A dual role of SNAP-25 as carrier and guardian of synaptic transmission

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Control of exocytotic neurotransmitter release is essential for communication in the nervous system and for preventing synaptic abnormalities. The function of synaptosomal-associated protein of 25 kDa (SNAP-25) as a crucial component of the core machinery required for synaptic vesicle fusion is well established, but evidence is growing to suggest an additional modulatory role in neurotransmission. In this issue of *EMBO reports*, Antonucci *et al* show that the efficacy of evoked glutamate release is modulated by the expression levels of SNAP-25—a function that might relate to the ability of SNAP-25 to modulate voltage-gated calcium channels and presynaptic calcium ion concentration [1]. Altered synaptic transmission and short-term plasticity due to changes in SNAP-25 expression might have direct consequences for brain function and for the development of neuropsychiatric disorders.

Communication between neurons is essential for brain function and occurs through chemical neurotransmission at specialized cell–cell contacts termed ‘synapses’. Within the nerve terminal of the presynaptic neuron electrical stimuli cause the opening of voltage-gated calcium channels (VGCCs), which results in the influx of calcium ions. This triggers the exocytic release of neurotransmitter by fusion of synaptic vesicles with the presynaptic membrane. Released neurotransmitter molecules are detected by specific receptors expressed by the postsynaptic neuron.

Calcium-induced synaptic vesicle fusion requires complex assembly between the soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) synaptobrevin 2, located on the synaptic vesicle, and the abundant plasma membrane SNAREs SNAP-25 and syntaxin 1, on the opposing presynaptic plasma membrane.

SNARE complex assembly is tightly regulated by Sec1/Munc18-like proteins [2]. Further regulatory factors such as the synaptic vesicle calcium-sensing protein synaptotagmin 1 couple the SNARE machinery to presynaptic calcium influx. SNARE-mediated neurotransmitter release occurs preferentially at the active zone—a presynaptic membrane domain specialized for exocytosis within which VGCCs are positioned close to docked synaptic vesicles through a proteinaceous cytomatrix and associated cell adhesion molecules [3,4].

Altered short-term plasticity due to changes in SNAP-25 expression might have direct consequences for brain function and for the development of neuropsychiatric disorders

An unresolved conundrum in synaptic transmission remains—the observation that SNARE proteins, such as SNAP-25, are among the most highly expressed, in copy number, presynaptic proteins, whilst only a handful of SNARE complexes are needed to drive the fusion of a single synaptic vesicle [5]. Why, then, are SNAREs such as SNAP-25 so abundant? One possible explanation might be that SNARE proteins, in addition to forming trans-SNARE complexes, assemble with other proteins, and such partitioning might regulate neurotransmission. For example, SNAP-25 has been shown to negatively regulate VGCCs in glutamatergic but not in GABAergic neurons [6]. A secondary regulatory function of SNAP-25 is also supported by its genetic association with synaptic abnormalities such as schizophrenia and attention deficit hyperactivity disorder

(ADHD) in humans [7]. SNAP-25 expression is reduced twofold in the hippocampus and frontal lobe from schizophrenic patients [8] and in animal models for ADHD [9]. Thus, SNAP-25 expression levels might crucially regulate normal synaptic function.

A new study in this issue of *EMBO reports* by Antonucci and colleagues investigates the consequences of reduced SNAP-25 expression on synaptic function in SNAP-25^{+/-} heterozygous (Het) mutant mice. By using patch clamp electrophysiology, Antonucci *et al* revealed a selective enhancement of glutamatergic but not GABAergic neurotransmission as a result of reduced SNAP-25 expression. Several other parameters including the amplitude and frequency of miniature excitatory and inhibitory currents were unaffected. These data indicate that reduced levels of SNAP-25, an essential component of the fusion machinery, selectively enhance evoked release of glutamate whilst synaptic connectivity and postsynaptic glutamate receptor sensitivity remain unaltered. Further electrophysiological experiments in hippocampal neurons in culture showed that elevated glutamatergic transmission was probably due to increased release probability rather than changes in the number of fusion-prone, so-called ‘readily releasable synaptic vesicles’. This effect was occluded by pharmacologically induced calcium entry bypassing VGCCs, suggesting that altered calcium influx might underlie the differences in evoked glutamate release between wild-type and SNAP-25 Het neurons. As schizophrenia and ADHD are associated with changes in short-term plasticity, a paradigm reflecting presynaptic function, Antonucci *et al* analysed neurotransmission by paired-pulse stimulation—a protocol whereby two closely paired stimuli are applied within a 50ms time interval. Wild-type neurons

Fig 1 | Effect of presynaptic SNAP-25 levels on calcium-induced glutamate release. Top: in wild-type (WT) neurons, SNARE-mediated calcium-triggered synaptic vesicle fusion is negatively regulated by complex formation between SNAP-25 and VGCCs. Bottom: reduced SNAP-25 expression in heterozygotes (Het;^{+/-}) partly releases VGCCs from SNAP-25-mediated clamping, resulting in elevated calcium influx through VGCCs and increased glutamate release through SNARE-mediated calcium-triggered synaptic vesicle fusion. Note that many key exocytotic proteins have been omitted for clarity. SNAP-25, synaptosomal-associated protein of 25 kDa; SNARE, soluble NSF attachment protein receptor; VGCC, voltage-gated calcium channel.

showed significant short-term facilitation, that is, a stronger response to the second stimulus as a result of increased calcium levels in the presynaptic compartment. By contrast, Het neurons had a reduced response to the second stimulus. Such paired-pulse depression is commonly viewed as a sign of increased release probability, which occurs when the first stimulus induces a partial depletion of release-ready synaptic vesicles during paired stimulation. As a consequence, the second stimulus evokes a comparably reduced response [3]. The switch from paired-pulse facilitation to depression was not fully reproduced in hippocampal slices from wild-type and Het mice, although facilitation seemed to be attenuated in SNAP-25 Het slices. One possible explanation for the apparent discrepancy between cultured neurons taken from newborn animals and acute slices from adult mice is the constant postnatal increase in SNAP-25 expression in SNAP-25 Het mice [10], which might partly counteract the defects caused by heterozygosity. Consistent with this explanation are data from rescue experiments by Antonucci *et al*, which showed that altered neurotransmission and defects in short-term plasticity in Het neurons can be gradually recovered in parallel with increased SNAP-25 expression. Moreover, cultured neurons show substantially higher levels of endogenous activity compared with acute slice preparations, leading to possible changes in the

partitioning of SNAP-25 between SNARE complexes and association with VGCCs. Further experiments are clearly required to resolve these issues. Irrespective of these potential caveats, the combined data support the hypothesis that alterations in SNAP-25 expression underlie regulatory changes in neurotransmission, resulting in altered short-term plasticity and possibly disease.

Many open questions remain. In particular, the precise mechanisms underlying elevated glutamatergic transmission and presynaptic plasticity under conditions of reduced SNAP-25 expression remain elusive. It has been shown before that free SNAP-25 inhibits Cav2.1-type VGCCs [6], an effect reversed by overexpression of syntaxin 1, which might associate with SNAP-25. Conversely, SNAP-25 occludes negative regulation of Cav2.2 VGCCs by free syntaxin 1 [3]. Hence, it is tempting to speculate that differential partitioning of SNAP-25 between free, SNARE-, syntaxin 1- and VGCC-complexed forms could regulate evoked neurotransmission (Fig 1). In this scenario, reduced SNAP-25 expression in Het animals and in schizophrenic and ADHD patients would be sufficient to sustain SNARE-mediated synaptic vesicle fusion but partially releases VGCCs from SNAP-25-mediated inhibition. This would result in elevated calcium influx and facilitated neurotransmission. Additional levels of regulation could be imposed by developmental switching

between alternatively spliced 'a' and 'b' isoforms of SNAP-25 [11], age-dependent alterations in presynaptic protein turnover and post-translational modifications.

Future studies need to address these possibilities, and their relationship to cognitive impairments and synaptic diseases, such as schizophrenia and ADHD.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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EMBO reports (2013) **14**, 579–580; published online 4 June 2013; doi:10.1038/embo.2013.74