

## Review

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# The Synaptic Function of $\alpha$ -Synuclein

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**Abstract.**  $\alpha$ -Synuclein is an abundant neuronal protein which localizes predominantly to presynaptic terminals, and is strongly linked genetically and pathologically to Parkinson's disease and other neurodegenerative diseases. While the accumulation of  $\alpha$ -synuclein in the form of misfolded oligomers and large aggregates defines multiple neurodegenerative diseases called "synucleinopathies", its cellular function has remained largely unclear, and is the subject of intense investigation. In this review, I focus on the structural characteristics of  $\alpha$ -synuclein, its cellular and subcellular localization, and discuss how this relates to its function in neurons, in particular at the neuronal synapse.

**Keywords:**  $\alpha$ -synuclein, synapse, membranes, SNARE, neurotransmitter release, synaptic vesicles

## HISTORY

$\alpha$ -Synuclein was named after its localization on synaptic vesicles and on nuclear envelopes isolated from the Torpedo electric organ [1]. In parallel,  $\alpha$ -synuclein was identified as the non-amyloid- $\beta$  component (NAC) found in amyloid plaques of Alzheimer's disease patients [2]. The discovery of  $\alpha$ -synuclein was soon followed by the identification of its close homologs  $\beta$ - and  $\gamma$ -synuclein [3–6]. Since then,  $\alpha$ -synuclein has been linked to various devastating diseases, including Parkinson's disease [7, 8], dementia with Lewy bodies [7, 8], multiple system atrophy [9–11], Alzheimer's disease [12, 13], pantothenate kinase-associated neurodegeneration (PKAN; a.k.a. neurodegeneration with brain iron accumulation type I; formerly Hallervorden-Spatz syndrome) [14–16], Pick's disease [17], diffuse Lewy body disease [18], Lewy body variant of Alzheimer's disease [19], amyotrophic lateral sclerosis (ALS) [20, 21], ALS-Parkinsonism-dementia complex of Guam [22, 23], pure autonomic failure [24], frontotemporal dementia [25, 26], progressive supranuclear palsy [27, 28],

corticobasal degeneration [29], and Krabbe disease [30], collectively termed "synucleinopathies". In addition, genome-wide association studies have identified a higher risk of sporadic Parkinson's disease for individuals with variations in the *SNCA* gene [31], highlighting  $\alpha$ -synuclein's genetic link to the disease. The physiological function of  $\alpha$ -synuclein, however, has remained enigmatic.

## $\alpha$ -SYNUCLEIN EXPRESSION & LOCALIZATION

$\alpha$ -Synuclein is a protein of 140 residues that is predominantly and ubiquitously expressed in the brain [4], in particular throughout the neocortex, hippocampus, olfactory bulb, striatum, thalamus, and cerebellum in the rat brain [32]. While initially described as a nuclear protein [33, 34], these reports have not been consistent. In contrast, the presynaptic localization of  $\alpha$ -synuclein has become well established (see below). Yet, although  $\alpha$ -synuclein is highly enriched in synaptic boutons which sprout from axons of different neurochemical phenotypes,  $\alpha$ -synuclein is not present in all synaptic terminals, and, curiously, not all terminals accumulate the protein in neurodegenerative disorders [35], suggesting selective expression, targeting, and pathogenic vulnerability in certain neuronal populations. Furthermore, although highly enriched in

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the nervous system [2, 4], its expression is not limited to nervous tissues: significant amounts of  $\alpha$ -synuclein have been detected in red blood cells [36], and low levels of expression have been found at mRNA and/or protein level also in other tissues [37–43], suggesting more general cellular functions in addition to its activity in the brain.

Out of the three synuclein family members,  $\beta$ -synuclein reveals the most brain-specific expression [44], and  $\gamma$ -synuclein the least [5]. Similar to  $\alpha$ -synuclein,  $\beta$ - and  $\gamma$ -synucleins localize to synaptic terminals [4, 45, 46], and overlap with expression of  $\alpha$ -synuclein in certain brain areas [5, 44, 47]. Although  $\beta$ - and  $\gamma$ -synuclein are absent from Lewy bodies, they co-localize with  $\alpha$ -synuclein in spheroid-like neuronal inclusions in Parkinson's disease, dementia with Lewy bodies and PKAN [7, 15]. The identification of polymorphisms in  $\beta$ - and  $\gamma$ -synuclein that predispose to dementia with Lewy bodies and diffuse Lewy body disease [18, 48], neurodegeneration in mutant  $\beta$ - and wild-type  $\gamma$ -synuclein transgenic mice [49–51], co-occurrence of  $\beta$ -synuclein in  $\alpha$ -synuclein-containing Pick bodies in frontotemporal dementia [17], and the link of  $\gamma$ -synuclein to ALS, Gaucher's disease, and Alzheimer's disease [52–54], suggests that all synucleins may be involved in neurodegenerative diseases.

Within the nervous system, the expression of  $\alpha$ -synuclein is developmentally regulated.  $\alpha$ -Synuclein mRNA expression begins in late embryonic stages in rodents, reaches a peak in the first few postnatal weeks, and is then reduced [55, 56].  $\alpha$ -Synuclein protein levels increase during development and remain high during

adulthood [56, 57], suggesting post-transcriptional regulation of its levels.  $\alpha$ -Synuclein distributes from the soma to presynaptic terminals during early weeks of development in rodents [58, 59] and in humans [60, 61], where it associates with synaptic vesicles [1, 62]. Although it is still unclear how  $\alpha$ -synuclein reaches the synapse, its preference for synaptic vesicle membranes [1, 62], and its affinity for the vesicular SNARE protein synaptobrevin-2 [63], synapsin III [64], or rab3A [65], may target it to presynaptic boutons. Strikingly, while highly concentrated in presynaptic terminals,  $\alpha$ -synuclein is among the last proteins to reach the synapse [58, 66]. Together with its presence only in vertebrates [67], this suggests that  $\alpha$ -synuclein has an activity required for a more complex cellular function that is not essential for basic neurotransmitter release or synapse development.

## STRUCTURE OF $\alpha$ -SYNUCLEIN

$\alpha$ -Synuclein has a remarkable and unique structure (Fig. 1). Its N-terminal sequence is divided into seven 11-mer repeats with a KTKGEV consensus sequence (residues 1–95), which, similar to apolipoproteins, form an amphipathic  $\alpha$ -helix with 3 turns, and mediate association of  $\alpha$ -synuclein with lipid membranes [68–72]. This region contains also the NAC domain (residues 60–95), an area believed to be responsible for  $\alpha$ -synuclein aggregation [2] and sensing of lipid properties [73]. Curiously, all identified mutations associated with synucleinopathies are located in this region: A30P, E46K, H50Q, G51D, A53E, and A53T [74–80], five of which cluster within

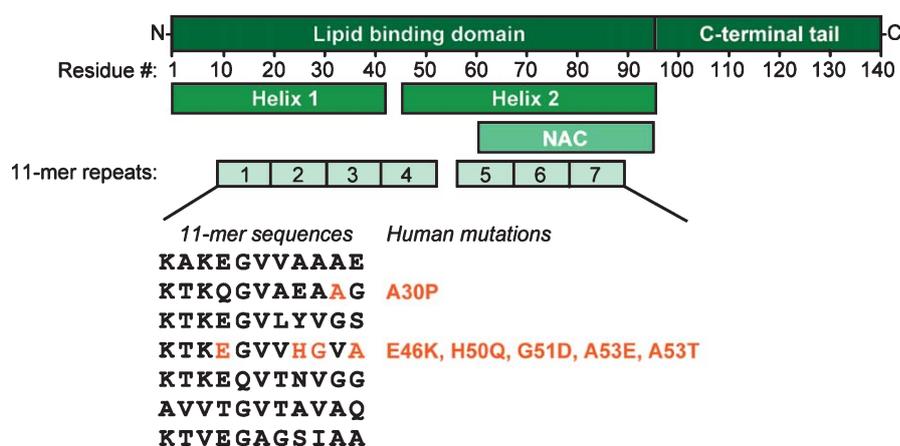


Fig. 1.  $\alpha$ -Synuclein domain structure. Upon binding to lipid membranes, the N-terminal domain of  $\alpha$ -synuclein folds into two amphipathic helices; the C-terminal tail of  $\alpha$ -synuclein does not contribute to membrane binding. The lipid binding domain can be divided into seven highly conserved 11-mer sequences. Helix 2 contains the aggregation-prone NAC-domain. All disease-linked mutations of  $\alpha$ -synuclein are located in the second and fourth 11-mer stretch.

eight residues, suggesting that lipid binding or lack thereof may be linked to  $\alpha$ -synuclein pathology. The C-terminus of  $\alpha$ -synuclein (residues 96–140) is highly acidic and largely unstructured [68, 69, 81], target of various post-translational modifications [82], and believed to be responsible for (i) interactions with proteins (see below), (ii) ion, polycation and polyamine binding [83–86], (iii) modulation of membrane binding of  $\alpha$ -synuclein [87, 88], and for (iv) protection of  $\alpha$ -synuclein from aggregation [89–91].

### INTRACELLULAR POOLS OF $\alpha$ -SYNUCLEIN

$\alpha$ -Synuclein exists in a dynamic equilibrium between a soluble state and a membrane-bound state, with its secondary structure depending on its environment. The interaction between  $\alpha$ -synuclein and lipid surfaces is believed to be key feature for mediating its cellular functions (Fig. 2). Soluble cytosolic  $\alpha$ -synuclein is intrinsically unstructured and behaves like a natively unfolded protein [71, 92–95]. A debate has recently developed around  $\alpha$ -synuclein's soluble state, due to a proposed metastable tetrameric form of  $\alpha$ -synuclein [96, 97]. While other studies have demonstrated that no such cytosolic tetramer exists in the central nervous system, in erythrocytes, mammalian cells, and in *E. coli* [94, 95, 98, 99], binding to cellular factors, such as lipids or membranes, can induce and stabilize such multimers [100], as endogenous multimers become unstable as the protein approaches purity [101].

In presence of lipid membranes, such as artificial liposomes, lipid droplets and lipid rafts, the N-terminal residues of  $\alpha$ -synuclein adopt an alpha-helical

structure which mediates binding of  $\alpha$ -synuclein to membranes [68–71, 102–104]. Membrane binding is likely a cooperative effect of the 11-mer sequences, as truncation of the N-terminal domain reduces lipid binding drastically, and requires acidic head groups [102–106], such as phosphatidylethanolamine, phosphatidylserine or phosphatidylinositol. This suggests an interaction of the membrane headgroups with lysines found on opposite sides of the  $\alpha$ -synuclein helix. Both, a single elongated alpha-helix, and a broken alpha-helix have been reported, depending on membrane curvature [68, 71, 72], and  $\alpha$ -synuclein is able to transition between these two states [81, 107]: Upon binding to membranes with larger diameter ( $\sim 100$  nm),  $\alpha$ -synuclein adopts an elongated helix [68, 108–111]. In contrast, in presence of small and highly curved vesicles,  $\alpha$ -synuclein adopts a broken helix conformation [71, 81, 112, 113], likely to adapt to the smaller liposome area.  $\alpha$ -Synuclein preferentially binds to vesicles of smaller diameter [69, 114], and as such associates with synaptic vesicles in the brain [1, 62].

Recently, it was found that  $\alpha$ -synuclein is N-terminally acetylated, mediated by attachment of an acetyl group to the alpha amino group of the first amino acid of  $\alpha$ -synuclein [94, 95, 115, 116]. N-terminal acetylation of  $\alpha$ -synuclein is seen both in healthy and Parkinson's disease individuals, and increases its helical folding propensity, its affinity for membranes, and its resistance to aggregation [115–118], suggesting that N-terminal acetylation of  $\alpha$ -synuclein could have important implications for both the native and pathological structures and functions of  $\alpha$ -synuclein [119]. In addition, phosphorylation of  $\alpha$ -synuclein regulates its structure, membrane binding, protein interactions, oligomerization, fibril formation, and neurotoxicity [120–125], although the exact kinases and phosphatases regulating (de)phosphorylation of  $\alpha$ -synuclein remain unknown. Other post-translational modifications, such as ubiquitination [126, 127], sumoylation [128, 129], glycation [130–132], glycosylation [133, 134], nitration [135–137], and proteolysis [12, 89, 138, 139], can result in changes in protein charge and structure. This may lead to altered binding affinities with other proteins and lipids, but their functional significance remains unknown and controversial.

$\alpha$ -Synuclein folding stabilizes and protects its target membrane [140], and membrane-binding protects  $\alpha$ -synuclein from aggregation [141–144], although membrane binding has also been reported to accelerate aggregation under oxidative stress [145]. Recently,

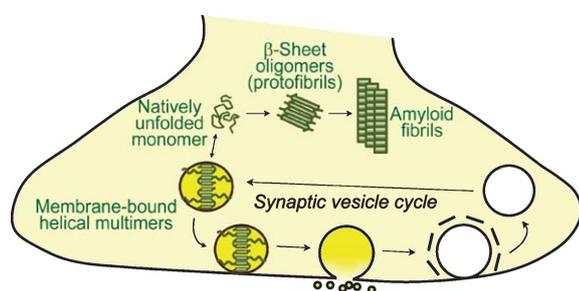


Fig. 2. Physiological and pathological conformations of  $\alpha$ -synuclein at the synapse. Cytosolic  $\alpha$ -synuclein is monomeric and natively unfolded. Upon binding to synaptic vesicles, the N-terminal residues of  $\alpha$ -synuclein adopt a helical structure. Membrane binding of  $\alpha$ -synuclein is associated with its multimerization, which is essential for its physiological function at the synapse. Pathologically, unfolded  $\alpha$ -synuclein in the cytosol can convert into  $\beta$ -sheet containing oligomers (protofibrils) which eventually form amyloid fibrils.

alpha-helical multimers of  $\alpha$ -synuclein have been reported upon binding of  $\alpha$ -synuclein to membranes, which are required for its physiological function at the synapse, and protect  $\alpha$ -synuclein from aggregation [100, 142, 146]. In contrast to these physiological conformations, in its pathologically relevant state,  $\alpha$ -synuclein adopts a beta-sheet rich conformation which is accompanied by aggregation and fibril formation, and deposition into Lewy bodies [147–151]. These cytosolic aggregates are likely derived from the less stable, natively unfolded conformations of cytosolic  $\alpha$ -synuclein [142].

### $\alpha$ -SYNUCLEIN FUNCTION AT THE SYNAPSE

The normal function of  $\alpha$ -synuclein remains enigmatic, despite more than 25 years of research. Assessing the normal function of  $\alpha$ -synuclein has been challenging, because: (i)  $\alpha$ -Synuclein is an intrinsically unstructured protein that cycles between a natively unfolded state in cytosol, and a helical multimeric state on membranes [71, 92–95, 100]; (ii) Overexpression of  $\alpha$ -synuclein triggers toxic effects in humans [152, 153] and in animal models [154–156], that are much worse than the effects caused by loss of  $\alpha$ -synuclein [157, 158]. This disconnection of the pathogenic activity of  $\alpha$ -synuclein from its physiological function [159] complicates findings in overexpression models; (iii) Potential compensation of  $\alpha$ -synuclein function by its isoforms  $\beta$ - and  $\gamma$ -synuclein complicate findings in knockout animals and necessitate simultaneous knockout of all isoforms or acute manipulation, such as done via viral injections. However,  $\alpha$ -synuclein's presynaptic localization and its interaction with highly curved membranes and synaptic proteins strongly suggests a regulatory function associated with the synapse, such as synaptic activity, synaptic plasticity, learning, neurotransmitter release, dopamine metabolism, synaptic vesicle pool maintenance, and/or vesicle trafficking (Fig. 3).

#### *Protein interactions*

$\alpha$ -Synuclein has been reported to interact with and affect a variety of proteins, mostly at the presynaptic terminal. This includes a controversial binding of phospholipase D [160–163], regulation of the membrane interaction of the G-protein rab3 [65], binding to the SNARE-protein synaptobrevin-2 and chaperoning SNARE-complex assembly [63, 159], binding and modulation of synapsin III [64], binding of VMAT2

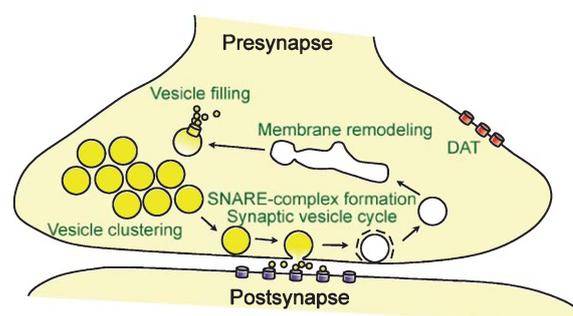


Fig. 3. Function of  $\alpha$ -synuclein at the synapse. Shown are the synaptic processes that  $\alpha$ -synuclein has been reported to affect, including membrane remodeling, modulation of the dopamine transporter DAT and vesicular monoamine transporter VMAT2, clustering of synaptic vesicles and maintaining synaptic vesicle pools, promoting SNARE-complex assembly, and modulating the release cycle of synaptic vesicles.

[164], dopamine and serotonin transporters [165–167], and regulation of tyrosine hydroxylase [168–170]. While these interactions are compatible with a function at the presynaptic terminal, the reported localization of  $\alpha$ -synuclein to mitochondria [171–173], endoplasmic reticulum [174, 175], Golgi [174, 175], and nuclei [1, 176] may arise from an altered subcellular distribution or spillover to other membranes, due to overexpression or during cell disruption. Overall, the functional significance of most of these findings remains unclear.

#### *Lipid transport, lipid packing and membrane biogenesis*

The similarity of  $\alpha$ -synuclein with class A2 apolipoproteins and decreased brain palmitate, phosphatidylglycerol and cardiolipin metabolism in absence of  $\alpha$ -synuclein [177–179] suggest a role in lipid metabolism, although lipidomic profiling of brains from synuclein transgenic and knockout mice revealed minimal effects of synuclein on lipid metabolism [180].  $\alpha$ -Synuclein has been reported to bind to fatty acids [181], and may thus serve as a fatty acid transporter between the cytosol and membrane compartments, while other studies suggest the contrary [182]. Furthermore,  $\alpha$ -synuclein has been shown to induce membrane curvature and convert large vesicles into highly curved membrane tubules, cylindrical micelles and vesicles [183–187], driven by binding affinity, partition depth, and interleaflet order asymmetry [188]. In addition,  $\alpha$ -synuclein has been reported to organize membrane components [189], to modulate phospholipid composition [190], and to be a specific inhibitor of phospholipase D1 and D2 *in vitro* and

*in vivo* [160–162]. This suggests that  $\alpha$ -synuclein may be involved in cleavage of membrane lipids and membrane biogenesis. Yet, the data on  $\alpha$ -synuclein and phospholipase D inhibition are controversial [163]. Last,  $\alpha$ -synuclein has been reported to sense lipid packing defects and to affect lipid packing [191, 192], and binding of  $\alpha$ -synuclein to synaptic vesicles may stabilize them via stabilizing their intrinsically tight curvature [193].

#### *Impact on dopamine metabolism and dopaminergic neurons*

While many types of neurons are affected in Parkinson's disease [194–196], a remarkable sign is the loss of dopaminergic neurons in the substantia nigra, and the resulting deficiency of dopamine signaling [197–199]. Despite tremendous strides in the understanding of  $\alpha$ -synuclein function and dysfunction, the increased vulnerability of dopaminergic neurons to  $\alpha$ -synuclein pathology remains unclear at the mechanistic level.  $\alpha$ -Synuclein has been proposed to regulate homeostasis of monoamines in synapses, via interaction with the serotonin transporter [165]. It binds to and regulates the targeting and the activity of the dopamine transporter DAT [166, 167, 200], although its mode of action remains controversial [201–203].  $\alpha$ -Synuclein inhibits dopamine synthesis by inhibiting the expression and activity of tyrosine hydroxylase [154, 168–170, 204], likely via reducing the phosphorylation state of tyrosine hydroxylase and stabilizing dephosphorylated inactive tyrosine hydroxylase [168, 205–207]. In agreement, aging-related increases in  $\alpha$ -synuclein expression in the substantia nigra negatively correlate to the expression of tyrosine hydroxylase [57]. In addition,  $\alpha$ -synuclein affects the vesicular dopamine transporter VMAT2: Knockdown of  $\alpha$ -synuclein increased the density of VMAT2 molecules per vesicle, while overexpression inhibits VMAT2 activity, interrupting dopamine homeostasis by causing increased cytosolic dopamine levels [164]. In agreement with a function in dopamine metabolism, absence of  $\alpha$ -synuclein causes decreased reuptake of dopamine in the dorsal striatum [208], a 36% reduction in striatal dopamine, accompanied by a reduction in tyrosine hydroxylase-positive fibers in the striatum, decreased striatal levels of tyrosine hydroxylase and dopamine transporter [209], and a decrease in the number of dopaminergic neurons in the substantia nigra [210, 211]. In addition,  $\alpha/\beta$ -synuclein double knockout mice display 20% reduced dopamine levels, with no change in dopamine uptake and release

[212], a two-fold increase in extracellular dopamine levels upon striatal stimulation, and hyperactivity in a novel environment, which is reminiscent of mice expressing reduced levels of the dopamine transporter [213]. Overall, this suggests that dopaminergic neurons may have both, a higher need for  $\alpha$ -synuclein function, and a higher susceptibility to  $\alpha$ -synuclein dysfunction. Yet, the presence of  $\alpha$ -synuclein in cells other than dopaminergic neurons suggests a more general activity in neuronal function.

#### *Molecular chaperone activity*

The biochemical structure of  $\alpha$ -synuclein predicts a function as a molecular chaperone capable of binding to other intracellular proteins. This hypothesis was strengthened by three observations: First,  $\alpha$ -synuclein shares structural and functional homology with the 14-3-3 family of molecular chaperone proteins [214]. Second, via its C-terminal domain,  $\alpha$ -synuclein suppresses the aggregation of thermally denatured proteins [215–219], and overexpression of  $\alpha$ -synuclein protects dopaminergic neurons from oxidative stress and apoptosis [220, 221]. Third,  $\alpha$ -synuclein rescues the lethal neurodegeneration caused by knockout of the co-chaperone CSP $\alpha$  in mice by chaperoning assembly of synaptic SNARE-complexes [63, 222]. This function of  $\alpha$ -synuclein is essential for long term functioning of neurons, since  $\alpha$ -,  $\beta$ -,  $\gamma$ -synuclein triple-knockout mice have reduced SNARE-complex assembly, show neuropathological signs and reveal shortened survival [63, 223, 224]. This chaperone function is consistent with the lack of an acute effect of  $\alpha$ -synuclein on cell survival and neurotransmitter release, and may become particularly important under stressful conditions and during the long life of a neuron.

#### *Neurotransmitter release and synaptic plasticity*

The presynaptic localization of  $\alpha$ -synuclein, its interaction with synaptic vesicles [1, 62] and synaptobrevin-2 [63], its SNARE-complex chaperoning activity [63], and its changes during periods of song-acquisition-related synaptic rearrangements in birds [225] strongly argues for a role in neurotransmitter release and synaptic plasticity, although its precise function remains unclear. Yet, absence of  $\alpha$ -synuclein in worms, flies and yeast suggests that  $\alpha$ -synuclein is not required for synaptic transmission or membrane trafficking in general. In agreement, knockout of  $\alpha$ -,  $\alpha/\beta$ -,  $\alpha/\gamma$ -, or  $\alpha/\beta/\gamma$ -synucleins does not induce morphological changes in the brain [63, 157, 212, 224],

although changes in synaptic protein levels [63, 212], changes in synapse structure and size [223], and impairments in survival [63, 223] have been reported in synuclein triple knockout mice. Together with neuromuscular pathology in mice lacking  $\alpha$ -synuclein [226], and reduced working and spatial memory learning in  $\alpha$ -synuclein knockout mice [227, 228], this suggests that  $\alpha$ -synuclein contributes to the long-term operation of a neuron.

The effect of  $\alpha$ -synuclein on neurotransmission and synaptic plasticity has been investigated both in knockout and under overexpressing conditions, where  $\alpha$ -synuclein has been reported to both promote and inhibit neurotransmitter release, or have no effect at all. While some studies reported a lack of effect of  $\alpha$ -synuclein on neurotransmitter release [63, 212, 229], others revealed an enhancement of synaptic transmission [223, 224, 230–234], or a decrease in release [157, 158, 213, 223, 235–237]. Two recent studies have reported an inhibitory effect of  $\alpha$ -synuclein on synaptic vesicle endocytosis during intense stimulation, but not under basal levels [238, 239], while another study reported an enhancement of clathrin-mediated endocytosis by  $\alpha$ -synuclein in neuronal and non-neuronal cells [240]. Whether the inconsistent results obtained for the effects of  $\alpha$ -synuclein on neurotransmission and synaptic plasticity could be ascribed to the experimental models used and the investigated brain regions, needs to be determined. It seems to be clear, though, that  $\alpha$ -synuclein is not required for basal neurotransmission, but plays an important role in maintaining neurons during intense neuronal activity and over their long lifetime.

How does  $\alpha$ -synuclein exert its effect on the neurotransmission machinery? Within the presynaptic terminal,  $\alpha$ -synuclein is highly mobile, as shown by photo-bleaching experiments, and  $\alpha$ -synuclein disperses from synaptic vesicles upon stimulation [241, 242], similar to synapsin I [243]. Facilitated by its dynamic membrane-binding, this suggests that  $\alpha$ -synuclein can be recruited to the site of high membrane-fusion activity, and that neural activity controls the normal function of  $\alpha$ -synuclein at the nerve terminal. Indeed,  $\alpha$ -synuclein attenuates the mobility of synaptic vesicle pools between presynaptic boutons and maintains the overall size of the recycling pools at individual synapses [244].

*In vitro*,  $\alpha$ -synuclein inhibits docking of synaptic vesicle mimics with plasma membrane mimics [245, 246]. This inhibition is not caused by interfering with the fusion process itself, but is due to clustering of synaptic vesicle mimics, a process strongly dependent

on  $\alpha$ -synuclein's ability to associate with lipids and synaptobrevin-2 [246].  $\alpha$ -Synuclein driven vesicle clustering has been initially reported in yeast [247, 248]. Recently,  $\alpha$ -synuclein has been reported to cluster synaptic vesicles in neurons [146], which is likely mediated by  $\alpha$ -synuclein's ability to form multimers on the vesicle surface [100, 146]. This clustering activity of  $\alpha$ -synuclein restricts synaptic vesicle motility [146], and thereby likely affects the kinetics of neurotransmitter release. Supportively,  $\alpha$ -synuclein associates with specific subpopulations of synaptic vesicles [100, 249], and cooperatively regulates synaptic function with synapsin III in dopaminergic neurons [64]. In addition,  $\alpha$ -synuclein knockout synapses reveal a selective deficiency of undocked vesicles without affecting docked vesicles [158], and knockdown of  $\alpha$ -synuclein leads to a significant reduction in the distal pool of synaptic vesicles [66].

How does clustering of synaptic vesicles by  $\alpha$ -synuclein multimers relate to increased SNARE-complex levels?  $\alpha$ -Synuclein induced vesicle clustering may increase the local concentration of synaptic vesicles and thereby of the SNARE protein synaptobrevin-2. This clustering of synaptic vesicles at the active zone would promote the formation of neuronal SNARE-complexes by constraining additional synaptic vesicles close to the active zone. Supportively, the SNARE-complex assembly deficit in  $\alpha/\beta/\gamma$ -synuclein triple knockout mice aggravates with increased synaptic activity [63].

Overall, the effect of  $\alpha$ -synuclein on neurotransmitter release is likely not mediated by directly acting on the release machinery, but by affecting the spatial organization of distinct synaptic vesicle pools within the presynaptic terminal, possibly via  $\alpha$ -synuclein multimerization, which is triggered by membrane binding and potentiates SNARE-complex assembly [100]. This activity of  $\alpha$ -synuclein contributes to the long-term operation of the nervous system, suggesting that alterations in the physiological function of  $\alpha$ -synuclein could promote the development of neuropathology in Parkinson's disease and related disorders.

## CONCLUSION

$\alpha$ -Synuclein is important for the normal function and integrity of synapses, and in the aging nervous system, dysfunction of  $\alpha$ -synuclein becomes a predisposing factor for synaptic dysfunction and the development of neuropathology. Overexpression of  $\alpha$ -synuclein triggers redistribution of the SNARE proteins SNAP-25,

syntaxin-1 and synaptobrevin-2 in an age-dependent manner in the striatum [250], impairs proper vesicle trafficking and recycling [175, 248, 251, 252], and large  $\alpha$ -synuclein oligomers inhibit SNARE-mediated vesicle fusion *in vitro* [253]. Furthermore, misfolded  $\alpha$ -synuclein, in the form of oligomers and aggregates, is believed to be toxic [254, 255], and recent studies have revealed propagation of misfolded  $\alpha$ -synuclein between neurons [256–259]. However, many questions remain unclear, including the causes of the selective vulnerability of dopaminergic neurons in Parkinson's disease, the triggers for  $\alpha$ -synuclein aggregation and pathology, and the role of aging in the pathogenesis of Parkinson's disease. Understanding how  $\alpha$ -synuclein localizes to and functions at the synapse, will provide a biological context to how it misfolds, which species of  $\alpha$ -synuclein are toxic, how these species are released and taken up by neurons, and how they nucleate new aggregates in a healthy cell. It is clear that either too little or too much  $\alpha$ -synuclein is deleterious for the brain. Thus, aiming at maintaining a healthy balance of  $\alpha$ -synuclein in the brain is a worthwhile target for preventing synucleinopathies, while maintaining its normal brain function.

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## CONFLICT OF INTEREST

The author has none to declare.

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