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FUNCTION AND PLASTICITY OF SYNAPSES

Transient Switching of NMDA-Dependent Long-Term Synaptic Potentiation in CA3-CA1 Hippocampal Synapses to mGluR1-Dependent Potentiation after Pentylenetetrazole-Induced Acute Seizures in Young Rats

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The mechanisms of impairment in long-term potentiation after *status epilepticus* (SE) remain unclear. We investigated the properties of LTP induced by theta-burst stimulation in hippocampal slices of rats 3 h and 1, 3, and 7 d after SE. Seizures were induced in 3-wk old rats by a single injection of pentylenetetrazole (PTZ). Only animals with generalized seizures lasting more than 30 min were included in the experiments. The results revealed that LTP was strongly attenuated in the CA1 hippocampal area after PTZ-induced SE as compared with that in control animals. Saturation of synaptic responses following epileptic activity does not explain weakening of LTP because neither the quantal size of the excitatory responses nor the slopes of the input-output curves for field excitatory postsynaptic potentials changed in the post-SE rats. After PTZ-induced SE, NMDA-dependent LTP was suppressed, and LTP transiently switched to the mGluR1-dependent form. This finding does not appear to have been reported previously in the literature. An antagonist of NMDA receptors, D-2-amino-5-phosphonovalerate, did not block LTP induction in 3-h and 1-d post-SE slices. An antagonist of mGluR1, FTIDS, completely prevented LTP in 1-d post-SE slices, whereas it did not affect LTP induction in control and post-SE slices at the other studied times. mGluR1-dependent LTP was postsynaptically expressed and did not require NMDA receptor activation. Recovery of NMDA-dependent LTP occurred 7 d after SE. Transient switching between NMDA-dependent LTP and mGluR1-dependent LTP could play a role in the pathogenesis of acquired epilepsy.

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Natural Toxins Inhibit Gating Pore Currents Underlying Rare Channelopathies

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Neurotoxins produced by venomous animals such as spiders or snakes are well-known selective ligands of ion channels and neuroreceptors. The specificity and potency of some neurotoxins with respect to particular ion channel isoforms exceeds that of available antibodies. This is why these substances are used routinely in molecular neurobiology to characterize their targets. We make a step forward by identifying toxins that modify selective properties of not just native ion channels but their mutant forms characteristic of genetic diseases.

Hypokalemic and hyperkalemic periodic paralyses are orphan diseases with symptoms of muscle weakness and paralysis exuberated by changes in serum potassium levels. The identified molecular pathomechanism is muscle membrane depolarization that leads to inactivation of voltage-gated ion channels and irresponsiveness to nerve stimuli. The depolarization is due to so-called gating pore currents in mutant ion channels such as the muscle-type sodium channel Nav1.4. We found that gating modifier toxins from spider venom inhibit gating pore currents through the mutant channels. Interestingly, toxins present differential activity and inhibit selectively gating pore currents through different domains of the channels. We conclude that spider neurotoxins represent the long sought for hits for development of drugs to treat hypokalemic and hyperkalemic periodic paralysis.

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Asynchronous Neurotransmitter Release at the Mouse Hippocampal Synapses between CCK+ Interneurons and CA1 Pyramidal Cells

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Asynchronous release (AR) of neurotransmitter is characteristic feature for the number of synapses in the CNS and in neuromuscular junctions. However, it is more pronounced during neurodegenerative disorders. Thus detailed study of the mechanisms underlying AR is necessary for better understanding of normal and pathological brain functioning. Although the molecular machinery involved in AR generation is relatively well studied, the Ca^{2+} source allowing prolonged vesicle fusion remains unclear. Therefore, the main goal of this project was to test the possible contribution of the different sources to the presynaptic Ca^{2+} elevation and generation of AR at synapses between CCK+ interneurons and CA1 pyramidal cells. All experiments conducted on acute mice hippocampal slices (P14-21) using simultaneous pair recordings from connected neurons. We found that AR at synapses between CCK+ interneurons and CA1 pyramidal cells does not require activation of calcium permeable NMDA receptor, channels, presynaptic vanilloid receptors (TRPV-1) or Ca^{2+} release from internal stores.

Our data suggest that the long lasting Ca^{2+}elevation results from reduction of Ca^{2+} extrusion rate due to low expression of Ca^{2+}-ATPase at this synapses. Remaining Ca^{2+} clearing presynaptic complex, Na^{+}/Ca^{2+} exchanger, fails to reduce intraterminal Ca^{2+} concentration to the values insufficient to trigger vesicle fission. Moreover high frequency stimulation leading to robust Na+ entry to the terminal might switch exchanger to the reverse mode which could prolong AR duration.

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Novel Cannabinoid Receptor-Mediated Synaptic Plasticity in the Hippocampus

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The endocannabinoid system plays a key role in regulating brain function and can modulate neurotransmitter release in various brain regions, including the hippocampus, where cannabinoids are known to affect memory function and have well-established presynaptic actions on both excitatory and inhibitory hippocampal synaptic transmission. Numerous studies have examined the modulatory effects of cannabinoids at hippocampal Schaffer collateral (SC)-CA1 synapses. However the effect of cannabinoids on the anatomically-distinct temporoammonic (TA) input to hippocampal CA1 neurons remains to be established.

Standard extracellular recordings were used to examine the effects of different selective agonists for CB1 receptors on excitatory synaptic transmission at juvenile TA-CA1 synapses. Recordings were made from transverse hippocampal slices (350μM) prepared from 12-18 day old rats, perfused with oxygenated aCSF.

Application of methanandamide (100nM; 15min) induced a long-term increase (LTP; to 148± 5% of baseline; n=4; $p<0.001$) in excitatory synaptic transmission via activation of CB1 receptors, as the CB1R- antagonist AM251 (10nM) blocked this effect (102 ± 1.1% of baseline; n=4; $p>0.05$). This CB1R-induced LTP had a postsynaptic locus of expression and was NMDA receptor-dependent. Furthermore, the PI3-kinase signaling pathway underlies this effect as treatment with the inhibitor of PI3-kinase, wortmannin (50 nM) blocked CB1R-induced LTP (100 ± 1.3% of baseline; n=4; $p>0.05$). These findings suggest that CBR1 activation can have marked, postsynaptic actions on hippocampal plasticity and that these effects are pathway-specific.

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Multiplex Imaging Relates Quantal Glutamate Release to Presynaptic Calcium Homeostasis *In Situ*

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Information processing by brain circuits depends on Ca^{2+}-dependent, stochastic release of the excitatory neurotransmitter glutamate. Whilst optical glutamate sensors have enabled detection of synaptic discharges, understanding presynaptic machinery requires simultaneous readout of glutamate release and nanomolar presynaptic Ca^{2+} *in situ*. Here, we find that the fluorescence lifetime of the red-shifted Ca^{2+} indicator Cal-590 is Ca^{2+}-sensitive in the nanomolar range, and employ it in combination with green glutamate sensors to relate quantal neurotransmission to presynaptic Ca^{2+} kinetics. Multiplexed imaging of individual and multiple synapses in identified axonal circuits reveals that glutamate release efficacy, but not its short-term plasticity, varies with time-dependent fluctuations in presynaptic resting Ca^{2+} or spike-evoked Ca^{2+} entry. Within individual presynaptic boutons, we find no nanoscopic co-localisation of evoked presynaptic Ca^{2+} entry with the prevalent glutamate release site, suggesting loose coupling between the two. The approach opens a new horizon in our understanding of release machinery at central synapses.

Neurotoxicity of Prenatal Hyperhomocysteinemia: Neuroprotective Effects of Hydrogen Sulfide

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Homocysteine is a sulfhydryl-containing amino acid derived from methionine. The cellular concentration of homocysteine is regulated by two key pathways: remethylation back to methionine or transsulfuration to cysteine with simultaneous production of hydrogen sulfide (H_2S). Homocysteine levels increased in different conditions including genetic factors, diet, life style or miscellaneous medication. Elevated levels of the homocysteine, called hyperhomocysteinemia (hHcy), are associated with higher risk of neurovascular diseases, dementia, developmental impairments or epilepsy. Oxidative stress is one of the common mechanisms of homocysteine induced disorders. H_2S as an established gasotransmitter, implicated in the regulation of numerous physiological functions is also well known for its neuroprotective potential. Recent data indicate that the level of H_2S decreased in hHcy conditions which may mediate homocysteine induced neurotoxicity. In our study we analyzed the early behavioral impairment and the level of oxidative stress in brain of offspring with prenatal hHcy and the protective role of H_2S. At the same time we elucidated some cellular mechanisms of acute and chronic application of homocysteine and possible mechanisms of H_2S mediating neuroprotection. The obtained data may underlie the new therapeutic approaches to prevent homocysteine-induced neurotoxicity.

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Activity of Fast-Spiking GABAergic Interneurons During Human Neocortex Complex Events

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Rodents, which are most commonly used experimental animals in the cellular neuroscience research show some differences to the human in operation of neocortical microcircuits. Supragranular layer of the human neocortex contains a subpopulation of glutamatergic pyramidal cells (PCs), which form strong multivesicular excitatory synapses specifically to GABAergic inhibitory interneurons. Similar connections have not been reported in the rodent supragranular neocortex. These synapses in the human comprise approximately 10% of excitatory connections between the layer 2/3 PCs and the interneurons in the frontal and temporal cortices. The pyramidal cells are called "master PCs", because they evoke suprathreshold excitatory postsynaptic potential in the interneurons. Consequently, a single master PC spike elicits temporally-structured firing of local GABAergic inhibitory cells. In this talk I will present data on the human neocortex master pyramidal cell circuits. In addition, I will demonstrate how the master PCs generate neuronal network activity by unitary spike, and how synaptic long-term plasticity controls the activity. Finally, I will present results showing robust self-inhibition in human fast-spiking basket cells through GABAergic autaptic connections and I will demonstrate how this contributes to the neuronal activity in the supragranular layer.

Calcium Dynamics and Information Coding at Hippocampal Mossy Fibre Terminals

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Mossy fiber terminals in the hippocampus form large complex terminals innervating pyramidal cells and interneurons. Presynaptic terminals are composed of several active zones, each supported by assigned pools of vesicles. The properties of synaptic transmission strongly depend on the location of the calcium sensors in relation to the source of calcium influx in the terminals. Spatial dynamics of calcium in individual presynaptic terminals will dictate the release of vesicles at specific release sites. However, the spatial compartmentalization of calcium signaling and its consequences on short-term synaptic facilitation remain largely unexplored.

We performed whole-cell patch-clamp recordings from visually-identified CA3 pyramidal cells in acute mouse hippocampal slices. Mossy fiber (MF)-mediated EPSCs were evoked by electrical minimal stimulation. The spatial dynamics of calcium elevations in mossy fiber boutons was probed using random-access two-photon microscopy in combination with single granule cell recordings.

Previously we unveiled a counting logic used mossy-fiber-pyramidal cell synapses that largely responsible for burst detection. We investigated whether mossy fiber-interneuron synapses behave similarly. We found two distinct interneuron populations, one that was insensitive to the presynaptic frequency and one that showed rate coding. The presented work will investigate the contribution of several factors to these coding strategies including receptor function and the molecular architecture of the synapses.

In addition to synchronously released neurotransmitters, action potentials also trigger delayed asynchronous release. Asynchronous release contribute to information transfer at synapses, including at the hippocampal mossy fiber to CA3 pyramidal cell synapse where it controls the timing of postsynaptic firing. Here, we investigated how different patterns of presynaptic firing control asynchronous release. We find that asynchronous release at MF-CA3 synapses is biphasic and lasts on the order of seconds following repetitive stimulation. While the first phase is limited to a few hundred milliseconds and demonstrates a high release rate, the second phase lasts on the order of seconds and demonstrates a much lower release rate. Elevating the stimulation frequency or the external Ca^{2+} concentration increased the total rate of asynchronous release, but had no impact on the biphasic nature of asynchronous release, suggesting the dependency of asynchronous release on presynaptic Ca^{2+} dynamics. Direct MFBs Ca^{2+} imaging revealed slow Ca^{2+} decay kinetics and that the peak amplitude of Ca^{2+} transients was invariant during trains of action potentials. Last, we observed that asynchronous release was preferentially mediated by P/Q-type voltage-gated Ca^{2+} channels and that increasing presynaptic Ca^{2+} buffering with EGTA-AM selectively reduced the rate but lengthened the total asynchronous release.

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Non-Canonical Role of Glutamatergic NMDA Receptor: from Physiology to Pathology

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Understanding cell communication in the brain is a major challenge in fundamental biology as well as in medicine since a deficit in transmission between neurons is likely at the origin of major neuropsychiatric disorders. Over the last decade, our understanding of the synapse, the main site of communication between neurons, has been expanded thanks to the development of super-resolution and single molecule imaging approaches. Indeed, the visualization in live brain cells of the synaptic material such as the neurotransmitter receptors revealed that these molecules are highly dynamic and are constantly diffusing within the plasma membrane. Here, I will first describe the non-canonical role of NMDA receptor, which play key roles in several physiological (e.g. learning and memory) and pathological (e.g. schizophrenia), using single molecule-based imaging approaches in hippocampal neuronal networks. Then, I will discuss how such a new level of regulation of the receptor trafficking is affected in models of psychosis.

Prenatal Hyperhomocysteinemia Induces Aging of Neuromuscular Synapses Mouse

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Homocysteine (HCY) is a sulfur containing amino acid synthesized from methionine (Veeranki and Tyagi, 2013). The impairments of the HCY metabolism induce an increase of HCY plasma level called hyperhomocysteinemia (hHCY). hHCY is often followed by skeletal muscle dysfunctions, evidenced by muscle weakness, less fatigue resistance due to oxidative stress, inflammation and endoplasmic reticulum stress in mice (Veeranki and Tyagi, 2015; Majumder et al., 2017; 2018a,b). Oxidative stress is one of the mechanisms of the damaging action of HCY known as a powerful oxidant, producing reactive oxygen species (ROS) such as superoxide anion (O2-) and hydrogen peroxide (H2O2) (Petras, 2014), which affect synaptic transmission at the central and peripheral nervous systems (Giniatullin et al., 2006). Recently it was shown that HCY sensitizes neuromuscular junction to the inhibitory effect of H2O2 via activation of NMDA receptors and nitric oxide (Bukharaeva et al., 2015).

Elevated levels of HCY during pregnancy (prenatal hHCY) impair fetus development, resulted in functional disabilities, learning deficits and skeletal muscle myopathy of the offspring (Gerasimova et al., 2017; Yakovlev et al., 2018; Yakovelva et al., 2018). Newborns are particularly vulnerable to oxidative stress due to high ability of ROS production and decreased antioxidant protection (Marseglia et al., 2014).

The aim of the present study was to analyze the processes of synaptic transmission in neonatal and adult rats with maternal hHCY.

Enhanced levels of homocysteine during pregnancy induce oxidative stress and contribute to many age-related diseases. In this study, we analyzed age-dependent synaptic changes in the developing neuromuscular synapses of rats with prenatal hyperhomocysteinemia (hHCY). One of the main findings is that the intensity and the timing of transmitter release in synapses of neonatal (P6 and P10) hHCY rats acquired features of matured synaptic transmission of adult rats. Thus, the amplitude and frequency of miniature end-plate currents (MEPCs) and evoked transmitter release analyzed by the quantal content of EPCs were higher in neonatal animals with hHCY compared to the control group. The morphology and the intensity of endocytosis of synaptic vesicles in motor nerve endings was assessed using the fluorescence dye FM1-43. Adult-like synapses were found in neonates with hHCY which were characterized by larger area of presynaptic terminals compared to controls. No difference in the intensity of FM1-43 fluorescence was observed between the two groups of animals. Prenatal hHCY resulted in reduced muscle strength assessed by the Paw Grip Endurance test. Using biochemical assays we found an increased level of H2O2 and lipid peroxidation products in the diaphragm muscles of hHCY rats. This was associated with a lowered activity of superoxide dismutase and glutathione peroxidase.

Our data indicate that prenatal hHCY induces oxidative stress and apparent faster functional and morphological "maturation" of motor synapses.

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