1 INTRODUCTION

1.1 SYNAPTIC TRANSMISSION AND PLASTICITY

The central nervous system is composed of more than 100 billion neurons, each forming from a few hundred to as many as 200 000 synaptic connections, thus creating a vastly complex network of interacting cells for information exchange. The three-dimensional map of this network is genetically pre-determined to a large extent and is very similar in all members of a given species. Its generation is developmentally regulated by mechanisms such as axonal pathfinding through extracellular guidance molecules and elimination of unwanted connections by neuronal competition and programmed cell death.

The main role of the central nervous system is to provide the control functions for the organism. It receives a vast amount of incoming signals from the different sensory organs and receptors and then integrates them to determine the responses to be made. The synapse is a specialised point of contact between neurons and is thus at an advantageous site for the control of signal transmission. Each synapse is individually regulated and shows selective action. While one type of synapse, for example, may respond to and amplify even very weak incoming signals, another synapse type may only respond to strong signals or when signals from different sources arrive simultaneously.

The synapse plays a pivotal role in the processes of learning and memory formation. In certain excitatory pathways of the hippocampus, for example, brief, high-frequency stimulation produces a long-lasting enhancement in the effectiveness of the stimulated synapses. This modification is known as long-term potentiation, or LTP, a form of synaptic plasticity that initiates long-term memory formation. At the synapse, information transfer changes from an electrical to a chemical mechanism involving the release of small chemical messengers called neurotransmitters from the presynaptic terminal. Classic neurotransmitters such as acetylcholine and amino acid transmitters are stored in uniform organelles known as small synaptic vesicles (SSV). When an action potential travelling along an axon arrives at the nerve terminal, voltage-gated calcium ion channels open and cause an influx of calcium ions. This influx triggers the fusion of synaptic vesicles with the plasma membrane, thus enabling the neurotransmitters to be released into the synaptic cleft, where they diffuse and subsequently bind to and activate specific receptors on the postsynaptic cell membrane. LTP

can be envisioned as a mechanism that increases the conductance of a synapse to signal transduction, thereby allowing a more efficient response to a given stimulus.

Two pathways lead to the enhanced synaptic activity that is implicated in LTP formation. One path involves an increased efficiency of the postsynaptic neurotransmitter receptors, possibly by a posttranslational modification of the receptor molecule combined with a more long-term upregulation of receptor protein translation. The other path involves the presynapse by increasing the amount of neurotransmitter released. It could be envisioned that this increased efficiency in neurotransmitter release at the presynapse is modulated at least in part at the level of the synaptic vesicle.

Subsequent to neurotransmitter release, synaptic vesicles are endocytosed and reloaded with neurotransmitter for another round of exocytosis. These cycles are repeated many times and can function independently of the nerve cell body, thus making the synapse an autonomous unit containing all elements necessary for vesicle exocytosis and recycling. Of course, the long-term changes involved in memory formation are coupled to changes in protein synthesis. Thus, although the synapse can function autonomously, synaptic plasticity requires communication of the synapse with the cell body and vice versa.

Apart from small synaptic vesicles many neurons also contain large dense-core vesicles (LDCV) so called because of their electron-dense core. These vesicles store peptide neurotransmitters, and, in contrast to SSVs, need to return to the Golgi network after each exocytotic event. Neuroendocrine (NE) cells which are responsible for the coordination between the nervous system and the various endocrine organs of the body, always possess LDCVs, which they use to store their peptide hormones. In addition NE cells also contain small synaptic-like vesicles analogous to SSVs. The exocytosis of large dense core vesicles and small synaptic (-like) vesicles of neurons and neuroendocrine cells is tightly regulated.

1.2 PROTEINS INVOLVED IN EXOCYTOTIC MEMBRANE FUSION

Signal transduction at the synapse involves the release of neurotransmitter molecules from the presynaptic terminal. Seeing that the functioning of an organism is controlled mainly by its nervous system, the regulation of synaptic transmission and thereby of neurotransmitter release is very important. In addition, changes in the efficiency of neurotransmitter exocytosis is one of the aspects that plays a significant role in learning and memory formation. Thus, regulation of exocytosis is extremely relevant for synaptic plasticity.

Neurotransmitters are exocytosed through an aqueous fusion pore formed when the synaptic vesicle membrane fuses with the plasma membrane. The basic mechanisms of membrane fusion have been conserved during evolution and are used by virtually every cell. Membrane fusion does not occur spontaneously in the aqueous environment of the cytoplasm because the repulsive energy between the two phospholipid membranes is too high at close distances. Specialised fusion proteins are therefore needed that bind to one another and thereby overcome the repulsive energy of the membranes. Three classes of proteins have been discovered that appear to be involved in all intracellular fusion reactions. At the synapse these fusion proteins are synaptobrevin 2 on the synaptic vesicle, and syntaxin 1a and SNAP 25 (for synaptosomal protein of 25 kDa) at the plasma membrane.

1.2.1 Synaptobrevin

The synaptic vesicle fusion protein synaptobrevin (or VAMP, for vesicle-associated membrane protein) 2, is a small 18 kDa protein with a single membrane-spanning domain. Synaptobrevin was initially discovered in the *Torpedo* nervous system (Trimble et al., 1988). It has since been cloned and sequenced in several species (Elferink et al., 1989; Südhof et al., 1989), and shows a very high degree of conservation in phylogenetically distant animals. Synaptobrevin is an abundant protein constituting more than 8 % of synaptic vesicle proteins. It consists of 116 amino acids in mammals and is made up of four domains. The cytoplasmic N-terminus consists of a proline-rich head but otherwise varies between different species. It is followed by a highly conserved hydrophilic core of 63 amino acids which is 80 % identical in *Drosophila* and cow. It is this core that comprises the domain for binding to the fusion complex. A well-conserved 20 amino acid transmembrane domain is then followed by a divergent C-terminus. Synaptobrevin 2 is ubiquitously expressed throughout the brain. Additional isoforms of synaptobrevin have been characterised, for example synaptobrevin 1 which is expressed at high levels in the spinal cord and localises to a limited number of nuclei in the brain, and cellubrevin which is present in all cells on membranes involved in the constitutive recycling of plasma membrane receptors (McMahon et al., 1993).

1.2.2 Syntaxin

The plasma membrane proteins that contribute to the fusion complex are syntaxin 1a and SNAP 25. Syntaxin 1a is a 35 kDa protein which, like synaptobrevin 2, contains a single transmembrane domain, and a cytoplasmic binding domain for the fusion complex. The amino-terminal region of syntaxin contains a separately folded domain. This domain probably plays a role in mediating additional protein-protein interactions and may also fold back onto

the fusion complex binding domain of syntaxin thereby inhibiting formation of the exocytotic fusion complex. Syntaxin was originally identified by a monoclonal antibody as a retinal antigen named HPC-1 (Barnstable et al., 1985), and was subsequently found to interact with the synaptic vesicle protein synaptotagmin (Bennett et al., 1992) and with N-type and P/Q-type calcium channels (Yoshida et al., 1992; Leveque et al., 1998). At the synapse, two isoforms (1a and 1b) have been cloned and characterised (Bennett et al., 1992). Additional isoforms have been identified which are expressed at other cellular localisations and in non-neuronal cells (all fusion protein isoforms and their sequences can be found at NCBI Entrez Web site: http://www3.ncbi.nlm.nih.gov/Entrez/)

1.2.3 SNAP 25

The plasma membrane fusion protein SNAP 25 is expressed exclusively in neurons and neuroendocrine cells. It does not have a transmembrane domain and is instead anchored to the membrane by a posttranslational fatty acid modification involving palmitoylation of cysteine residues. SNAP 25 contains two domains for fusion complex binding connected by a linker region that forms a loop to allow the domains to be aligned in parallel in the fusion complex. Thus, SNAP 25 contributes two binding domains to the fusion complex. Two isoforms of SNAP 25 (a and b) have been identified so far (Mollineda and Lazo, 1997) which differ in their distribution and developmental regulation. In addition, SNAP 23, a novel SNARE protein which is 59 % identical to SNAP 25, is expressed in non-neuronal tissues (Ravichandran et al., 1996). Syntaxin 1a and SNAP 25 are found along the entire axonal membrane (Galli et al., 1995; Garcia et al., 1995). Surprisingly, large pools of the two proteins are also present on synaptic vesicles (Walch-Solimena et al., 1995; Otto et al., 1997).

1.3 CHARACTERISATION OF THE ASSEMBLED FUSION COMPLEX

The complex formed by the fusion proteins synaptobrevin, SNAP 25, and syntaxin is extremely stable. This is why cells have evolved a specialised chaperone whose function it is to dissociate the complex under ATP hydrolysis. In neurons, this chaperone is known as NSF, or N-ethyl maleimide sensitive factor. NSF acts in conjunction with adaptor proteins called SNAPs, for soluble NSF attachment proteins (no relation to the fusion protein SNAP 25). Interestingly, NSF and SNAPs were discovered prior to the fusion proteins as a prerequisite for exocytosis. The fusion proteins were subsequently identified as receptors for NSF and SNAPs. SNAPs need to bind to the fusion complex before NSF can bind and stimulate

ATPase activity. The fusion complex is therefore also known as the SNARE (SNAp REceptor) complex, and its constituent proteins as SNARE proteins.

The essential role that the SNARE proteins play in exocytosis was revealed not only when they were identified as NSF receptors, but additionally when they were discovered to be targets of botulinum and tetanus neurotoxins. These toxins represent clostridial proteases that cleave the SNARE proteins and thereby prevent the assembly of a stable fusion complex. Nerve terminals that are poisoned with clostridial neurotoxins appear morphologically unchanged, but no vesicle exocytosis can occur. This indicates that the SNARE complex is important for fusion but not for attachment of synaptic vesicles to the plasma membrane. Synaptobrevin 2 is cleaved by tetanus toxin and botulinum toxins B, D, F and G, syntaxin 1a by botulinum toxin C1, and SNAP 25 by botulinum A, and E (for review, see Ahnert-Hilger and Bigalke., 1995). All cleavage sites map to two narrow bands in the C-terminal region of the fusion complex (Fasshauer et al., 1998). This is surprising because the assembled complex is resistant to neurotoxin proteolysis. Probably the target structure and not the individual cleavage sites were conserved during evolution of the toxins.

The assembled fusion complex is resistant not only to neurotoxin proteolysis, but also to denaturation by heat, or by the detergent SDS. The high stability of the SNARE complex is due to the formation of a very tightly bundled helix at the core of this complex. Fusion proteins share a homologous domain referred to as the SNARE motif that consists of approximately 60 very highly conserved amino acids. These SNARE motifs exclusively mediate the binding of the fusion proteins into the tight core complex. The crystal structure of the synaptic core complex comprising the SNARE motifs of synaptobrevin 2, syntaxin 1a and SNAP 25 has recently been solved (Sutton et al., 1998). It consists of a 12 nm twisted four-helical bundle with all chains aligned in parallel. A core complex formed by the isolated SNARE complexes exhibits most of the biophysical and biochemical properties of complexes formed by the intact proteins. In addition to their SNARE motifs, fusion proteins contain flanking regions that either attach them to membranes and / or mediate further protein-protein interactions, a few of which have been characterised (discussed in Section 1.4). Interestingly, the surface of the assembled SNARE complex contains four shallow grooves with hydrophobic and charged regions. It is possible that these grooves provide binding domains for regulatory proteins. (for review, see Jahn and Südhof, 1999).

1.4 ROLE OF SYNAPTOTAGMIN IN REGULATED EXOCYTOSIS

Formation of the SNARE complex is necessary but not sufficient for membrane fusion. It leads to a state of hemifusion in which only the proximal phospholipid monolayers of the vesicular and plasma membranes are connected by a highly bent stalk, while the two distal monolayers converge towards each other but are still separated. Completion of the fusion reaction with the formation of a fusion pore is probably triggered by the synaptic vesicle calcium-binding protein synaptotagmin upon calcium entry into the synapse. How is the calcium signal transferred into a signal for neurotransmitter exocytosis? Calcium ions (Ca^{2+}) enter the synapse through voltage-gated Ca^{2+} channels within 200 microseconds of the action potential arriving at the synaptic terminal, causing the local Ca^{2+} concentration to increase from 100 nM to greater than 200 µM. Exocytosis requires a threshold Ca^{2+} concentration of 20-50 µM, and a concentration of 190 µM for half-maximal activation (Sheng et al., 1996). SNARE complexes have been shown to bind directly to the N-type and P/Q-type calcium channels (Martin-Moutot et al., 1996; Seagar and Takahashi, 1998). This arrangement facilitates rapid exocytosis by high local concentrations of Ca^{2+} and minimum diffusion time. Presumably Ca^{2+} ions induce conformational changes in synaptic proteins that catalyse membrane fusion. Increasing evidence supports the idea that the synaptic vesicle protein synaptotagmin 1 is the Ca^{2+} receptor in exocytosis, although other sensors may be involved as residual neurotransmission persists in synaptotagmin-null mutants (Di Antonio et al., 1993). The putative role of synaptotagmin as a signal transducer during exocytosis is substantiated by its calcium ion-regulated interaction with the SNARE protein syntaxin (Chapman et al., 1995).

Synaptotagmin is an abundant and highly conserved synaptic vesicle protein that is composed of a short intravesicular N-terminal sequence, a single transmembrane region, and a 40 kDa cytoplasmic domain (Perin et al., 1990; Perin et al.; 1991a, Perin et al., 1991b). Most of the cytoplasmic domain is formed by two C_2 domains. C_2 domains are regulatory sequences that occur widely in nature and often play a role in membrane trafficking or signal transduction. The first C_2 domain (called C_2A) binds syntaxin in a Ca^{2+}-dependent manner. Although the interacting surfaces of syntaxin and the C_2A domain are probably complementary in shape, in the absence of Ca^{2+} their interaction is inhibited by the presence of negative charges on both surfaces that repel each other. Upon binding of two Ca^{2+} ions, the electrostatic potential of the Ca^{2+}-binding region of the C_2A domain becomes strongly positive, causing an attraction of the two surfaces (Shao et al., 1997). Synaptotagmin thus acts

as an electrostatic switch during Ca^{2+}-induced exocytosis. Binding of synaptotagmin to the SNARE complex via syntaxin probably induces a structural rearrangement in the fusion machinery, thereby triggering the opening of the fusion pore and neurotransmitter release. Empty synaptic vesicles are subsequently endocytosed within one to five seconds by way of clathrin-coated pits which become coated vesicles. Assembly of clathrin coats is initiated by binding of the AP2 adaptor protein complexes to membrane receptors, shown to be synaptotagmins (Zhang et al., 1994). Thus, synaptotagmin probably functions both in the last step of exocytosis and the first step of endocytosis.

1.5 REGULATION OF SNARE COMPLEX FORMATION

SNARE proteins function in most if not all cellular fusion events. At the synapse, however, formation of the fusion complex has to be tightly regulated because timing and efficiency of neurotransmitter release are of utmost importance to survival and functioning of the organism. In addition, the regulation of neurotransmitter release from the presynapse plays a central role in synaptic plasticity. Because each synapse is a highly specialised unit, its proper functioning requires a mechanism whereby the efficiency of SNARE complex formation can be individually regulated.

How is the extreme reactivity of the SNARE proteins (Otto et al., 1997) reconciled with the need for a highly regulated form of exocytosis at the synapse? It can be imagined that a range of control proteins exists that bind to the SNARE proteins and regulate their availability to the exocytotic fusion complex. In this scenario, the control proteins would lead their SNARE binding partners into the fusion complex prior to exocytosis and back out of the fusion complex subsequent to exocytosis. A few SNARE protein binding partners have been identified. In most cases, however, the regulatory significance of the discovered binding proteins has yet to be elucidated.

For syntaxin, possible regulatory proteins could be Munc-18 and tomosyn. Munc-18, a protein which is essential for exocytosis, binds to the N-terminal domain of syntaxin including the adjacent part of the SNARE motif. Syntaxin does not simultaneously bind to Munc-18 and SNAP 25, presumably because their binding sites partially overlap (Hata et al, 1993). Munc-18 has been shown also to bind to two C_2 domain proteins named Doc2A and Doc2B (Orita et al., 1995; Kojima et al., 1996), and to a family of proteins called Mints (Okamoto and Südhof, 1997) which in turn bind to cellular junction proteins. Possibly Munc-

18 binds syntaxin preceding SNARE assembly in order to stabilise intermediate stages. The regulatory protein tomosyn binds to syntaxin via a C-terminal SNARE motif related to synaptobrevin. Tomosyn is capable of dissociating Munc-18 from syntaxin and forming a complex with syntaxin, SNAP 25 and synaptotagmin, suggesting that it may regulate SNARE complex formation (Fujita et al., 1998). Other proteins have been shown to interact with syntaxin, including Munc-13 (Brose et al., 1995) which apart from binding Doc2 (Orita et al., 1997) binds syntaxin when it is part of the fusion complex (Betz et al., 1997).

Hrs-2 (for "hepatocyte growth-factor regulated tyrosine kinase substrate", Komada and Kitamura, 1995) is an ATPase enzyme that binds to SNAP 25. This interaction is inhibited by calcium in the concentration range that supports neurotransmission (Bean et al., 1997), suggesting that Hrs-2 plays a regulatory role prior to membrane fusion.

For the vesicular SNARE protein synaptobrevin, the only known non-SNARE binding partner is the vesicular membrane protein synaptophysin. Synaptophysin was one of the first synaptic proteins to be characterised, yet its function has remained controversial (see below). The interaction of synaptophysin with synaptobrevin is specific and exclusive. Thus, synaptobrevin that is bound to synaptophysin cannot enter the SNARE complex and, conversely, synaptobrevin that is part of the SNARE complex cannot interact with synaptophysin (Calakos and Scheller, 1994; Edelmann et al., 1995). These findings have led to the proposal that synaptophysin regulates the availability of synaptobrevin to the SNARE complex prior to fusion (Edelmann et al., 1995). This regulatory role, however, still needs to be confirmed experimentally.

1.6 SYNAPTOPHYSIN: AN ABUNDANT VESICLE PROTEIN WHOSE FUNCTION HAS REMAINED CONTROVERSIAL

The regulation of neurotransmitter release is an important part of synaptic plasticity. How is this regulation achieved? Do control mechanisms already exist at the level of the transmitter-storing synaptic vesicles? The only known non-SNARE binding partner of the vesicular SNARE protein synaptobrevin is synaptophysin. Therefore, synaptophysin is also the only known protein that may regulate the availability of synaptobrevin to its plasma membrane SNARE partners SNAP 25 and syntaxin. Although synaptophysin was the first vesicle protein to be detected and studied in detail, its exact function has remained controversial. Since formation of the SNARE complex is a prerequisite for neurotransmitter exocytosis, the interaction of synaptophysin with synaptobrevin may well play a regulatory role in

neurotransmitter release at the level of the synaptic vesicle. However, direct evidence for such a role still needs to be obtained.

What do we know about synaptophysin?

1.6.1 Localisation and structure of synaptophysin

Because of its high immunogenicity synaptophysin was discovered independently by several laboratories using immunochemical methods (Bock and Helle, 1977 [who named it synaptin but later found it to be identical to synaptophysin: Gaardsvoll et al., 1988]; Jahn et al., 1985 [initially terming it p38]; Wiedenmann and Franke, 1985 [who coined the name synaptophysin]). It is an abundant protein in the brain, corresponding to approximately 7 % of synaptic vesicle protein (Jahn et al., 1987). Synaptophysin is expressed almost exclusively on small synaptic vesicles and is therefore a common marker for nerve terminals. In addition, synaptophysin is expressed by a variety of peptide-secreting neuroendocrine cells and is an established marker for these cells as well as for tumours derived from the neuronal and neuroendocrine systems (Wiedenmann and Huttner, 1989). It is controversial whether synaptophysin is also present, albeit at a 100-times lower concentration, on large dense core vesicles, or whether the synaptophysin that is detected in large dense core vesicle fractions is due to microvesicle contamination (Jahn and De Camilli, 1991). Synaptophysin is not detected on LDCVs when using light and electron microscopy, immunocytochemistry and immunochemistry (Navone et al, 1988).

Synaptophysin is an acidic (pI of 4.8) protein with a molecular weight ranging from 38 kDa in neurons (Jahn et al., 1985; Wiedenmann and Franke, 1985) to between 40 kDa and 42 kDa in neuroendocrine cells depending on its state of glycosylation. Synaptophysin is N-glycosylated in the vesicle interior (Leube et al., 1987). It is phosphorylated on its tyrosine residues by the vesicular tyrosine kinase c-src on the first intravesicular loop (Pang et al., 1988). Synaptophysin contains four membrane-spanning domains made up of approximately 24 amino acid residues each. The amino and carboxy termini face the cytoplasmic side of the vesicle, and two loops are formed in the vesicle lumen. Each of these intravesicular loops is stabilised by a disulphide bridge formed between two cysteine residues. The amino terminus is very short (approximately 18 amino acids) and contains no recognisable signal transduction mechanism, while the carboxy terminus is longer (approximately 90 amino acids) and carries ten copies of a glycine-rich pentapeptide repeat similar to that found in rhodopsin

(Ovchinnikov et al., 1988). Most of synaptophysin's antigenic sites are situated on this carboxy terminus.

Synaptophysin is highly conserved in the mammalian species. When comparing bovine, rat and human synaptophysin, there is an 88 % identity between all three species (Johnston et al., 1989). Only 3 % of the amino acids are substituted in the transmembrane regions, and most changes occur in the intravesicular loop, where 22 % of the amino acids are substituted. The longest stretches of identical amino acids are at the aminoterminal region of the fourth transmembrane domain (14 residues) and in the first intravesicular loop (12 residues). In contrast, *Torpedo* synaptophysin is more divergent and shows only 62 % amino acid similarity with rat synaptophysin (Cowan et al., 1990).

Synaptophysin forms homomeric complexes (Rehm et al., 1986; Thomas et al., 1988) that are maintained by noncovalent interactions between the subunits. Using chemical cross-linking in intact cells, complexes of no more than 4 subunits are obtained (Johnston and Südhof, 1990). Higher molecular weight subunits that have been described are most likely due to artefacts arising during detergent purification which may promote disulphide cross-linking of synaptophysin by facilitating disulphide exchange reactions.

1.6.2 Proposed functions of synaptophysin

Much has been speculated about the function of synaptophysin. It was reported that the cytoplasmic tail binds calcium ions (Rehm et al., 1986) thus making it a possible candidate for transducing the calcium signal into exocytosis of synaptic vesicles. This interaction could however not be confirmed in a later study (Brose et al., 1992). Synaptophysin incorporated into planar lipid membranes led to the formation of a hexameric homo-oligomer that functioned as a voltage-sensitive ion channel (Thomas et al., 1988). The formation of such a channel has however never been shown to occur in vivo and is unlikely to play a role in exocytosis (see Südhof and Jahn, 1991).

It has been suggested that synaptophysin may play a structural role in maintaining the high curvature of small synaptic vesicles. Because of their small size (diameter of approximately 45 nm in the mammalian CNS), synaptic vesicles have a high degree of curvature, which requires asymmetric packing of phospholipids and proteins. Synaptophysin cannot be reconstituted in an inside-out orientation in liposomes (Jahn and Südhof, 1994). In addition, peripherin, a protein of similar structure to synaptophysin, but no sequence homology, probably functions in a similar manner in maintaining the highly curved rim

region of photoreceptor discs (Connell and Molday, 1990; Arikawa et al., 1992). It has also been suggested that synaptophysin may play a role in vesicle genesis. When nonneuroendocrine cells are forced to express synaptophysin, the protein is integrated into a novel type of vesicle (Leube et al., 1989).

The discovery of the synaptophysin-synaptobrevin interaction led to the proposal of a regulatory role for synaptophysin during fusion events (Edelmann et al., 1995). However, data elucidating the nature of the synaptophysin-synaptobrevin interaction and its mode of regulation still need to be obtained.

1.7 PERSPECTIVE

Neurotransmission at the synapse is a highly regulated event that involves a range of control mechanisms. Here, the incoming electrical signals are changed to chemical signals which lead to neurotransmitter release. Each synapse is individually regulated and shows selective action in response to an incoming signal. This so-called synaptic plasticity is achieved by presynaptic and postsynaptic changes which affect the efficiency of neurotransmission. At the presynapse, the efficiency of neurotransmitter release is probably altered by fine-tuning the regulation of SNARE complex formation. At the level of the synaptic vesicle, synaptophysin is the only known non-SNARE binding partner of a SNARE protein. Seeing that neurotransmitters are stored in and released from synaptic vesicles, it is quite possible that the synaptophysin-synaptobrevin interaction plays a role in synaptic plasticity.

It was the aim of the present study to increase our knowledge concerning the function of synaptophysin and of the synaptophysin-synaptobrevin complex, also with respect to SNARE complex formation. The following questions were specifically addressed:

Is the interaction of synaptophysin with synaptobrevin developmentally regulated?

Does the synaptophysin-synaptobrevin complex play a role in all synaptobrevin-mediated membrane fusion events where synaptophysin is present?

Are there indications that the synaptophysin-synaptobrevin complex adds to synaptic plasticity in the adult brain?