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within S1 arising from other cortical areas and the output signal of cortical circuits.

PP.172

Influence of glucose deprivation on membrane potentials of different membranes in rat brain synaptosomes.

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Hypoglycemia can cause neuronal cell death similar to that of glutamate-induced cell death. We investigated the effect of glucose removal from incubation medium on changes of mitochondrial and plasma membrane potentials in rat brain synaptosomes using the fluorescent dyes DiSC3(5) and JC-1. We also monitored pH gradients in synaptic vesicles and their recycling by the fluorescent dye acridine orange. Glucose deprivation was found to cause an inhibition of K+-induced Ca2+-dependent exocytosis and a shift of mitochondrial and plasma membrane potentials to more positive values. The sensitivity of these parameters to the energy deficit caused by the removal of glucose showed the following order: mitochondrial membrane potential > plasma membrane potential > pH gradient in synaptic vesicles. The pH-dependent dye acridine orange (AO) was used to investigate synaptic vesicle recycling. However, the compound's fluorescence was shown to be enhanced also by the mixture of mitochondrial toxins rotenone (10 μM) and oligomycin (5 μg/mL). This means that AO can presumably be partially distributed in the intermembrane space of mitochondria. Glucose removal from the incubation medium resulted in a 3.7-fold raise of AO response to toxins suggesting a dramatic increase in the mitochondrial pH gradient. Our results suggest that the biophysical characteristics of neuronal presynaptic endings do not favor excessive non-controlled neurotransmitter release in case of hypoglycemia. The inhibition of exocytosis and the increase of the mitochondrial pH gradient, while preserving the vesicular pH gradient, are proposed as compensatory mechanisms.

PP.173

Long-lasting response changes in deep cerebellar nuclei in vivo correlate with low-frequency oscillations

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The deep cerebellar nuclei (DCN) have been suggested to play a critical role in sensorimotor learning and some forms of long-term synaptic plasticity observed in vitro have been proposed as a possible substrate. However, till now it was not clear whether and how DCN neuron responses manifest long-lasting changes in vivo. Here, we have characterized DCN unit responses to tactile stimulation of the facial area in anesthetized mice and evaluated the changes induced by theta-sensory stimulation (TSS), that is known to induce plasticity in the cerebellar cortex in vivo. DCN units responded to tactile stimulation generating bursts and pauses, which reflected combinations of excitatory inputs most likely relayed by mossy fiber collaterals, inhibitory inputs relayed by Purkinje cells, and intrinsic rebound firing. Interestingly, initial bursts and pauses were often followed by stimulus-induced oscillations in the peri-stimulus time histograms (PSTH). TSS induced long-lasting changes in DCN unit responses. Spike-related potentiation and suppression (SR-P and SR-S) were correlated with stimulus-induced oscillations. Fitting with resonant functions suggested the existence of peaks in the theta-band. Optogenetic stimulation of the cerebellar cortex altered stimulus-induced oscillations suggesting that Purkinje cells play a critical role in controlling DCN oscillations and plasticity. This observation complements those reported before on the cerebellar cortex supporting the generation of multiple distributed plasticities in the cerebellum following naturally patterned sensory entrainment. The unique dependency of DCN plasticity on circuit oscillations discloses a potential relationship between cerebellar learning and activity patterns generated in the cerebellar network.

Poster Session III (2/3)

Neurophysiology – Memory formation, storage and recall

PP.174

The Parkinson-related E193K LRRK2 variant impacts neuronal vesicles dynamics through perturbed protein interactions.

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The Leucine-Rich Repeat Kinase 2 (LRRK2) is a complex protein, expressed in neurons and implicated in Parkinson disease (PD). LRRK2 contains a dual enzymatic activity and several structural domains that constitute a versatile platform for multiple protein interactions at the synapses. In this study, we characterize the functional role of the N-terminal Armadillo repeats domain of LRRK2 and the impact on synaptic vesicle (SV) dynamics of a novel variant, E193K, harboured within this domain and identified in an Italian family affected by PD. Using a genetically encoded sensor of recycling, synaptopHluorine, and total internal reflection fluorescence microscopy, we visualized SV trafficking in the N2A neuroblastoma cells expressing the wild type LRRK2 protein, a mutant lacking the Armadillo domain (ΔN LRRK2) or the E193K variant. We found that expression of the ΔN construct increased the frequency and the amplitude of spontaneous synaptic events. A similar phenotype was detected in the presence of the E193K variant, suggesting that this mutation behaves as a loss-of-function mutation. A domain-based pulldown approach demonstrated that the LRRK2 N-terminus binds to cytoskeletal (β-actin and -tubulin) and SV (synapsin I) proteins and the E193K substitution alters strength and quality of LRRK2 interactions. The results support a role of the Armadillo domain in interaction with synaptic proteins and suggest that the E193K mutation affects LRRK2 function via perturbation of its physiological network of interactors, resulting in impaired vesicular trafficking. These findings may have important implications for understanding the role of LRRK2 at the synapses and the pathophysiological mechanism for LRRK2-linked diseases.

PP.175

Characterization of the potentiated synapse-specific PSD-95 interactome via activity-dependent in vivo expression of a proteomic probe

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The acquisition of new memories relies on synaptic plasticity. Changes in the quality and quantity of the protein content of dendritic spines, also via local translation, play a paramount role in this process (Nakahata & Yasuda, Front Syn Neurosci 2018; Aug 29). However, current approaches are inadequate for a systematic analysis of the in vivo potentiation-specific synaptic proteome (Dietrich & Kreutz, Mol Cell Proteomics 2016;15, 368). Recently, we developed the “SynActive” strategy to express any reporter protein specifically at potentiated synapses (Gobbo et al., Nat Comm 2017;8, 1629). Here, we exploited SynActive to express proteomic reporter baits to study the PSD-95 interactome – a hub for dendritic spine plasticity - of in vivo potentiated hippocampal synapses. We constructed a SynActive-controlled, FLAG-tagged PSD-95, which was expressed in the mouse hippocampus via AAV. Exposing mice to contextual fear conditioning triggered structural plasticity and concurrent production of SynActive-FLAGged PSD-95 at potentiated spines. This allowed immunoprecipitation of the potentiation-specific PSD-95 interactome, which was characterized by mass spectrometry. In a parallel control experiment we used constitutively expressed, FLAGged PSD-95. Comparative bioinformatics analysis of the two datasets allowed to isolate the molecular fingerprint of post-synaptic plasticity serving learning of a new behaviour, the first example of potentiation-specific interactome.Our results provide the proof of concept of a new approach to characterize the structural features of synaptic plasticity, with promising implications for advancing our knowledge not only on the physiology of learning and memory, but also on activity-dependent synaptic alterations at early stages of neurodegeneration.

PP.176

Quantifying barcode information content in dendritic spines of the rodent brain

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The study of the relationship between dendritic spine plasticity and memory employs spine counting methods or dynamic spine motility analysis. There are theoretical and practical limitations in this approach, mainly linked to the lack of information on the spine's distribution along the dendrite, that in turn dictates the pattern of connections between neurons. This protocol describes the application of non-linear techniques to extract the information content of dendritic spines from still images: the resulting new morphological parameter, entropy