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pathways) for further studies on complement-bound synaptic/neuronal preparations. Most notably, we identified neuronal pentraxin 1, which was described before as an important player regulating synaptic plasticity, presumably interplaying with the local complement system in the brain.

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Ras and Rab interactor 1 (RIN1) controls dendritic arborisation and filopodial motility in a protein kinase D-dependent manner

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Actin cytoskeleton plays a pivotal role in fine-tuning cellular shape and morphological plasticity in neurons. Ras and Rab interactor 1 (RIN1) is predominantly expressed in the nervous system, especially in forebrain structures, with a known role in synaptic plasticity. Recently, RIN1 was proved to be a direct target of protein kinase D (PKD), possessing serine 292 (S292) as a PKD-specific phosphorylation site. S292 phosphorylation results in 14-3-3 binding and the sequestration of active RIN1 from the cytoplasm. PKD has been shown to regulate dendritic arborisation and actin dynamics in dendritic spines in an activity-dependent manner. Therefore, we aim to investigate the importance of RIN1 as a downstream target of PKD in relation to dendritic and/or spine morphology and motility.

To reveal the importance of PKD-mediated S292 phosphorylation, RIN1 mutants possessing S292A or S292E point mutations – hindering or mimicking constant phosphorylation, respectively – were created. RIN1 has the ability to signal through two downstream pathways, Abl-mediated cytoskeletal remodelling and Rab5-mediated endocytosis. Accordingly, E574A and RIN1QM point mutants, which selectively impair the interactions of RIN1 with Rab5 and Abl kinase, respectively, have been also created.

Neuronal cultures were prepared from RIN1ko embryonal hippocampi and cultured neurons were transfected with fluorescently labelled wild type or mutant RIN1 constructs. Dendritic arborisation was analysed by the Sholl analysis Fiji plugin on confocal microscopic images recorded from transfected pyramidal neurons. According

to our results, RIN1 overexpression increased the extent of the dendritic tree, which was completely blocked by mutating the S292 site of RIN1. These results indicate that PKD-mediated phosphorylation of serine 292 is required for the RIN1-dependent regulation of dendritic arborisation.

Live cell imaging recordings were also made from transfected neurons. Filopodial and dendritic spine motility in the transfected neurons was investigated by an Image J plugin developed recently in our laboratory (Dendritic Filopodia Motility Analyser). Using this tool, we show that overexpression of RIN1 as well as its phospho-mimetic mutant form (S292E) significantly increased motility. This effect was completely blocked if the non-phosphorylatable S292A RIN1 mutant was expressed. To reveal the importance of Rab5 versus Abl kinase mediated RIN1 pathways, analyses on the RIN1 double point mutants are currently in progress. Research was supported by the KTIA_NAP_13-2014-0018 grant and by the Hungarian Scientific Research Foundation grant K81934 to KS, by the PF247/13-1 DFG project to AH and KS and by the MÖB-DAAD and Erasmus travel exchange programs.

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Protein kinase D regulates homeostatic plasticity in hippocampal cell cultures

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Homeostatic plasticity stabilizes the properties of neuronal circuits by adjusting the responsiveness of postsynaptic neurons according to the strength of the trigger inputs. Chronic tetrodotoxin (TTX) treatment precludes the generation of action potentials and eliminates spike-evoked synaptic transmission. Within 48 hours of TTX treatment, neurons adapt to reduced synaptic input by increasing the surface amount of excitatory neurotransmitter receptors. This phenomenon is called homeostatic upscaling, but the underlying regulatory cascades are still far from understanding. Protein kinase D (PKD) is a serine/threonine kinase, which is highly expressed in CNS neurons. It has been proven that PKD promotes neuronal plasticity via regulating actin dynamics within dendritic spines. However, in non-neuronal cells PKD also plays a role in Golgi-to-cell-surface transport processes by controlling the biogenesis of specific transport carriers. The aim of our study is to investigate whether PKD regulates the intrinsic biophysical properties of neurons during homeostatic plasticity through regulating plasma membrane localized ion channels.

Homeostatic upscaling was evoked in primary dissociated mouse hippocampal cultures by 48h TTX treatment, while endogenous PKD functions were blocked by a specific PKD inhibitor (kNB 142-70). Neuronal responsiveness was measured by the whole-cell current clamp technique and IV protocols were analyzed. Cells were labelled with biocytin to allow further characterization of the recorded cells. Glutamic acid decarboxylase (GAD) 65 and 67 immunoreactivity was used to define GABAergic neurons, while glutamatergic neurons were characterized based on chicken ovalbumin upstream promoter transcription factor-interacting proteins 2 (CTIP2) and prospero homeobox protein 1 (PROX1) expression.

According to our results, 48h of TTX treatment did not change cell viability or resting membrane potential, but significantly increased T-current mediated postinhibitory rebound firings in the recorded neurons. The addition of PKD-specific inhibitor completely abrogated this effect, indicating that T-type Ca-channel expression (CaV3) is upregulated during homeostatic upscaling in a PKD-dependent manner. Interestingly, long term kNB 142-70 treatment selectively increased the frequency of H-current mediated voltage sags in the recorded neurons, suggesting increased activity of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Importantly, this effect was independent from the TTX treatment. Our results already show that PKD selectively modulates the occurrence of certain currents and the excitability and firing properties of neurons and participates in homeostatic upscaling. Analyses at the mRNA and protein levels are currently in progress to define whether PKD directs transcriptional, transport or modulatory role in case of CaV3 and HCN channels.

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Protein kinase D regulates neurotransmitter receptor turnover in hippocampal neurons

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Synaptic plasticity is known to underlie memory formation and learning. Synaptic strength is modulated in an activity-dependent manner via altering the actin network in dendritic spines or by regulating neurotransmitter receptor recycling in the postsynaptic membrane. It is proven that protein kinase D (PKD) promotes neuronal plasticity via regulating actin network remodeling in dendritic spines. PKD also controls

the integrity of Golgi apparatus and mediates the somatodendritic distribution of transferrin receptor. Our aim is to investigate whether PKD modulates neuronal plasticity via neurotransmitter receptor turnover, as well.

PKD activity was modified genetically or pharmacologically, either via overexpressing a constitutively active PKD mutant or by the application of a selective PKD inhibitor. Fluorescently labeled transferrin uptake was analyzed to follow receptor-mediated endocytosis in cultured hippocampal neurons. Endosomal compartments were identified with EGFP-tagged Rab endosomal marker proteins. Kinetics of transferrin receptor endocytosis and colocalization between internalized transferrin molecules and Rab positive vesicles were analyzed by confocal microscope. Our results show that PKD has an effect on the early steps of endocytosis, directs vesicular trafficking at the plasma membrane and stimulates the recycling endosomal system. To extend our investigations on PKD-regulated neurotransmitter receptor turnover, the relative amount of the AMPA receptor GluA1 subunit within the plasma membrane was analyzed in relation to modulating endogenous PKD activity. Cell surface biotinylation was used to selectively precipitate plasma membrane inserted GluA1 via labeling its extracellular region with membrane impermeable biotin. During antibody feeding, anti-GluA1 antibody was added to living neurons for a short period. After fixation, postsynaptic areas were highlighted by Shank2 immunoreactivity and the surface-bound anti-GluA1 signal was analyzed by confocal microscopy. Our results indicate that long-term inhibition of PKD decreases, whereas short-term PKD inhibition increases the amount of plasma membrane inserted postsynaptic AMPA receptors. Increased PKD activity, on the other hand, reduces surface expression of GluA1 subunit in the dendrites. Thus, PKD presumably regulates rapid neurotransmitter receptor endocytosis directly at the plasma membrane while it also affects the secretion and plasma membrane insertion, albeit with a slower kinetics.

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Classification of neuronal phenotypes based on static physiological parameters and firing responses – contrasting observations

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One of the highest priority goals of today's neuroscience is to achieve the most 'complete' and accurate classification of neurons in the mammalian brain. Researchers