Complex regulatory mechanisms converge on the exocytotic apparatus to regulate transmitter release and ensure precise control of signaling events. One mechanism by which presynaptic GPCRs have been shown to modulate synaptic transmission is through fast, membrane-delimited inhibition of exocytosis such as that occurring through the direct interaction between Gα subunits and SNAP25. SNAP25 is a key downstream effector of G protein subunits; we have shown that proteolytic cleavage of SNAP25 by botulinum toxin A (BoNT/A) reduces the ability of Gα to compete with the calcium sensor synaptotagmin 1 (Syt1) for binding, to SNAP-25 in a calcium-dependent manner. These truncated SNAP25 proteins sustain a low level of exocytosis but are unable to support serotonin-mediated inhibition of exocytosis in lamprey spinal neurons. Mutagenesis studies narrowed down the final 3 amino acids on SNAP25 as critical for G binding and inhibition of exocytosis. A transgenic mouse containing the SNAP25Δ3 mutation was generated using CRISPR/Cas9 technology. We have found a variety of behavioural abnormalities in these mice, suggesting that inhibition of exocytosis through this mechanism is widespread and pervasive. We postulate that this dynamic regulation of vesicle fusion properties plays an important role in presynaptic integration.