

**Progressive Overfilling of Readily Releasable Pool Underlies Short-term Facilitation at Recurrent Excitatory Synapses in Layer 2/3 of the Rat Prefrontal Cortex**

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Short-term facilitation of recurrent excitatory synapses within the cortical network has been proposed to support persistent activity during working memory tasks, yet the underlying mechanisms remain poorly understood. We characterized short-term plasticity at the local excitatory synapses in layer 2/3 of the rat medial prefrontal cortex and studied its presynaptic mechanisms. Low-frequency stimulation induced slowly developing facilitation, whereas high-frequency stimulation initially induced strong depression followed by rapid facilitation. This non-monotonic delayed facilitation after a brief depression resulted from a high vesicular fusion probability and slow activation of  $\text{Ca}^{2+}$ -dependent vesicle replenishment, which led to the overfilling of release sites beyond their basal occupancy. Pharmacological and gene knockdown (KD) experiments revealed that the facilitation was mediated by phospholipase C/diacylglycerol signaling and synaptotagmin 7 (Syt7). Notably, Syt7 KD abolished facilitation and slowed the refilling rate of vesicles with high fusion probability. Furthermore, Syt7 deficiency in layer 2/3 pyramidal neurons impaired the acquisition of trace fear memory and reduced c-Fos activity. In conclusion,  $\text{Ca}^{2+}$ - and Syt7-dependent overfilling of release sites mediates synaptic facilitation at layer 2/3 recurrent excitatory synapses and contributes to temporal associative learning.