

Review

 α -Synuclein – Regulator of Exocytosis, Endocytosis, or Both?

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α -Synuclein is known as a presynaptic protein that binds to small synaptic vesicles. Recent studies suggest that α -synuclein is not only attracted to these tiny and therewith highly curved membranes, but that in fact the sensing and regulation of membrane curvature is part of its physiological function. Moreover, recent studies have suggested that α -synuclein plays a role in the endocytosis of synaptic vesicles, and have provided support for a function of α -synuclein during exo- and endocytosis in which curvature sensing and membrane stabilization are crucial steps. This review aims to highlight recent research in the field and adds a new picture on the function of α -synuclein in maintaining synaptic homeostasis upon intense and repetitive neuronal activity.

Structure to Function

From the time of its discovery, α -synuclein (see [Glossary](#)) has been known as a presynaptic protein [1]. The protein has attracted much interest because it is one of the major constituents of Lewy bodies [2], and mutations as well as gene duplications and triplications are linked to familial Parkinson's disease [3–6]. Nevertheless, how much more do we know about this elusive protein today, nearly 30 years later? Structurally, α -synuclein is a 140 amino acid (aa) protein with three main regions, the N-terminal region, which forms an α -helix and is essential for binding to phospholipid vesicles, the NAC (non-A β component) region, relevant for aggregation, and the acidic C-terminal region with chaperone-like activity [7]. Nuclear magnetic resonance (NMR) studies have recently determined the exact aa residues involved in membrane binding. The first 25 N-terminal residues of α -synuclein are designated as the membrane anchor region, binding very tightly to membranes. On the other hand, aa 26–97 define a region that binds less tightly and is inferred to regulate the affinity of α -synuclein binding to membranes [8].

Recently, it has been proposed that α -synuclein is a **curvature-sensing and stabilizing** protein [9,10], and it has been added to the class of **amphipathic helix**-containing proteins that sense and generate membrane curvature [11,12]. While these proteins are unstructured in solution, they start to fold their amphipathic helix under specific physicochemical conditions, a process that is strongly favored by lipid packaging defects in curved membranes. In this way, such proteins are capable of sensing the curvature of membranes. In addition, upon insertion of the amphipathic helix into the lipid surface, the membrane curvature itself becomes stabilized (Figure 1). Although the amphipathic helix of α -synuclein is structurally very different from the helices of other **amphipathic lipid-packaging sensor (ALPS) proteins**, α -synuclein seems to act in a similar way. Indeed, with its small hydrophobic residues and a more pronounced polar face, the structure of α -synuclein appears to be inherently designed to bind to negatively

Trends

Membrane remodeling processes are crucial steps for the exo- and endocytosis of synaptic vesicles.

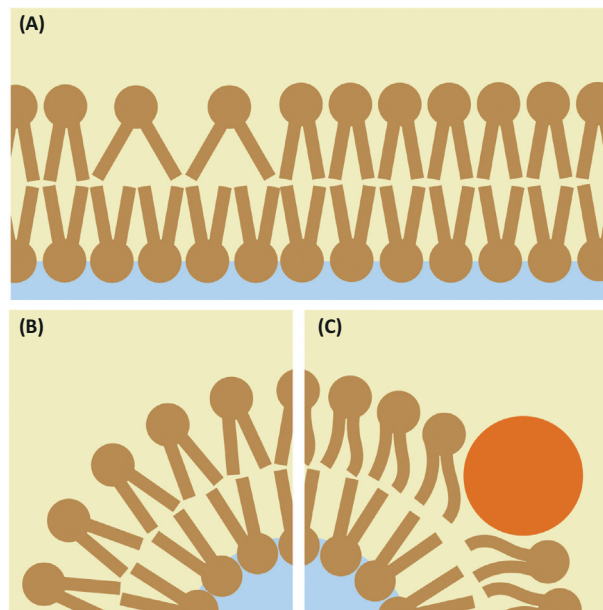
From structure–function relationships, α -synuclein has been proposed to be a curvature sensing and regulating protein, thus proposing a role in mechanisms of exo- and endocytosis.

A function in exocytosis has been suggested (i) via mediating SNARE-complex assembly, (ii) curvature stabilization, thus preventing premature fusion of vesicles, and (iii) in tethering of synaptic vesicles via lipid binding of α -synuclein with both its N- and a more C-terminal region.

Latest studies indicate a function of α -synuclein in synaptic vesicle endocytosis. (i) A possible function in clathrin-mediated endocytosis has been proposed, but a function also seems likely in (ii) fast endocytotic pathways such as kiss-and-run, or in (iii) the recently discovered ultrafast endocytosis process.

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Figure 1. Lipid Packaging Defects and Membrane Curvature Sensing. In planar phospholipid bilayer membranes lipid packaging defects can occur when there is an imbalance between cylindrical and conical lipids (A). On the other side, bending of membranes can also induce less-occupied regions because cylindrical lipids are not able to fill the outer, larger radius (B). Proteins with an amphipathic lipid-packaging sensor (ALPS) fold their amphipathic helix if favored by the presence of such lipid packaging defects and are thereby able to sense and stabilize membrane curvature (C).

charged lipids and to interfere with small lipid packaging defects, occurring notably in synaptic vesicles with a high content of polyunsaturated lipids (an excellent review on the topic is given in [13]).

In the past α -synuclein has been viewed simply as a protein bound to vesicles. The current structure–function relationship presages a potential paradigm shift from the traditional view of α -synuclein in that it suggests that the protein is not only attracted to highly curved membranes but also that the sensing and stabilization of curved membranes is an inherent part of its physiological function. The presynaptic terminal is a place of sustained membrane remodeling as synaptic vesicles constantly approach the active zone of the presynaptic plasma membrane and release their neurotransmitter content. Subsequently, both membrane material as well as proteins must be recycled, on the one hand, to clear the active zone from excess material and, on the other, to form new synaptic vesicles to be loaded again with neurotransmitter molecules. Current efforts analyzing the role of α -synuclein in the regulation of exocytosis point to a regulatory function, rather than a direct involvement in the actual release machinery, such that the lipid-binding properties come into play. Recently, NMR studies found that α -synuclein is able to bind via its N-terminal region (aa 1–25) as well as with its aa 65–97 region to lipid membranes, and a double-anchor mechanism was proposed in which α -synuclein tethers either two vesicles to one another, or vesicles to the plasma membrane, possibly to facilitate processes required for **exocytosis** and **endocytosis** [14]. Moreover, general stabilization of membrane curvature could have a modulating effect on exocytosis.

α -Synuclein in Synaptic Vesicle Exocytosis

α -Synuclein and SNARE-Complex Assembly

A function of α -synuclein in synaptic vesicle docking and fusion has been indicated by studies showing that α -synuclein has a role in the assembly of the **SNARE complex**, a process

Glossary

Amphipathic helix: α -helical protein structure segregating hydrophobic and polar residues.

Amphipathic lipid-packaging sensor (ALPS) proteins: proteins containing an ALPS motif that fold into an amphipathic helix if favored by the presence of lipid packaging defects; the amphipathic helix is inserted into the lipid membrane, thereby stabilizing membrane curvature.

Bulk endocytosis: membrane uptake by large membrane invagination.

Clathrin-mediated endocytosis (CME): endocytosis which is characterized by a coating of vesicles with the clathrin-triskelion scaffold.

Curvature sensing and stabilization: curvature of biological membranes is a premise for many cellular processes. Major mechanisms of curvature sensing and stabilization include: classic coats such as clathrin, the arc-shaped BAR domain proteins, and proteins with an ALPS motif/amphipathic helix.

Endocytosis: uptake of molecules from the extracellular space into the cell by forming cell invaginations. In the case of endocytosis at the synaptic terminal, endocytosis is deemed to retrieve excess membrane rather than molecules.

Exocytosis: fusion of a vesicle with the plasma membrane leading to release of the vesicle contents into the extracellular space. In the case of synaptic vesicles, exocytosis leads to the release of neurotransmitter into the synaptic cleft.

Kiss-and-run: a mechanism of exocytosis in which the synaptic vesicle opens only a small fusion pore. This pore reseals easily afterwards and the vesicle is thus kept intact. This in contrast to the full fusion event in which the synaptic vesicle collapses and the vesicle and presynaptic membrane fuse together.

Rab proteins: proteins that are centrally involved in membrane trafficking, including vesicle formation, transport, and fusion. To date over 70 different Rab proteins have been identified.

SNARE complex: the soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex is a multiprotein complex that brings

essential for many membrane fusion events. For exocytosis of synaptic vesicles, the SNARE proteins from the plasma membrane, **syntaxin** and **synaptosome-associated protein 25** (SNAP-25), and the SNARE protein anchored on the synaptic vesicle, **vesicle-associated membrane protein 2** (VAMP2), assemble together to form a complex with four helices, which then zips the vesicles onto the plasma membrane, ready to undergo fusion following synaptic stimulation. There is still some controversy on how α -synuclein might act on this SNARE-complex. While one major study indicates increased SNARE-complex assembly [15], others argue for a negative regulatory function of α -synuclein [16,17]. A direct interaction of α -synuclein with VAMP2, the vesicular SNARE protein, has been described, but also the incorporation of α -synuclein within the four-helical SNARE protein complex has been discussed (Figure 2A).

α -Synuclein and Fusion of Lipid Vesicles Complemented with SNARE Proteins

What can we learn from *in vitro* experiments studying the effect of α -synuclein on vesicle fusion? In assays reconstituting fluorescently labeled lipid vesicles with syntaxin and SNAP-25 in one case, and with VAMP2 in the other, α -synuclein was clearly shown to inhibit vesicle fusion in a concentration-dependent manner [18–20]. However, two of these studies argue that no direct binding of α -synuclein to the SNARE complex is needed for this inhibition, and that inhibition is merely due to the lipid interaction of α -synuclein [19,21]. On the other side, for α -synuclein oligomers, binding to VAMP2 was found to be a crucial step for inhibiting vesicle fusion [20]. Together, these data draw again a controversial picture of α -synuclein function in SNARE-complex formation, but, at least for α -synuclein monomer, a mechanism that relies on membrane curvature stabilization, thus preventing premature fusion, seems likely. Studies analyzing pure lipid vesicles lend further support to this hypothesis and clearly show the ability of α -synuclein to integrate into the lipid surface, which then enables stabilization of lipid packaging defects of vesicles and thereby of their membrane curvature [21,22] (Figure 2B).

In addition to a role in curvature sensing and stabilization, α -synuclein could potentially promote exocytosis through its double-anchor mechanism, binding to lipid membranes not only with the N-terminal region (aa 1–25), but also with the region aa 65–97 [14]. Therewith α -synuclein would be able to tether synaptic vesicles to the plasma membrane or even the active zone, which would influence exocytosis (Figure 2C). Furthermore, α -synuclein could this way influence the proximity of synaptic vesicles to voltage-gated calcium channels in the plasma membrane, which has been shown to regulate the mode of exo- and endocytosis by influencing the local calcium environment [23].

α -Synuclein – Inhibitory or Facilitating Function in Vesicle Fusion?

Does α -synuclein inhibit or facilitate vesicle fusion? The aforementioned *in vitro* studies largely point to an inhibitory role of α -synuclein. Furthermore, this is strengthened by experiments performed in cells in which α -synuclein overexpression has been shown to inhibit the docking and fusion of endoplasmic reticulum (ER) vesicles with Golgi membranes [17,24,25]. However, on the other side, there is strong evidence that α -synuclein is a positive modulator of SNARE-complex assembly, as shown in mice with a knockout of the cysteine-string protein α (CSP α). In these mice a severe neurodegenerative phenotype is seen that has been linked to a decrease in the chaperoning of the SNAP-25 protein, which in turn decreases the efficacy of the SNARE-complex assembly and exocytosis [26–28]. Intriguingly, there was an almost complete rescue of this toxic phenotype when α -synuclein was overexpressed, and a positive regulatory effect of α -synuclein on exocytosis was therefore proposed. A simple substitution of CSP α chaperone activity by α -synuclein was not seen, and it was therefore suggested that α -synuclein might instead be able to stabilize SNAP-25 within the SNARE complex and thus make the protein less prone to degradation [27]. However, other and more subtle downstream effects could also come into play. The role of α -synuclein in stabilizing the curvature of synaptic vesicles could

two membrane surfaces into close proximity, zipping them together and preparing for fusion of the two compartments. This mechanism is not only involved in the fusion of synaptic vesicles with the presynaptic membrane but also, for example, in the fusion of vesicles from the ER with Golgi membranes.

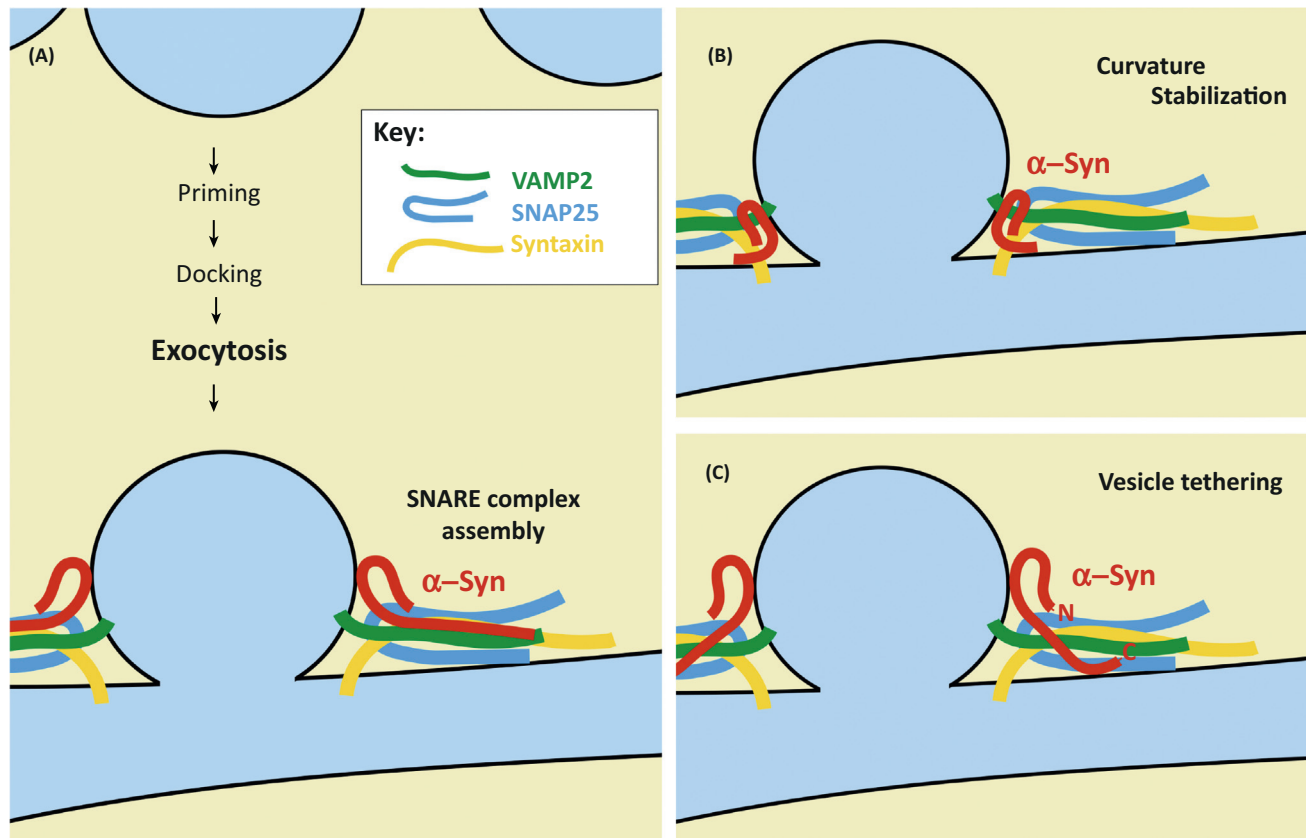
Synaptosome-associated protein 25 (SNAP-25): SNARE protein of the presynaptic membrane contributing two α -helices to the SNARE complex.

Syntaxin: SNARE protein anchored in the presynaptic plasma membrane that contributes one α -helix to the SNARE complex.

Ultrafast endocytosis: endocytosis at the presynaptic membrane with very fast initial membrane retrieval in the 100 ms time-frame. Vesicle recovery by endosomal budding occurs in about 5–6 s.

Vesicle-associated membrane protein 2 (VAMP2): also named synaptobrevin 2, a SNARE protein that is anchored in the synaptic vesicle and which contributes one α -helix to the SNARE complex.

α -Synuclein: presynaptic protein and main constituent of Lewy bodies in Parkinson's disease. The isoforms β - and γ -synuclein show high homology to α -synuclein but are not associated with the disease.



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Figure 2. α -Synuclein in Exocytosis. The current picture of the function of α -synuclein (α -Syn) points to a regulatory role in maintaining synaptic homeostasis upon intense neuronal activity. A function in exocytosis has been suggested via (A) mediating SNARE-complex assembly, (B) curvature stabilization thus preventing premature fusion of vesicles, and (C) a double-anchor mechanism tethering synaptic vesicles to the plasma membrane through two lipid-binding regions.

reduce the capacity of vesicles to fuse fully with the plasma membrane. Similarly, faster membrane and protein retrieval after exocytosis could improve overall protein homeostasis. These hypotheses could bring at first glance contradicting results into one line of thought. On one side α -synuclein is inhibiting vesicle fusion via stabilization of the curvature of synaptic vesicles. On the other, α -synuclein improves the recycling of synaptic vesicles, thus maintaining the exocytotic machinery in good condition, overcoming the loss of CSP α chaperoning.

Function of α -Synuclein on Synaptic Activity and Transmitter Release

What is the bottom line of studies looking at synaptic activity and transmitter release in α -synuclein animal or cell models? Recent work in Parkinson's disease models with overexpression of wild-type α -synuclein show mainly an inhibitory effect of α -synuclein on neurotransmitter release [29–34], whereas two other studies come to the opposite conclusions [35,36]. In one of the latter increased synaptic activity was found in cultured hippocampal neurons acutely injected with α -synuclein. In the other, paired pulse facilitation was seen in α -synuclein overexpressing mice (these studies are summarized in Table 1). What is happening upon α -synuclein knockout? Here studies report decreased exocytosis [33,35,37,38], no change [36,39], or even increased exocytosis [40–42] (Table 2). However, these studies need to be interpreted with care because α -synuclein is not the only synuclein isoform, and loss of physiological function could possibly be compensated by β - or γ -synuclein. Studies in mice with knockout of α -, β - and γ -synuclein (triple knockout mice) so far show an increase in

Table 1. Exocytosis – Studies on α -Synuclein Overexpression Models^a

Publication	Models	Primary results		Research methods	Ref.
Liu 2004 [35]	Neurons with α -syn injection	Synaptic activity	↑	Glutamate-evoked mEPSCs, cultured hippocampal neurons	[00]
Larsen 2006 [31]	α -Syn overexpression in PC12 and chromaffin cells	Evoked dopamine release Exocytotic events per stimulus 'docked' vesicles	↓ ↓ ↑	HPLC, PC12 cells, KCl stimulation Amperometric recordings, chromaffin cells EM, PC12 cells	[00]
Gureviciene 2007 [33]	α -Syn overexpressing mice	Basal synaptic transmission Paired-pulse facilitation	– ↓	fEPSP slope, PP, hippocampal slices, mice aged 4–5 months	[00]
Watson 2009 [36]	α -Syn overexpressing mice	Basal synaptic transmission Paired-pulse facilitation	– ↑	fEPSP, PP, corticostriatal slices, mice aged 2–6 months	[00]
Scott 2010 [32]	Neurons from GFP α -syn overexpressing mice	Spontaneous synaptic activity Endocytotic uptake Vesicle density, vesicle diameter Presynaptic proteins	↓ ↓ ↓/↑ ↓	mEPSC, cultured hippocampal neurons FM4-64 loading EM, cultured hippocampal neurons Immunocytochemistry staining	[00]
Nemani 2010 [29]	α -Syn KO neurons with α -syn overexpression/ α -syn overexpressing mice	Exocytosis Synaptic transmission Recycling pool of synaptic vesicles	↓ ↓ ↓	vGLUT1–pHluorin, cultured hippocampal and VM neurons fEPSP slope, hippocampal slices, mice aged 3–5 weeks vGLUT1–pHluorin and FM4-64, cultured hippocampal neurons	[00]
Wu 2010 [30]	α -Syn overexpressing mice	Spontaneous synaptic activity	↓	EPSCs, coronal slices, mice 3 months	[00]
Janzic 2013 [34]	α -Syn overexpressing mice on an α -syn KO background	Dopamine release in dorsal striatum Dopamine content dorsal striatum Synaptic activity Vesicle clustering	↓ – –/↓ ↑	FCV, striatal slices, mice 3–4 months, 12 months, 18 months HPLC, mice 3–4 months, 12 months, 18 months Mean firing rate, dopamine neurons, mice 3–4 months, 18–22 months EM, dorsal striatum, mice 3 months	[00]

^aAbbreviations and symbols: ↑, increase; ↓, decrease; –, no change; EM, electron microscopy; EPSC, excitatory postsynaptic current; FCV, fast-scan cyclic voltammetry; fEPSP, field excitatory postsynaptic potential; HPLC, high performance liquid chromatography; mEPSC, miniature EPSC; P, paired-pulse measurements; α -syn, α -synuclein.

Table 2. Exocytosis – Studies on Synuclein Knockout Mice^a

Publication	Models	Primary results		Research methods	Ref.
α-Synuclein knockout (KO)					
Abeliovich 2000 [40]	α-Syn KO mice	Dopamine release Paired-pulse depression Dopamine/DOPAC level in the striatum	– ↓ ↓/–	FCV, PP, striatal slices, mice aged 6–8 weeks HPLC	[00]
Cabin 2002 [38]	α-Syn KO mice	Synaptic transmission Paired-pulse facilitation Synaptic response on prolonged repetitive stimulation Synaptic vesicles	– – ↓ ↓	fEPSP slope, PP, hippocampal slices, mice 4–5 weeks fEPSP slope, hippocampal slices, mice 4–5 weeks EM, cultured hippocampal neurons, mice 2 months	[00]
Liu 2004 [35]	Neurons from α-syn KO mice	Synaptic activity	↓	Glutamate-evoked mEPSCs, cultured hippocampal neurons	[00]
Martin 2004 [37]	α-Syn KO mice	Basal synaptic transmission Paired-pulse facilitation Synaptic efficacy	– – ↓	EPSC amplitude, hippocampal slices, mice 4–7 weeks 4-AP-induced EPSCs	[00]
Yavich 2004 [42]	α-Syn KO mice	Dopamine release	↑	FCV in 12–14 month old mice	[00]
Gureviciene 2007 [33]	α-Syn KO mice	Basal synaptic transmission Paired-pulse facilitation	– ↓	fEPSP slope, PP, hippocampal slices, mice 4–5 months	[00]
Watson 2009 [36]	α-syn KO mice	Paired-pulse facilitation	–	PP, corticostriatal slices, mice 2–6 months	[00]
Synuclein double knockout (DKO)/triple knockout (TKO)					
Senior 2008 [41]	αγ-Syn DKO mice	Dopamine release Striatal dopamine content	↑ –	FCV, striatal slices, mice 10–12 weeks HPLC, striatum, mice 6 months	[00]
Chandra 2004 [39]	αβ-syn DKO mice	Dopamine and GABA release Paired-pulse facilitation Dopamine/DOPAC/5-HT content	– – ↓/–/–	Synaptosomes (³ H-dopamine/ ¹⁴ C-GABA), KCl, sucrose stimulation PP, hippocampal slices, mice 3–5 weeks HPLC, dorsal striatum, mice 2–4 months	[00]
Greten-Harrison 2010 [43]	αβγ-Syn TKO mice	Basal synaptic transmission Paired-pulse facilitation Synapse size	↑/– – ↓	fEPSP slope, PP, hippocampal slices, mice 3 and 12 months EM, hippocampal sections, mice 3 months	[00]
Anwar 2011 [44]	αβγ-Syn TKO mice	Dopamine release in dorsal striatum Dopamine/DOPAC/5-HT content Synaptic vesicles SNARE complexes	↑ ↓/↓/↓ – –	FCV, striatal slices, mice 10–14 months HPLC, striatum, mice 4 months EM, dorsolateral striatum Coimmunoprecipitation of VAMP2 with SNAP-25	[00]

^aAbbreviations and symbols: ↑, increase; ↓, decrease; –, no change; DOPAC, 3,4-dihydroxyphenylacetic acid (dopamine metabolite); EM, electron microscopy; EPSC, excitatory postsynaptic current; FCV, fast-scan cyclic voltammetry; fEPSP, field excitatory postsynaptic potential; HPLC, high performance liquid chromatography; 5-HT, 5-hydroxytryptamine/serotonin; mEPSC, miniature EPSC; PP, paired-pulse measurements.

synaptic transmission only in young (3-months-old) but not in adult mice [43]. However, a more recent study demonstrated an increase in dopamine release in 1 year old triple knockout mice [44], which again argues for a negative regulatory function of α -synuclein in exocytosis (Table 2).

Several of the studies above also looked at the effect of α -synuclein on the maintenance of the synaptic vesicle pool. Knockout of α -synuclein was shown to decrease the number of synaptic vesicles, especially of the distal pool, which reduces their availability upon intense stimulation [38,45]. Other effects have also been reported upon α -synuclein overexpression. One study found an increase in synaptic vesicle size, another a decrease of vesicle re-clustering after endocytosis, and two other studies report decreased motility of synaptic vesicles [29,32,46,47]. Interestingly, α -synuclein overexpression has also been shown to increase the amount of vesicles docked to the membranes [31], which would be in line with the recently published double-anchor function of α -synuclein [14]. In summary, these studies show that α -synuclein seems to affect the vesicle pool, but a uniform picture of the underlying mechanisms is still lacking.

α -Synuclein in Synaptic Vesicle Endocytosis

α -Synuclein and Its Emerging Role in Synaptic Vesicle Endocytosis

The first indication that α -synuclein can lead to disturbances in the endocytosis of synaptic vesicles comes from studies applying lipophilic dyes for the visualization of exocytosis in live synapses. A reduction in presynaptic loading efficacy was reported upon α -synuclein overexpression, which suggested a reduction of endocytosis [32,46].

Intriguingly, recent efforts now reveal that α -synuclein plays a role in the endocytosis of synaptic vesicle [48–50]. In mice with simultaneous knockout of α -, β -, and γ -synuclein, synaptic vesicle exo- and endocytosis was monitored using a fluorescent sensor reporting on the recycling of synaptic vesicles. These data show a clear slowing of vesicle endocytosis, while there was no observed change in the rate of synaptic vesicle exocytosis. Expression of each individual synuclein isoform on the triple-knockout background was found to compensate for this endocytotic failure, demonstrating the complementary function of the synuclein isoforms. A decrease in the rate of endocytosis was evident after acute stimulation, but also at the basal level, as reported by retrograde labeling which revealed a diminished number of lately endocytosed synaptic vesicles [48].

A second study gives further confirmation of the role of α -synuclein in endocytotic membrane retrieval using patch-clamp capacitance measurements. The membrane capacity is a read-out for the expansion of the presynaptic membrane upon fusion events, and this was found to be similar for synaptic terminals from control mice and mice expressing the A53T mutant α -synuclein at the calyx of Held, indicating that there is no difference in the level of exocytosis. However, membrane retrieval was delayed in A53T α -synuclein-expressing calyces. Furthermore, acute short-term whole-cell dialysis of A53T as well as wild-type α -synuclein delayed membrane retrieval, indicating a direct interaction with, and influence on, membrane properties by α -synuclein [49].

A third study again analyzed the vesicle pool in lamprey synapses upon electrical stimulation [50]. When synapses acutely injected with α -synuclein protein were stimulated at 20 Hz, severe changes in the synaptic vesicle pool were seen, small synaptic vesicles were rare, and bulk membranous structures appeared within the presynaptic terminal. Controls exhibited a normal vesicle pool upon this stimulation protocol, and synapses acutely injected with α -synuclein also showed a normal vesicle pool when stimulated at 5 Hz. This indicates that α -synuclein is inducing a failure of synaptic vesicle endocytosis; however, this seems to be dependent on neuronal activity – in other words endocytotic failure only occurs upon increased synaptic

vesicle turnover [50]. This would be supportive for a regulatory function of synuclein, perhaps not being essential for basal neurotransmission levels but only upon intense repetitive stimulation.

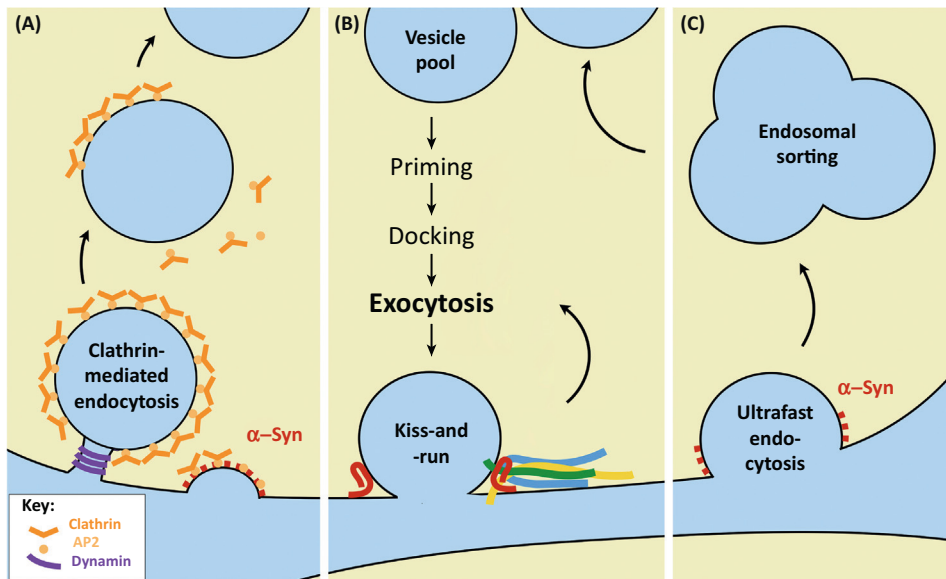
Mechanisms of Synaptic Vesicle Endocytosis

From these studies the concept solidifies that α -synuclein has a function in endocytosis. There are four major modes of endocytosis to differentiate, referred to as: **kiss-and-run** of synaptic vesicles, estimated to occur in <1 s time-frame [51]; **clathrin-mediated endocytosis** (CME), occurring in a 10–20 s time-frame [52–55]; **ultrafast endocytosis** followed by endosomal budding (initial membrane retrieval 100 ms, vesicle recovery 5–6 s) [56]; and **bulk endocytosis** [57]. Mechanisms of clathrin-mediated endocytosis are well established and many key players are known today; however, mechanisms for the kiss-and-run mode are under sustained debate and a molecular identity involved in the process is still missing. Kiss-and-run endocytosis is also known as ‘flicker-fusion’ because the synaptic vesicle does not fully collapse upon membrane fusion, and instead the neurotransmitter is released through a small fusion pore. In dopaminergic neurons, the neurons vulnerable in Parkinson’s disease, kiss-and-run-mediated exocytosis has been clearly shown [58,59]. However, in hippocampal neurons some studies argue that clathrin-mediated endocytosis is the main or even the only mechanism [54], while others report up to 60% of kiss-and-run endocytosis [60]. Clathrin-mediated endocytosis is one major mechanism of endocytosis, but there is still controversy on the involvement and relative importance of other mechanisms during ‘normal’ synaptic vesicle endocytosis, and it is not yet known if there are variations for different neuronal subtypes. Mechanisms of membrane retrieval might not be different between neuronal types *per se*, but it is possible that neurons rely on different mechanisms of endocytosis, depending on their electrophysiological activity and the accompanied need in vesicle recycling.

α -Synuclein – A Possible Role in Slow and Fast Endocytosis

As discussed, it is generally accepted that α -synuclein binds to synaptic vesicles. The evidence on its role in sensing and stabilizing the curvature of these tiny organelles is on the other hand much more recent and still emerging [9–11]. Regarding the function of α -synuclein in vesicle endocytosis, different views are emerging. On the one hand, α -synuclein is thought to be associated with clathrin-mediated endocytosis of synaptic vesicles (Figure 3A) because triple synuclein knockout led to changes in CME protein expression in mice and because increased numbers of clathrin-coated pits have been seen after stimulation of triple synuclein knockout synapses [48]. In addition, early studies on receptor-mediated endocytosis of transferrin suggested a role for α -synuclein in CME [61–63]. Furthermore, the study in lamprey synapses displayed disturbances of CME and increased bulk endocytosis [50]. However, because these changes have only been seen upon intense stimulation, another interpretation would suggest that this is not a direct effect on CME, and instead suggests that there is compensatory upregulation of CME when α -synuclein-related fast endocytosis mechanisms are impaired. Interestingly, endophilins, proteins well known for membrane curvature sensing and their function in CME, have been found to be upregulated in triple synuclein knockout mice [11], supporting the idea that α -synuclein and endophilins have partially complementary functions during endocytosis. Endophilin A, for example, is able to generate membrane tubulation [64], a phenomenon that has also been reported for α -synuclein [9,65], thus again pointing to a possible function of α -synuclein in inducing membrane curvature for vesicle endocytosis. However, whether this is part of CME or a clathrin-independent membrane retrieval mechanism remains open because endophilins have also been proposed to have clathrin-independent functions [66,67].

On the other hand, a role for α -synuclein in fast endocytosis mechanisms such as kiss-and-run must also be considered. α -Synuclein could play a role of stabilizing the curvature of small



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Figure 3. α -Synuclein in Endocytosis. Latest studies indicate a role of α -synuclein (α -Syn) in synaptic vesicle endocytosis. As a protein with curvature-sensing properties, the function of α -synuclein can be associated with several membrane-forming processes. Clathrin-mediated endocytosis was the first to be proposed (A). However, a role in fast endocytotic pathways such as kiss-and-run (B) or the recently discovered ultrafast endocytosis process (C) also seem likely, and are in accordance with a role of α -synuclein during intense and repetitive neuronal firing.

vesicles [11,21], thus making a full vesicle collapse during exocytosis less likely, as is required for kiss-and-run. In addition, the proposed double-anchor mechanism could influence the tethering of vesicles to the plasma membrane, thereby regulating the likelihood of exocytosis and thus preventing the full collapse of synaptic vesicles [14] (Figure 3B). For the mechanism of ultrafast endocytosis, it has yet to be understood how membrane fission can occur in a timescale of tens of milliseconds, while normal fission is reported to be in the range of seconds or even tens of seconds [68]. Facilitation by additional factors including actin, endophilins, and membrane lipids has been discussed, raising the question of whether α -synuclein might also be involved in this process (Figure 3C).

It has been proposed that different endocytotic pathways can coexist in one single synapse [69]. It therefore seems that different pathways are perhaps not different for neuronal subtypes in general, but that use of CME or fast membrane retrieval may be dependent on synaptic activity. Neurons with a high rate of exocytosis, such as the dopaminergic neurons of the substantia nigra with their continuous pacemaker activity [70], may rely on fast endocytosis, while other neurons rely mainly on CME and are less affected when fast mechanisms of endocytosis are disturbed. The results of capacitance measurements show that α -synuclein is possibly directly involved in the step of membrane retrieval [49]. However, rather than speaking of fast and slow endocytosis, which makes sense when looking at the timescale, one might more appropriately classify the processes into endocytosis upon intense stimulation and endocytosis upon moderate stimulation. While endocytosis can be maintained by CME at low-frequency firing, membrane-saving or fast retrieval mechanisms such as the kiss-and-run or ultrafast endocytosis are probably necessary to maintain the structure of the presynaptic membrane upon intense and repetitive stimulation because only then are further exocytotic events possible. When these fast mechanisms fail, CME can still maintain presynaptic homeostasis to a degree, and this leads to slowing down of endocytosis [48], or, as seen in lamprey synapses upon intense stimulation, bulk endocytosis will occur [50].

Intriguingly, overexpression of **Rab proteins** has been shown to rescue α -synuclein toxicity [24,25,71]. Rab proteins are key players in the endosomal sorting of vesicles, and they are therefore crucial for retrieving new functional synaptic vesicles. Whether there is a direct interaction between Rab proteins and α -synuclein or a general compensation for vesicle transport deficits remains elusive; however, this outlines once more the important role of α -synuclein in maintaining the synaptic vesicle pool.

LRRK2 –Role in Synaptic Vesicle Endocytosis

Mutations in the leucine-rich repeat kinase 2 (*LRRK2/PARK8*) gene are established as the most common genetic cause of Parkinson's disease [72,73]. Intriguingly, cumulating evidence also indicates a role of LRRK2 in synaptic vesicle endocytosis because LRRK2 knockout as well as the common G2019S LRRK2 mutant slow down synaptic vesicle endocytosis [74]. Similar observations were made in *Drosophila* LRRK2 loss-of-function mutants [64], and recently also in neurons from LRRK2 knockout mice [75]. Furthermore, inhibitors of LRRK2 kinase activity seem to reduce synaptic vesicle endocytosis [76]. Different mechanisms have been discussed, and LRRK2 has been found to phosphorylate mammalian endophilin A1 [75] as well as Rab5b [74,77]. However, although there is still controversy on the mechanism, a link to α -synuclein interaction with endophilin A or Rab proteins may reveal interesting new evidence related to Parkinson's pathology.

Concluding Remarks

The current picture on the function of α -synuclein points to a regulatory role in maintaining synaptic homeostasis upon intense neuronal activity. Because inhibitory neurons lack α -synuclein expression [78,79], and no synuclein homologs are expressed in invertebrates [80], α -synuclein does not appear to be essential for synaptic transmission *per se*. In fact, studies in songbirds indicate a possible function of α -synuclein during song development, pointing to a crucial role during intense synaptic activity [81].

The neurons most severely affected in Parkinson's disease, the dopaminergic neurons of the substantia nigra, exhibit an autonomous pacemaker activity, meaning that they undergo continuous cycles of exo- and endocytosis all throughout their life [70]. Instead of a direct effect of α -synuclein on the basic machinery of exocytosis itself, α -synuclein might maintain the synapse in good 'shape' upon prolonged and intense synaptic activity.

Neurons with a high rate of exocytosis, such as the dopaminergic neurons, may rely on fast endocytosis while other neurons can rely on CME, or at least are less affected when the fast mechanisms of endocytosis are impaired.

When fast mechanisms fail, CME will probably still be capable of maintaining presynaptic homeostasis to a certain level, and this is what one might see as a slowing down of endocytosis/membrane retrieval [48,49], or bulk endocytosis may take over, as seen in lamprey synapses upon intense stimulation [50]. Although these studies clearly show a disruption of endocytosis, they do not point to any specific 'pathway' that α -synuclein is potentially involved in. One might presume a function of α -synuclein in the fast mechanisms of endocytosis, such as kiss-and-run or ultrafast membrane retrieval. In this case α -synuclein could act upon curvature stabilization or via the double-anchor mechanism, making full collapse of synaptic vesicles less likely, and thus favoring kiss-and-run. On the other hand, α -synuclein may assist ultrafast endocytosis by influencing membrane curvature, as membrane tubulation properties similar to those of endophilin proteins have been shown for α -synuclein [9].

However, despite this exciting recent progress in the field, the exact role of α -synuclein is still far from understood and many details are yet to be clarified (see Outstanding Questions).

Outstanding Questions

α -Synuclein seems to have a major function in sensing and regulating membrane curvature. Is this function related to exocytosis via stabilization of synaptic vesicles to prevent premature fusion? Would this simultaneously speak for a function in kiss-and-run? Or, on the other side, could this function be related to endocytotic membrane retrieval at the presynaptic terminal?

Would α -synuclein be able to initiate new budding events of vesicles, or does it only facilitate the bending of membranes – or even only stabilize existing curved membranes?

Molecular key players of kiss-and-run and ultrafast endocytosis are still missing; how then could one prove and visualize a possible overlap of α -synuclein with these elusive mechanisms. Would it be possible to demonstrate a decrease of kiss-and-run events in presynaptic dopaminergic terminals, while clathrin-mediated endocytosis is upregulated?

How much do pathways of endocytosis coincide within one synaptic terminal? How do different mechanisms work together? Are there different needs in different neuronal subtypes according to their firing patterns?

Are dopaminergic neurons especially vulnerable because of their autonomous pacemaking activity? Continuous cycles of exo- and endocytosis, where the active zone must be cleared from excessive membrane immediately and in time for the next round of exocytosis, would put a particular burden on and special needs for fast endocytosis mechanisms.

Nevertheless, intracellular mechanisms relying on reshaping and stabilization of membrane curvature seem to be interesting targets in the context of improving our understanding of the physiological implications of α -synuclein.

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