

## SNAP-25 PHOSPHORYLATION ON SERIN 187 MEDIATES NEGATIVE MODULATION OF NEURONAL CALCIUM DYNAMICS

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SNAP-25 is a SNARE protein, mainly expressed in excitatory neurons, which regulates neurotransmission directly, via formation of a fusion complex with the partner SNAREs syntaxin 1 and VAMP2, and indirectly, by reducing calcium responses induced by depolarisation. The region of the protein responsible for the negative modulation of calcium dynamics is located at the C-terminal of the protein, between residues 180-197 (Verderio et al 2004). This region contains a residue, serine 187, which can be phosphorylated by PKC activity. We have investigated whether the phosphorylation of serine 187 is involved in the regulation of calcium dynamics by exogenously expressing two mutants in hippocampal neurons: the mutant 187A, which mimicks the phosphorylated form of SNAP-25 and the mutant 187E which mimicks the non-phosphorylated form of the protein. By using calcium imaging, we found that only the mutant 187E, mimicking serine phosphorylation, is effective in reducing calcium responses evoked by 50 mM KCl. Moreover, whole cell recordings of calcium currents indicated that mutant 187E reduces the peak calcium current. We have also found that SNAP-25 phosphorylation at serine 187 is an activity-dependent process. Indeed, by increasing the activity of neuronal network by different pharmacological agents (100 µM bicuculline, 100 µM glutamate), the levels of the phosphorylated form of SNAP-25 increased. An increase in SNAP-25 phosphorylation was also observed in an animal model of epilepsy, i.e. rats intraperitoneally injected with Kainate to induce epileptic seizures. Kainate –induced SNAP-25 phosphorylation peaked at 30 minutes after injection and went back to basal level 60 minutes later. All together these findings demonstrate that phosphorylation of SNAP-25 on serine 187 negatively modulates calcium dynamics by inhibiting voltage-dependent calcium channels. Given SNAP-25 phosphorylation is enhanced by neuronal activity, the protein can mediate a negative feedback modulation of neuronal activity during intense activation.

### Reference:

Verderio C., Pozzi D., Pravettoni E., Inverardi F., Schenk U., Coco S., Proux-Gillardeaux V., Galli T., Rossetto O., Frassoni C., and Matteoli M. (2004) *Neuron*. 41: 599-610