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SYNAPTIC TRANSMISSION

# Closer encounters

> under conditions of elevated cAMP, depolarization preferentially induces the release of tightly coupled vesicles

Some forms of synaptic plasticity involve changes in the probability of presynaptic synaptic vesicle release, but the mechanisms remain poorly understood. In this study, Midorikawa and Sakaba suggest that at hippocampal mossy fibre boutons (hMFBs), depolarization-induced facilitation of vesicle release results from increased coupling between vesicle-release machinery and its associated $Ca^{2+}$ channels.

Synapses between hMFBs and CA3 pyramidal cells express presynaptic forms of long-term and short-term synaptic plasticity that involve $Ca^{2+}$- and cAMP-dependent mechanisms. To investigate the spatiotemporal dynamics of depolarization-induced vesicle release at these synapses, the authors used a variety of techniques applied to dissociated hMFBs and acute brain slices. The time course of vesicle release was monitored using capacitance measurements of the presynaptic membrane, where vesicle fusion produces a step-like increase in membrane capacitance. Individual vesicle fusion events were monitored by sparsely labelling vesicles with the fluorescent dye FM1-43, which is taken up into vesicles and can be detected by total internal reflection fluorescence (TIRF); on exocytosis, the dye is released along with the vesicle contents, and the fluorescence signal disappears. Lastly, the authors used concurrent presynaptic stimulation and measurement of excitatory post-synaptic currents (EPSCs) in CA3 neurons to monitor changes in synaptic transmission.

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First, the authors confirmed that depolarization of dissociated hMFBs and brain slices resulted in jumps in membrane capacitance and the disappearance of fluorescently labelled vesicles, which was attenuated by tetanus toxin (which blocks vesicle fusion) and was indicative of vesicle fusion. Depolarization of hMFB also evoked EPSCs in CA3 pyramidal cells. Together these findings indicate that transmitter release from hMFBs can be monitored accurately by combining multiple methods.

The induction of long-term potentiation (LTP) at hMFBs involves a presynaptic cAMP-dependent mechanism. The authors mimicked this by introducing cAMP to the presynaptic terminal via the patch pipette or by extracellular application of forskolin. Capacitance jumps increased compared with the control condition (without cAMP), indicating facilitation of vesicle release. Blocking PKA, a downstream target of cAMP, abolished this facilitation. Interestingly, $Ca^{2+}$ currents were similar in the presence and absence of cAMP, suggesting that exocytosis is more sensitive to $Ca^{2+}$ in the presence of cAMP. Indeed, TIRF monitoring of depolarization-induced exocytosis events showed that these events increased in number and speed in the presence of cAMP, consistent with a change in sensitivity of the vesicles to $Ca^{2+}$.

Docked and primed synaptic vesicles can be loosely or tightly coupled to $Ca^{2+}$ channels, such that under the same $Ca^{2+}$ concentration, tighter coupling results in greater evoked exocytosis. In the presence of EGTA (which blocks loosely coupled vesicles only) but an absence of cAMP, depolarization-induced capacitance jumps were strongly inhibited, whereas in the presence of cAMP, capacitance jumps were unaffected. These findings suggest that under conditions of elevated cAMP, depolarization preferentially induces the release of tightly coupled vesicles.

Together, these findings suggest that at hMFBs, facilitation of release is caused by tighter coupling between presynaptic $Ca^{2+}$ channels and vesicle-release machinery, thus leading to potentiation of synaptic transmission, which might play a role in LTP induction at these synapses.

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**ORIGINAL ARTICLE** Midorikawa, M. and Sakaba, T. Kinetics of releasable synaptic vesicles and their plastic changes at hippocampal mossy fiber synapses. *Neuron* http://dx.doi.org/10.1016/j.neuron2017.10.016 (2017)