RIM binding protein 2 in hippocampal mossy fiber synaptic transmission and plasticity

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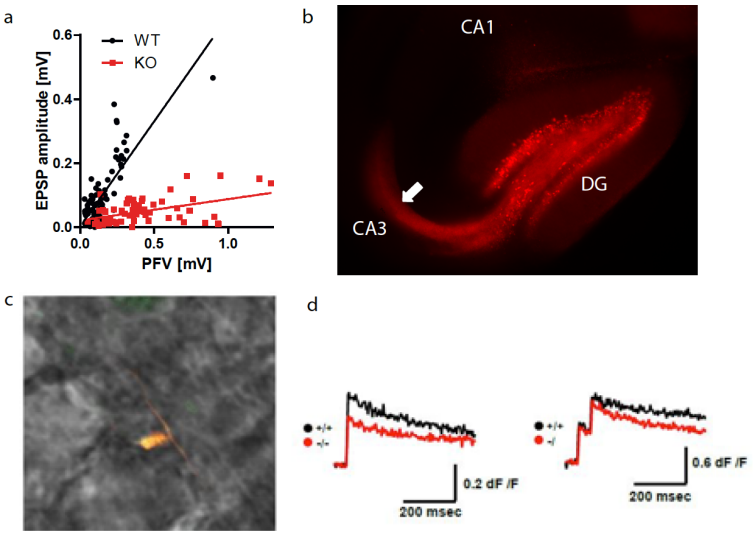
Abstract:

The precise and exact subsynaptic localization of active zone components is of utmost importance for correct synaptic transmission as well as synaptic plasticity. Rim binding proteins (RBP 1/2) are crucial for orchestrating the interplay of various components of the synaptic release apparatus such as voltage dependent calcium channels and the active zone proteins Rim and Munc13. In this project we intend to investigate the significant role of RBP2 in transmission and plasticity at hippocampal mossy fibers (mf), a prototypical central synapse especially well suited for presynaptic accessibility and manipulations. Unpublished experiments from our laboratory (Fig. 1a) revealed a severe impairment of basal mossy fiber synaptic transmission in RBP2 KO mice.

Superresolution imaging and electron microscopy experiments performed by the Rosenmund and Sigrist laboratories revealed a reduced number of synaptic vesicles at the active zone of mossy fibers, supporting a central function for RBP2 in the active zone of these synapses. Currently, the underlying molecular mechanism responsible for this dramatic phenotype compared to other glutamatergic synapses in the hippocampus (Grauel et al 2016) is unclear.



We want to pursue two lines of experiments in order to investigate two key aspects of RBP2 function at mossy fiber synapses:
Aim 1: the role of RBP2 in calcium secretion coupling.
Aim 2: An optogenetic structure function analysis of RBP2 at mossy fiber synapses that helps revealing the key molecular interaction partners responsible for the phenotype.

Aim 1: We plan to study the influence of RBP2 on calcium-influx into single presynaptic terminals/boutons by means of two-photon-calcium-imaging through individual calcium indicator loading of granule cells (Fig 1c,d). This method allows uncovering gross impairments of action-potential driven calcium influx. For a detailed analysis of potential impairments in the calcium secretion coupling in RBP2 KO mice, we plan to probe the coupling distance between calcium source and sensor: how is this coupling influenced by RBP2 loss, both under basal conditions as well as after induction of mf long-term plasticity? A dynamic switch has recently been described from loose to tight nanodomain coupling in the process of plasticity expression (Midorikawa and Sakaba, 2017), which can be readily tested by the application of calcium chelators with different binding kinetics while recording synaptic transmission.
Aim 2: We are currently establishing an optogenetic strategy for light-triggered mossy fiber synaptic transmission using channelrhodopsin expression in granule cells (Fig 1b). Using lentiviral particles generated in collaboration with the Rosenmund lab, we will express Channelrhodopsin and full-length or truncated RBP2 variants from a single bicistronic vector in RBP2 KO mice. In acute hippocampal slices of these mice, brief flashes of light will trigger synaptic transmission selectively from synapses expressing the RBP2 variants. In collaboration with the group of Stefan Sigrist, we will design RBP2 variants lacking crucial interaction domains, including one or more of the three Src-homology 3 (SH3) domains, or the Fibronectin III (FNIII) repeats. Channelrhodopsin-driven mossy fiber transmission in wildtype mice will serve as a control for these experiments.

References

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Midorikawa,M., and Sakaba,T. (2017). Kinetics of Releasable Synaptic Vesicles and Their Plastic Changes at Hippocampal Mossy Fiber Synapses. Neuron *96*, 1033-1040.