation of theta oscillations. GABA uptake or enhancement of tonic GABAA current with allopregnanolone modulate the epochs of the theta rhythms.

Interpreting Ca Activity in Astrocytes: from Raw Data to Events to Models

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Analyzing spontaneous or evoked astrocytic Ca activity is different from that of neuronal activity. In astrocytes, there are no fixed spatial sources of signals, as Ca transients can originate in many places and tend to spread in different directions.

We present some open-source solutions for motion correction, estimation of dynamic baseline fluorescence level and detection of Ca^{2+} events. In many steps we rely on dimensionality reduction techniques to extract meaningful dynamics. We also follow patch-oriented localized analysis with selective grouping of time signals to keep details of the dynamics.

We further discuss approaches to interpret the observed spatiotemporal patterns of Ca^{2+} events by converting the dynamics into symbolic form or fitting simplified data-driven models for modal description of the spatiotemporal patterns.

Astroglia-Controlled Activity of Cells Within The Neurovascular Unit

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Neurovascular unit (NVU) consists of brain microvessel endothelial cells (BMECs), pericytes, astrocytes, neurons, and microglia. Structural and functional integrity of blood-brain barrier (BBB) which is a part of NVU is coordinated by various types of intercellular communication within the NVU. It is generally accepted that functioning of NVU/BBB is dramatically compromised in almost all the types of brain pathology (neurodevelopmental disorders, neurodegeneration, trauma, ischemia, neuroinflammation, neuroinfection etc.), therefore deciphering intercellular communications in NVU might shed light on novel approaches to manipulating NVU activity and recovery.

Perivascular astroglial cells may have central regulatory position within this multicellular ensemble. Contribution of astrocytes to the regulation of neuroplasticity or BBB integrity is based on the secretion of gliotransmitters, cytokines, release and uptake of metabolites. Thus, atrsoglia coordinates neuron-astrocyte metabolic coupling, establishment and functioning of the astroglial networks, and astrocyte-endothelial interactions, thereby resulting in adjustment of local blood flow to actual neuronal needs.

Such regulatory role of astroglia is important in neurogenic niches where vascular scaffold controls all the stages of neural stem cells (NSCs) and neuronal progenitor cells (NPCs) development. Actually, neurogenic niches represent the well-coordinated platform for tight integration of neurogenesis, vascular support, and angiogenesis based on specific properties of BMECs and perivascular cells critical for adjusting the local microenvironment to current metabolic needs of NSCs/NPCs.

We used original static and dynamic NVU/BBB *in vitro* models to study regulatory potential of astroglial cells on NSCs/NPCs development and BMECs activity in normal conditions and in the Alzheimer's type of neurodegeneration. We found that intercellular interactions within the NVU/BBB are partially controlled by perivasculr astroglia, and these mechanisms are compromised in cerebral amyloid angiopathy. Thus, astrocytes could be considered as target cells for controlling neurogenesis and endothelial-driven mechanisms of neuroplasticity.

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Role of Inorganic Polyphosphate in Neuron-Glia Interaction

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Inorganic polyphosphate (polyP) is present in every cell and is highly conserved from primeval times. In mammals, polyP plays multiple roles in including control of cell bioenergetics and signal transduction. In the brain, polyP mediates signalling between astrocytes via activation of purinergic receptors. Recently we identified of polyP-containing vesicles and the main triggers for polyP release from these vesicles in cortical astrocytes. In cultured astrocytes, polyP was found to be localized predominantly within the intracellular vesicular compartments which express vesicular nucleotide transporter VNUT (putative ATP-containing vesicles), but not within the compartments expressing vesicular glutamate transporter 2 (VGLUT2). Release of glutamate reduces glutamate- and AMPA- but not a NMDA- induced calcium signal. Effect of polyP on glutamate and specifically AMPA receptor was dependent on the presence of P2Y but not P2X receptor inhibitors. Pre-incubation cortical neurons with polyP reduced as initial calcium peak and reduced the number of neurons with delayed calcium deregulation in response to high concentrations of glutamate that results to protection of neurons against glutamate-induced excitotoxicity. Thus, activation of P2Y receptors by polyP reduced calcium signal in AMPA receptors that protecting neurons against glutamate excitotoxicity.

Homeostatic Control of Hippocampal Excitatory Transmission by Astrocytes

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The circadian rhythmicity with which mammals sleep, feed, regulate their body temperature and engage in reproductive behaviors is controlled by the main circadian master clock, the supra-chiasmatic nucleus SCN of the hypothalamus, and by the coordinated activity of other oscillators distributed throughout the brain. Astrocytes are thought to be competent circadian oscillators, because they are capable of changing the expression level of different clock genes. What it not known is how these phenomena affect the structural and functional properties of these cells, including their ability to modulate excitatory synaptic transmission among neurons. By combining electrophysiology recordings with 3D axial STEM tomography and protein retention expansion microscopy, we show that astrocytes undergo daily changes in their morphology which alter their glutamate uptake capacity. This has profound consequences for modulation of synaptic integration and plasticity in neurons. Together, these findings indicate that the brain undergo regular remodeling processes largely mediated by astrocytes.

Activity-Dependent Astroglial Control of Supra-Linear Dendritic Integration via NDMAR Co-Agonist Supply

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Dendrites of hippocampal CA1 pyramidal cells (PCs) integrate local synaptic input. Supra-linear integration of spatially clustered CA3-CA1 input depends on amplification by voltage-gated sodium channels and N-methyl-D-aspartate receptors (NMDARs). Our previous work demonstrated that NMDAR function depends on astroglial supply of the NMDAR co-agonist D-serine. This observation implies that NMDAR-dependent supra-linear integration is also controlled by NMDAR co-agonist supply. We used whole-cell patch clamp recordings combined with micro-iontophoretic glutamate application and two-photon excitation fluorescence microscopy to test this hypothesis in acute hippocampal slices. We found that, indeed, application of exogenous D-serine reduces the threshold of dendritic spikes and increases their amplitude. Endogenous D-serine supply depends on astrocyte Ca^{2+} signaling, which can be triggered by activation of astrocyte endocannabinoid receptors (CB1R). As expected, application of a CB1R agonist induced prominent Ca^{2+} transients in astrocytes and boosted supra-linear integration. Also, endogenous release of endocannabinoids triggered by stimulation of CA1 PCs axons resulted in an enhanced dendritic integration. This effect was not seen in the presence of saturating concentrations of D-serine, DAAO (D-serine degrading enzyme) or when CB1Rs were inhibited. Moreover, an astrocyte-specific knockout of CB1Rs prevented the effect. Interestingly, the boost of integration could only be observed when CA1 PC axons were stimulated at 10Hz and 20Hz, but not at 4Hz or 40Hz. In summary, we reveal a novel signaling pathway that creates a frequency-dependent positive feedback of CA1 PC population activity on their supra-linear dendritic integration involving astrocyte and CB1R-dependent NMDAR co-agonist supply.

Cellular Mechanisms of Neuropsychiatric Symptoms Induced by Thyroid Dysfunction

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Thyroid hormones (THs) are essential not only for the development of the central nervous system (CNS) but also for matured brain function. In the CNS, circulating thyroxine (T4) crosses blood-brain barrier via specific transporters and is taken up to astrocytes, becomes L-tri-iodothyronine (3, 3', 5–triiodothyronine; T3), an active form of TH, by type 2 de-iodinase (D2). T3 is released to the brain parenchyma from astrocytes (glioendocrine system). In adult CNS, both hypo- and hyper-thyroidism, the prevalence in female being >10 times higher than that in male, may affect psychological condition and potentially increase the risk of cognitive impairment and neurodegeneration including Alzheimer's disease (AD). We have reported that non-genomic effects of T3 on microglial functions and its signaling [1] and sex- and age-dependent effects of THs on glial morphology in the mouse brains of hyperthyroidism [2, 3]. Behavioral changes and spine density in hippocampus also showed sex-dependence. Recently we analyzed the opposite thyroid dysfunction, hypothyroidism, and found sex- and age-dependent changes in glial morphology and animal behavior as well. These results may help to understand physiological and/or pathophysiological functions of THs in the CNS and how hyper- and hypothyroidism affect psychological condition and cognition.

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The Role of Astrocytes in Seizure Generation: Insights from a Novel *In Vitro* Seizure Model Based on Mitochondrial Dysfunction

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Approximately one-quarter of patients with mitochondrial disease experience epilepsy. Their epilepsy is often severe and resistant towards conventional antiepileptic drugs. Despite the severity of this epilepsy, there are currently no animal models available to provide a mechanistic understanding of mitochondrial epilepsy. We conducted neuropathological studies on patients with mitochondrial epilepsy and found the involvement of the astrocytic compartment. As a proof of concept, we developed a novel brain slice model of mitochondrial epilepsy by the application of an astrocytic-specific aconitase inhibitor, fluorocitrate, concomitant with mitochondrial respiratory inhibitors, rotenone and potassium cyanide. The model was robust and exhibited both face and predictive validity. We then utilized the model to assess the role that astrocytes play in seizure generation and demonstrated the involvement of the GABA-glutamate-glutamine cycle. Notably, glutamine appears to be an important intermediary molecule between the neuronal and astrocytic compartment in the regulation of GABAergic inhibitory tone. Finally, we found that a deficiency in glutamine synthetase is an important pathogenic process for seizure generation in both the brain slice model and the human neuropathological study. Our study describes the first model for mitochondrial epilepsy and provides a mechanistic insight into how astrocytes drive seizure generation in mitochondrial epilepsy.

Effect of High-Fat Diet to Morphofunctional Characteristics of Astrocytes in Mouse Hippocampus

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Over 1.9 billion adults over 18 years of age are overweight. Of these, over 650 million were obese. But how chronic high-fat diet (HFD) effects to brain function is unclear. We studied 3 months age animals after one month of HFD. Astrocytes were recorded in whole-cell voltage-clamp mode for electrophysiological analysis and simultaneously loaded with Alexa Fluor 594 for two-photon microscopy morphological study. HFD induced significant remodeling in astrocytic distal branches as well as an increase in volume fraction of perisynaptic astrocytic leaflets unresolved with diffraction limited optical microscopy. No significant difference after HFD in the number of cells coupled through the gap-junctions in the astrocytic syncytium and in the length constant of coupling were found. The activity-dependent facilitation of glutamate transporter current and facilitation of synaptically-induced K^+ current were not different in control and astrocytes after HFD. However, the activity-dependent increase in transporter current decay time was abolished after HFD. These findings suggest that enhanced astrocytic coverage of synapses may prevent glutamate spillover after HFD. Then we performed confocal Ca^{2+} imaging in astrocytes in CA1 *str. radiatum*. Spontaneous Ca^{2+} event size decreased in the astrocytic network after HFD. However, the events duration was increased, as well as Ca^{2+} event integral. The long-term potentiation was significantly enhanced in CA3-CA1 synapses after HFD. Thus, along with astrocyte remodeling and enhanced Ca^{2+} signaling, temporary HFD promotes synaptic plasticity in young mice. Our results are consistent with the beneficial effects of high fat intake in young age.

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Astrocyte Remodeling and Enhanced Synaptic Plasticity are Induced by One Month of Caloric Restriction in Mice

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Caloric restriction (CR), without deficiency of essential nutrients, increases average and maximal lifespan. But, how it affects to morphofunctional characteristics of astrocytes is not completely clear. In this study, one month of calorically restricted diet (CR) induced morphological plasticity of astrocytes in the *stratum (str.) radiatum* of hippocampal CA1 area in three-months old mice. The volume fraction of distal perisynaptic astrocytic leaflets increased whereas the number of coupled astrocytes through gap-junction decreased. Reduced astrocyte coupling found in CR was not associated with changes in the expression of connexin 43. Uncoupling and morphological remodeling may affect spontaneous Ca^{2+} activity in the astrocytic network. Indeed, Ca^{2+} events were longer, whereas their spread was reduced in CR compared with control mice. Such change in the pattern of astrocytic Ca^{2+} activity may increase spatial resolution of the information encoding in the astroglial network. Consistent with expanded synaptic enwrapping by the astroglial processes, spillover of synaptically released K^+ and glutamate was diminished after CR. However, no significant changes in the expression of astrocytic glutamate transporter (GLT-1/EAAT2) were observed, although the level of glutamine synthetase was decreased. Glutamate uptake is known to regulate the synaptic plasticity. Indeed, the magnitude of long-term potentiation (LTP) in the glutamatergic CA3-CA1 synapses was significantly enhanced after CR diet. Our findings highlight an astroglial basis for improved learning and memory reported in various species exposed to CR.

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Noradrenergic Regulation of Astroglial Morphology and Function in Normal and Pathologic States

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Impairment of the main noradrenergic nucleus of the human brain, the *Locus coeruleus* (LC), which has been discovered in 1784 by Félix Vicq-d'Azyr (1748–1794), a French physician and neuroanatomist, represents one of defining factors of neurodegenerative diseases progression. Projections of LC neurons release noradrenaline, which stimulates astrocytes, heterogeneous, homeostatic neuroglial cells enriched with adrenergic receptors. There is a direct correlation between the reduction of noradrenergic innervations and cognitive decline associated with ageing and neurodegenerative diseases. It is, therefore, hypothesised that the resilience of LC neurons to degeneration influences the neural reserve that in turn determines cognitive decline. Deficits in the noradrenergic innervation of the brain might be reversed or restrained by increasing the activity of existing LC neurons, transplanting noradrenergic neurons, and/or using drugs that mimic the activity of noradrenaline on astroglia. In this lecture these strategies will be discussed along with presenting how the activation of adrenergic receptors modulate the morphology (cytotoxic edema), aerobic glycolysis and vesicle-based signaling in astrocytes. In particular we will address how fingolimod and ketamine, two established drugs used to treat neuroinflammation in multiple sclerosis and major depression, respectively, affect astrocytes.

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Perisynaptic Astrocytic Leaflets Remodeling after *Status Epilepticus* in the Rat Hippocampus

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Previously we demonstrated that rat Li^+/Pilocarpine-induced *status epilepticus* (SE) induces atrophy of astrocytic branches and astrocyte uncoupling in the hippocampus. However, the volume fraction (VF) of perisynaptic astrocytic leaflets (PALs) does not change. This finding does not rule out re-arrangement of PALs within synaptic microenvironment. Here we analyzed 3D reconstructions of astrocytic processes and dendritic spines obtained from serial section electron microscopy images of CA1 hippocampal neuropil in 3 weeks old Sprague-Dawley rats in 3 months after SE induction. We found a significant reduction in the density of dendritic spines compared with controls. Astrocytic branches had a similar surface-to-volume ratio (SVR) after SE, while SVR of PALs was significantly decreased. This suggests that PALs became thicker, which may reflect their retraction from the synapses. Indeed, although overall VF of the PALs did not change, it significantly decreased in the immediate vicinity of dendritic spines. The overall surface of the direct contact of PALs with a spine also significantly decreased after SE. In agreement with astrocytic branches atrophy, the integral VF of astrocytic branches became smaller. The reduction of astrocytic coverage of synapses correlated with enhanced glutamate spillover, which was detected as activity-dependent prolongation of glutamate transporter currents measured in whole-cell voltage-clamped CA1 astrocytes in response to extracellular stimulation of Shaffer collaterals. In addition to retraction of PALs, enhanced spillover may occur due to impaired glutamate uptake or glutamate-glutamine shuttle. Thus, we analyzed the subcellular distribution of astrocytic glutamate transporter (GLT-1) and glutamine synthetase (GS) with immunocytochemical staining. Our results suggest that SE induces retraction of PALs and promotes glutamate spillover which may make the tissue prone to epileptiform activity.

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