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α -Synuclein and dopamine at the crossroads of Parkinson's disease

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Abstract

α -Synuclein is central to the Lewy body neuropathology of Parkinson's disease (PD), a devastating neurodegenerative disorder characterized by numerous motor and non-motor manifestations. The cardinal motor symptoms are linked to death of dopaminergic neurons in the nigrostriatal pathway. Here we ask why these neurons are preferentially susceptible to neurodegeneration in PD and how α -synuclein is involved. To address these questions we bring together recent findings from genome-wide association studies, which reveal the involvement of α -synuclein gene variants in sporadic PD, with recent studies highlighting important roles for α -synuclein in synaptic transmission and dopaminergic neuron physiology. These latest advances add to our understanding of PD etiology and provide a central link between the genetic findings and neurodegeneration observed in sporadic PD.

Introduction

PD is a progressive and devastating neurodegenerative disorder, affecting 1% of individuals over 60 years old [1]. The three cardinal clinical features of PD are rigidity, resting tremor and bradykinesia, and these occur when approximately 50% of dopaminergic neurons projecting from the substantia nigra pars compacta (SNc) to the striatum are lost [1]. The neuropathological hallmark of the disease is the presence in surviving SNc neurons of intracellular inclusions known as Lewy bodies (LBs), which are composed mainly of the protein α -synuclein (Box 1) [1,2]. The pathology of PD is not solely confined to the nigrostriatal pathway because LBs are also found in the cortex, amygdala, locus coeruleus and peripheral autonomic system [3,4]. Dysfunction of these extranigral neuronal populations and the presence of LBs correlate with the non-motor manifestations of PD, including autonomic, sleep and olfactory dysfunctions, and these can precede the appearance of motor symptoms [3,4]. Although there is increasing awareness of the importance of these non-motor symptoms, the nigrostriatal dopaminergic pathway remains a focus for research and therapeutic intervention in treating the debilitating motor symptoms of PD.

PD is an excellent example of a neurological disorder in which a complex mix of aging, genetic susceptibility and environmental insult converge to varying degrees along a

spectrum to cause neurodegeneration and disease. At the extreme genetic end of the spectrum is familial PD caused by mutations in one of twelve known loci that together constitute 10% of PD cases [5]. At the extreme environmental-exposure end are a few cases of PD associated with the potent neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [6]. However, it is likely that the majority of sporadic PD cases result from a complex interaction between genes and environment, played out against the background of age, which remains the greatest risk factor. A multiple-hit hypothesis has recently been proposed for dopaminergic neuron loss in PD [7], suggesting the preferential neuronal death results from a combination of toxic cellular insults, from mitochondrial dysfunction or dopamine oxidation, and an impaired stress-induced protective response. Here we discuss the latest research on the contribution of α -synuclein to dopaminergic neurodegeneration in PD, bringing together recent genetic evidence supporting the involvement of α -synuclein gene variants in PD etiology with key findings emphasizing the role of α -synuclein in regulating dopamine metabolism and neuro-transmission.

The α -synuclein gene plays a role in both familial and sporadic PD

The first genetic evidence for the involvement of the α -synuclein gene, *SNCA*, in PD was the identification of three missense mutations (A30P, E46K and A53T) which segregated with the disease in unrelated families and caused PD with high penetrance [8–10]. Duplications and triplications of the wild-type *SNCA* locus have also been associated with autosomal dominant PD [11–14]. The presence of multiple copies of the gene was directly correlated with an increase in α -synuclein mRNA expression in the frontal cortex and in protein levels in whole blood samples [15]. Interestingly, and of importance when considering *SNCA* as a central link between familial and sporadic PD, the degree of overexpression was found to correlate with the severity of the disease. Individuals with *SNCA* triplication developed an early-onset form of PD with rapid cognitive decline and more severe non-motor symptoms, more widespread neurodegeneration and faster disease progression compared to patients carrying a duplication of the gene [11,14].

The role of the *SNCA* locus has now been extended from the rare familial form of PD to the common sporadic form of PD. Recent findings from the largest two genome-wide association studies (GWAS; see Glossary) performed to date on sporadic PD patients demonstrate a strong association between common single nucleotide polymorphisms (SNPs) within the *SNCA* locus and the disease [16,17] in two populations of different ancestry ($P = 2.24 \times 10^{-16}$ for a European population [16] and $P = 7.35 \times 10^{-17}$ for a Japanese population [17]), validating previous studies which found that variation at the *SNCA* locus increases PD susceptibility [18,19]. More recent GWAS identified the same *SNCA* variant found in the above European population study [16] in a different population ($P = 6.74 \times 10^{-8}$) [20]. Although the genetic association is highly statistically significant, the SNPs associated with the disease show an odds ratio (OR) of between 1.2 and 1.4, indicating a low increased disease relative risk and a low effect size. This illustrates one of the limitations of the GWAS approach (reviewed in Ref. [21]): because it is based on the principle that common variation underlies common disease, GWAS is designed to identify common variation of small effect and will miss rare variants of large effect. Nevertheless, the fact that the association at the *SNCA* locus was independently replicated in all three GWAS strongly indicates a potential role for α -synuclein in sporadic PD instead of being confined only to very rare familial forms of PD, a notion consistent with the α -synuclein aggregation pathology of LBs observed in all cases of PD.

Potential *SNCA*-associated disease mechanisms

The likely impact of disease-associated *SNCA* variants identified by GWAS [16,17,20] includes altered control of the level of transcription, regulation of alternative splicing, or altered stability of mRNA through post-transcriptional mechanisms (Figure 1).

Transcriptional regulation of *SNCA*

One mechanism underlying disease susceptibility could be altered transcriptional regulation of *SNCA*. REP1 is a polymorphic dinucleotide repeat site located 10 kb upstream of the start site of *SNCA* transcription, for which five alleles have been identified [22,23]. Interestingly, a risk allele of REP1, previously associated with PD [18,19,24] and shown to regulate *SNCA* expression in neuronal cell culture [25] and in a transgenic mouse model of PD [26], is in linkage disequilibrium (LD) with the risk alleles of the SNPs identified at the 3'-end of the *SNCA* locus [16]. It therefore remains unclear whether REP1-mediated transcriptional control, or mRNA stability regulated by the 3'-untranslated region (UTR) (see below), is the real functional genetic mechanism underlying disease susceptibility.

Although duplication and triplication of the *SNCA* locus elevate α -synuclein expression and cause familial PD [11,14], the broader question of whether α -synuclein expression is elevated in brains of sporadic PD patients remains unclear. In post-mortem sporadic PD midbrain tissue, total α -synuclein mRNA levels were found to be increased by 4-fold on average compared to control brains [27], although the variability between PD cases was very high. Contradictory results showing a reduction of α -synuclein mRNA in surviving neurons from the same brain region have also been reported [28,29]. Such comparisons are hard to perform given the massive neuronal cell loss and accompanying pathological processes in the midbrain of a PD patient. Thus these discrepancies could be attributed not only to different experimental designs, but more importantly to the limitations of measuring end-stage α -synuclein expression rather than assessing subtle changes in α -synuclein mRNA levels during disease progression [28].

Regulation of alternative splicing

A second mechanism by which SNPs can affect gene function is by regulation of alternative splicing. Several studies (reviewed in [30]) now report alternative isoforms of *SNCA* caused by alternative splicing of exons 3 and 5 to produce four variants of different amino acid (aa) length: exon 3⁺5⁺ (140 aa; SNCA140), exon 3⁻5⁺ (126 aa; SNCA126), exon 3⁺5⁻ (112 aa; SNCA112) and exon 3⁻5⁻ (98 aa; SNCA98) (Figure 1b). SNCA112 is reported to occur only in brains from patients with LB disease and is upregulated in cell culture models by the parkinsonian neurotoxins MPP⁺, the active metabolite of MPTP, or rotenone [31]. It is tempting to speculate that PD-associated polymorphic variants could affect *SNCA* splicing as a potential disease mechanism.

Regulation by microRNA binding

In the European population, three of the four associated SNPs with the most significant *P*-values were clustered around the 3'-UTR of *SNCA* [16], suggesting that post-transcriptional regulatory mechanisms involving mRNA stability and processing, possibly involving binding to regulatory microRNAs, underlie a third potential mechanism of disease susceptibility. The 3'-UTR of *SNCA* is known to contain target binding sites for two microRNAs, mir-7 and mir-153, which are expressed predominantly in neurons and induce downregulation of α -synuclein expression [32,33]. Genetic variability in the 3' region of *SNCA* has recently been implicated in the regulation of α -synuclein levels in the substantia nigra and cerebellum of human post-mortem tissue [34].

The emerging GWAS data demonstrate that variation at the *SNCA* locus is robustly associated with common, sporadic PD. This, combined with the previous findings that missense mutations and locus multiplications in *SNCA* cause rare, familial disease, and that α -synuclein accumulates in LBs, the defining protein inclusion found in PD, places both the gene and the protein at the centre of the molecular mechanisms of disease. It is, however, likely that the SNPs within the *SNCA* locus found associated with PD in the GWAS analyses are not actually themselves the functional polymorphisms but lie in LD with the functional variant. Indeed, discovery of the true functional variants will require extensive genomic DNA resequencing studies in PD cases followed by analysis in genomic DNA expression models.

α -Synuclein regulates the key stages of dopamine homeostasis

Although α -synuclein is widely expressed in the brain, PD-associated degeneration occurs preferentially in specific regions of the CNS, with the major pathology and the greatest loss of cells in the SNc leading to the principal motor symptoms. Understanding exactly how the regulation of dopamine homeostasis is affected by genetic variation at the α -synuclein locus is a major question in PD which remains to be answered.

Dopamine synthesis and content

α -Synuclein has been shown to regulate the production of dopamine in cultured cells through its interaction with tyrosine hydroxylase (TH), the rate-limiting enzyme responsible for converting tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) in the dopamine synthesis pathway [35,36] (Figure 2). Overexpression of α -synuclein in cells reduces the activity of the TH promoter [37], leading to reduced levels of TH mRNA and protein [38,39]. In addition, α -synuclein has been shown to bind TH, preventing its phosphorylation and inhibiting its activation by promoting the activity of protein phosphatase 2A (PP2A) [35,40]. Conversely, suppression of α -synuclein in cell culture models leads to increased phosphorylated TH and consequently increased TH activity [36]. Consistent with a role for α -synuclein in downregulating dopamine biosynthesis in cell culture models, reduced TH activity has been observed in several mouse models overexpressing wild-type α -synuclein [41,42]. α -Synuclein has also been proposed to interact with L-aromatic amino acid decarboxylase (AADC), the enzyme which catalyzes the conversion of L-DOPA to dopamine [43] (Figure 2). Overexpression of α -synuclein reduced serine phosphorylation and decreased AADC activity in dopaminergic cell models, possibly by altering PP2A activity [43].

As well as measuring enzyme activity, several studies have reported on striatal dopamine content. In studies of young adult α -synuclein null mutant mice, either a moderate decrease (~18%) [44] or no change [45–47] in striatal dopamine content has been reported. Loss of dopamine is more marked in aged α -synuclein null mice (24–26 months old), where striatal dopamine was significantly reduced (~36%) compared to their wild-type littermates [45,48]. This decline was accompanied by decreased expression of TH and dopamine transporter (DAT) in the striatum but no progressive reduction in the number of TH-positive neurons in the SNc [48].

These studies link α -synuclein with dopamine synthesis and content in both cell culture and animal models. It seems that a combined effect of α -synuclein levels and aging affect striatal dopamine levels and TH activity and, more importantly, induce synaptic dysfunction in the nigrostriatal system [48]. However, evidence for an effect on dopamine synthesis is not as compelling as the major role emerging for α -synuclein in synaptic vesicle function.

Synaptic vesicle function and dopamine release

The evidence for a link between α -synuclein and synaptic vesicle function comes mainly from analysis of non-dopaminergic hippocampal neurons. Although such experiments are not conducted in the dopaminergic midbrain neurons relevant to PD, these studies have informed on the role of α -synuclein in vesicle trafficking at neuronal synapses more generally (Figure 3). Mice lacking α -synuclein show an impairment in hippocampal synaptic responses to prolonged stimulation that is expected to deplete the docked and reserve pool of vesicles, as well as impairments in replenishment of docked pools from the reserve pool [49]. Consistent with a role for α -synuclein in regulating synaptic vesicles at presynaptic terminals, suppression of α -synuclein using antisense oligonucleotides in primary cultured hippocampal neurons decreased the availability of a 'distal' or reserve synaptic vesicle pool [50].

Transgenic mice overexpressing human α -synuclein have recently been shown to have impaired synaptic vesicle exocytosis in hippocampal neurons [52], and rat ventral midbrain dopaminergic neurons transfected with α -synuclein have shown a similar result [51]. Overexpression of α -synuclein in hippocampal neurons affected the reclustering of synaptic vesicles following endocytosis, causing a reduction in the size of the synaptic vesicle recycling pool [51]. Additionally, overexpression of α -synuclein carrying the A53T or E46K mutations (which retain the ability to bind membranes), but not by A30P (for which ability to bind membranes is dramatically reduced) in hippocampal cultures caused an inhibition of exocytosis, which is consistent with an important role for the N-terminal membrane binding domain of the protein in the function of α -synuclein [51]. Whether or not α -synuclein fulfils similar roles in dopaminergic neurons *in vivo* remains unknown, but these findings are nevertheless the first to associate modest overexpression of α -synuclein in the range seen in PD patients with a marked defect in synaptic transmission and neurotransmitter release [51]. Findings that the level of α -synuclein protein expression directly affects synaptic function suggest a mechanism whereby genetic polymorphisms found within regulatory elements controlling *SNCA* expression could influence PD susceptibility.

Moving to dopaminergic neurons, an increased rate of refilling of the readily releasable pool has been reported in mice lacking α -synuclein [52]. This result is in line with an increase in recovery from paired-pulse depression (PPD) that has been reported in striatal slices in one study [44], although another study found no such change in PPD [53]. Mice lacking either α - or γ -synuclein alone do not show changes in the levels of electrically evoked dopamine release in the striatum [53], but double knockout mice lacking both α - and γ -synuclein have a two-fold increase in evoked dopamine release. With no change in DAT activity or striatal dopamine content [53], these findings have been attributed to an increase in dopamine releasability, possibly due to changes in vesicle fusion or a redistribution of vesicles between reserve vesicle pools and the readily releasable pool [53]. The fact that absence of either α - or γ -synuclein individually fails to provide a phenotype which only becomes apparent in a combinatorial knockout model supports the concept of functional redundancy within the synuclein family. The surprising observation that mice lacking members of the synuclein family (α -, β - and γ -) individually or in combinations display only slight abnormalities in synaptic functions and no sign of neurodegeneration [54] could be an example of developmental compensation occurring during embryonic development in a global knockout mouse. Importantly, acute focal suppression of α -synuclein expression in the rat SNc using RNA interference induces nigrostriatal degeneration, suggesting a crucial role for α -synuclein in dopaminergic neuronal survival [55].

Overexpression of α -synuclein has been reported to decrease the rate of dopamine release both in mouse and cell culture models, but this is not attributable to changes in dopamine

levels or clearance/uptake mediated by DAT [56,57]. A reduction in the vesicle recycling pool due to reclustering of vesicles following endocytosis, as reported to occur in hippocampus [51], would go some way to explain this finding in dopaminergic neurons. Alternatively, α -synuclein could interfere with a late step in exocytosis [57] because α -synuclein overexpression in yeast inhibits vesicle docking and fusion to Golgi membranes [58], leading to impaired endoplasmic reticulum-to-Golgi vesicular trafficking [59].

The evidence from dopaminergic systems that deletion of synucleins elevates dopamine release [52,53], and that overexpression of α -synuclein leads to a decrease in dopamine release [51,57], suggests that α -synuclein can act as a negative regulator of dopamine release, perhaps by modulating vesicle fusion upon synaptic stimulation and distributing vesicles between the ready releasable and reserve synaptic pools. However, more studies in dopaminergic neurons are required to understand how α -synuclein interacts with dopaminergic vesicles to regulate dopamine release. Interestingly, α -synuclein overexpression partially rescued the progressive neurodegeneration caused by impaired SNARE (soluble NSF attachment receptor) complex assembly in mice lacking cysteine-string protein- α (CSP α), a synaptic vesicle protein [60]. Although the mechanism remains unclear, these results suggest a physiological role for α -synuclein in protecting presynaptic terminals against neurodegeneration.

DAT-mediated uptake of dopamine

In the striatum, the actions of dopamine are halted by its diffusion away from the synapse and reuptake by DAT into the presynaptic neuron. Thus, the activity of DAT plays a crucial role in governing dopamine signaling. Several *in vitro* studies show α -synuclein to be a regulator of DAT function, specifically controlling the rapid shuttling of the transporter to and from the cell membrane and hence its availability to take up dopamine [61–65]. On the other hand, mice lacking α -synuclein, or both α - and γ -synuclein, have no observable changes in DAT function [52,53,66]. It should be noted that cell culture studies assay DAT function in the cell body instead of in the presynaptic *in vivo* environment, where DAT could be handled and regulated differently by synucleins.

Unique properties of dopaminergic neurons account for preferential neurodegeneration

Mesostriatal dopaminergic neurons have several properties that could be factors in their susceptibility to neurodegeneration. Single nigrostriatal dopamine neurons in the rat brain have been shown to have a total cumulative axonal length as great as 70 cm [67], and it has been estimated that these axons could form 200 000–400 000 release sites or synapses in the striatum [68,69]. Thus, dopaminergic neurons have extraordinary high metabolic demands and turnover, which might in turn help to explain the generally elevated susceptibility of these neurons to oxidative stress. Interestingly, high levels of mitochondrial DNA deletions were found in SNc neurons in aged controls and PD patients, and these were shown to cause mitochondrial respiratory chain deficiency, leading to SNc cell death [70,71].

Within cells dopamine is a potentially dangerous neurotransmitter, easily undergoing oxidation to form reactive oxygen species and reactive quinones when in the cytoplasm, and it is therefore normally rapidly sequestered into vesicles by the action of the vesicular monoamine transporter 2 (VMAT2). A defect in synaptic vesicle formation or function could predispose neurons to, or even cause, some of the oxidative damage observed in PD, due to cytoplasmic accumulation of dopamine. Specific inhibition of VMAT2 by reserpine has been shown to lead to a sustained increase in the formation of dopamine autooxidation products, known as dopamine adducts, in the cytoplasm [72].

Other recent advances in our understanding of the physiology of dopaminergic neurons are providing evidence of molecular mechanisms which operate differently in those subsets of dopaminergic neurons which are susceptible versus those which are resistant to PD [7]. The main dopaminergic midbrain subpopulation affected in PD is a subpopulation of 'A9' nigrostriatal neurons, which are calbindin D28K-negative but G-protein-coupled inwardly-rectifying potassium channel 2 (GIRK2)-positive, and that originate in the ventrolateral SNc and project to the dorsal striatum [73–77]. Conversely, calbindin-positive/GIRK2-negative neurons including the 'A10' dopaminergic neurons of the ventral tegmental area (VTA) are largely spared. Therefore, several cellular processes could account for the differences in neuronal susceptibility which have been previously reviewed as part of a 'multiple hit' hypothesis for PD [7]. For example, the pacemaker activity of adult SNc dopaminergic neurons is driven by L-type CaV1.3 calcium channels, in contrast to sodium channels in VTA neurons [78], which could place SNc neurons under higher risk from calcium-dependent neurotoxic processes than those of VTA [79]. A decreased ability to buffer cytosolic calcium levels can augment degeneration triggered by various external factors, and this could be consistent with the specific degeneration of calbindin-negative dopaminergic neurons in PD. Second, cell-type-specific potassium channel activation has been suggested to contribute to specific dopaminergic subpopulation vulnerability seen in PD [7]. K-ATP channels are selectively activated in response to parkinsonism-inducing toxins in vulnerable SNc neurons, but not in spared VTA neurons [80], and dysfunction of homomeric GIRK2 channels in mice leads to death of SNc neurons [81]. Third, dopaminergic neurons from SNc have a higher DAT/VMAT2 ratio and are more susceptible to cell death than those containing a lower ratio, such as VTA neurons [82,83]. This is consistent with the crucial role of VMAT2 in maintaining low cytosolic concentrations of dopamine. Also, changes in the DAT/VMAT2 ratio can severely affect the susceptibility of dopaminergic neurons to parkinsonism-inducing neurotoxins [84]. Interestingly, α -synuclein has been shown to regulate VMAT2 expression [63]. Suppression of α -synuclein in human neuronal cells increased the density of VMAT2 transporters per vesicle and decreased the total number of intracellular vesicles [62].

These differences in molecular physiology at the level of dopaminergic soma are far from being the only ones to delineate dopaminergic neuron function in SNc versus VTA (e.g. Refs. [85,86]). They do not, for example, take into account the whole host of mechanisms which might differently govern dopaminergic neuron function and handling of dopamine itself at the level of the presynaptic dopaminergic bouton (e.g. Refs. [87–89]), but they serve to give examples of mechanisms that can be shown to be linked to preferential neurodegeneration of one subclass of dopaminergic neuron over another.

α -Synuclein dysfunction tips dopaminergic neurons over the edge

If one were to extrapolate the results obtained in primary neuronal cultures to native neurons in the brain, it is possible that the preferential vulnerability of a subgroup of dopaminergic neurons in the SNc arises from the convergence of different cellular risk factors, in particular the interaction between increased cytosolic dopamine concentrations and α -synuclein expression (Figure 4). High cytoplasmic calcium levels are likely to lead to increased cytosolic dopamine concentrations in neurons in the SNc but not in the VTA, probably by affecting regulation of TH and AADC activities [90]. Alterations in the levels of functional α -synuclein protein impair synaptic vesicle docking and fusion, preventing recycling of vesicles and leading to accumulation of dopamine in the cytosol. Furthermore, the high DAT/VMAT2 ratio characteristic of SNc neurons might allow dopamine to enter the presynaptic cell at a higher rate than it is incorporated into vesicles, causing an increase in cytoplasmic dopamine concentration.

In addition to the effects of α -synuclein on dopamine biology, dopamine and related auto-oxidation species can inhibit the final steps of α -synuclein aggregation (i.e. fibrillation). This promotes the accumulation of protofibrillar structures believed to be cytotoxic [91], possibly by causing perforation of the vesicular membranes [92], resulting in dopamine leakage and accumulation in the cytoplasm. This elevates the generation of reactive oxygen and nitrogen species [93], further enhancing the formation of dopamine reactive species and perpetuating a toxicity loop, which could eventually lead to neuronal cell degeneration.

Concluding remarks and future directions

Recent evidence from large GWAS involving many thousands of PD patients, together with new experimental data on dopamine cellular physiology, point to α -synuclein as a key link between familial and sporadic PD, and as a crucial regulator of dopamine homeostasis. A number of key questions remain to be answered (Box 2), including two main challenges. First, to further dissect the important roles that α -synuclein plays in regulating dopaminergic neurotransmission and in defining the unique cellular and molecular characteristics of dopaminergic neurons in the SNc that lead to their preferential neurodegeneration in PD. Second, we must relate the genetic polymorphisms at the *SNCA* locus associated with PD to the function of the α -synuclein protein and its role in dopamine homeostasis through understanding the role of genetic variants on transcriptional levels, RNA stability and processing, and on the control of alternative splicing. Overall, the recent data reviewed here suggest that α -synuclein dysfunction and dopamine physiology are intimately linked and are likely to be causal determinants of both familial and sporadic PD.

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Glossary

Genome-wide association studies (GWAS)

a powerful approach to identify common genetic variants of weak effect that underlie the risk of common disease. A GWAS is a case-control genetic association study scanning across the entire genome using densely distributed genetic markers to compare genetic variation between affected and unaffected individuals. Stringent statistical correction procedures, such as the Bonferroni correction for multiple testing, are generally adopted when analyzing the GWAS data, and P values $< 5 \times 10^{-8}$ are required to cross the rigorous threshold for genome-wide significance. The effect of allelic association with disease is measured by the odds ratio (OR). When comparing alleles in cases and controls, an OR of 1 implies that the allele is equally likely to occur in both groups; an OR > 1 implies that, for a given SNP, one of the alleles is more likely to be found in the case group than in the controls.

Lewy bodies (LBs)

the pathological hallmark of PD. These structures are intracytoplasmic eosinophilic spherical bodies with a dense core surrounded by a halo, composed mainly of α -synuclein. LBs also contain many other components, for example ubiquitin, neurofilament proteins and the Alzheimer's disease associated proteins amyloid precursor protein and A-beta peptides.

Linkage disequilibrium (LD)	a non-random association of alleles at different loci. Two alleles are in LD if they occur together with frequencies significantly different from those predicted from their individual allele frequencies. In other words, LD describes the tendency for a particular set of polymorphisms to avoid recombination and remain together during meiosis, and usually indicates that the two alleles of the SNP are physically close on the DNA.
Paired-pulse depression (PPD)	a phenomenon observed at dopaminergic terminals (and other synapses) during electrical stimulation of release using close pairs of stimulus pulses. Briefly, dopamine release is less (i.e. depressed) at a second stimulus pulse than after a first pulse.
Single nucleotide polymorphism (SNP)	a DNA sequence variation which occurs at a single base pair in the genome and is present in >1% of the population, although SNPs used in GWAS typically have a minor allele frequency of >5%.

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Box 1**Structural properties of α -synuclein and its implications in PD pathology**

The human gene encoding α -synuclein (*SNCA*) lies on chromosome 4q21.3-q22 and spans a region of 111 kb (Figure 1a). *SNCA* comprises seven exons, five of which correspond to a coding region (Figure 1b) that is highly conserved between vertebrate species. Structurally, α -synuclein is a 140 amino acid protein and its sequence can be divided into three regions with distinct structural characteristics (Figure 1c) [30]. The highly conserved N-terminal domain encodes for a series of imperfect 11 amino acid repeats with a consensus motif of KTKEGV reminiscent of the lipid-binding domain of apolipoproteins, which in certain conditions forms amphipathic helices [94]. The three missense mutations known to cause familial PD (A30P, E46K and A53T) lie in the amphipathic region, suggesting an important function for this region of the protein. The central hydrophobic region (non-amyloid- β component or NAC domain) of α -synuclein is associated with an increased propensity of the protein to form fibrils [95]. The acidic C-terminal tail contains mostly negatively charged residues and is largely unfolded.

Although the mechanisms by which the genetic variants affect protein pathology remain to be resolved, the processes by which α -synuclein protein can become pathological are better understood. Under certain conditions, α -synuclein monomers interact to form prefibrillar α -synuclein aggregates or protofibrils, which in turn can form insoluble fibrils [65,91,96]. A widely accepted hypothesis for α -synuclein toxicity proposes that protofibrils of α -synuclein are cytotoxic, whereas the fibrillar aggregates of the protein could represent a cytoprotective mechanism in PD [97,98]. Supporting this hypothesis, α -synuclein protofibrils are increased in the brains of patients with PD and dementia with Lewy bodies (DLB) [99], and have been associated with neurotoxicity in α -synuclein-overexpressing cells and mouse models [41,65].

Post-translational modifications in the C-terminal region of α -synuclein, such as oxidation, nitration and phosphorylation, influence the propensity of α -synuclein to aggregate *in vivo* [100–102]. For example, phosphorylation at serine 129 increases α -synuclein propensity to fibrillize, whereas phosphorylation at tyrosine 125 prevents α -synuclein fibrillation [103]. Similarly, C-terminal truncation of α -synuclein accelerates aggregation of the protein *in vitro* [104,105]. Truncated α -synuclein has been detected in Lewy bodies in PD and DLB [106,107]. Transgenic mice overexpressing C-terminally truncated α -synuclein (1–130) have substantial cell loss in the substantia nigra pars compacta (SNc) but not ventral tegmental area (VTA) [108], suggesting a toxic function of truncated α -synuclein in SNc dopaminergic neurons.

Figure I. Schematic representation of human α -synuclein depicting: (a) SNCA gene structure, (b) mRNA, and (c) protein domains.

Box 2**Outstanding questions**

- What is the biological significance of the common *SNCA* variants identified by GWAS? Are they themselves the true causative variants or in LD with the functional variants?
- What is the role of α -synuclein in regulating dopamine neurotransmission, specifically at the level of synaptic vesicle function and dopamine release in SNc dopaminergic neurons?
- What is the role of α -synuclein in other areas of the brain and its role in non-motor symptoms of PD?
- How do the cellular and molecular characteristics of SNc neurons account for the preferential susceptibility of this region to neurodegeneration in PD?
- Age is a key player in neurodegeneration. How do changes in the aged brain combine with α -synuclein dysfunction to lead to the preferential neurodegeneration of dopaminergic SNc neurons?
- Is PD a disorder of synaptic dysfunction or protein aggregation? Does synaptic dysfunction precede α -synuclein fibrillization, aggregation and the formation of LBs, or is synaptic dysfunction caused by protein aggregation?

Figure 1.

Possible *SNCA*-associated disease mechanisms. **(a)** Recent genetic studies have identified common variants at the *SNCA* locus which are associated with sporadic PD [16,17,20]. The three single nucleotide polymorphisms (SNPs) that were found to be most highly associated with PD (rs2736990, rs3857059 and rs11931074) are indicated by green arrows. Such variants could affect α -synuclein function and contribute to PD etiology by altering levels of gene transcription, altering mRNA stability (via altered miRNA binding) or by altering the generation of alternative splice isoforms. Some of the 3' SNPs identified are in linkage disequilibrium (LD) with the REP1 dinucleotide repeat in the promoter region (indicated in red), previously found to regulate *SNCA* expression [25,26]. **(b)** Alternative splicing of exons 3 and 5 generates four *SNCA* isoforms of different lengths, one of which (SNCA112; exon 3⁺5⁻) has been implicated in Lewy body formation and neurotoxicity [31].

Figure 2.

Effects of α -synuclein on dopamine homeostasis in the presynaptic terminal. Dopamine is synthesized in the cytoplasm by the action of tyrosine hydroxylase (TH) and amino acid decarboxylase (AADC). (i) α -Synuclein has been shown to regulate the activity of these enzymes [35,36,43]. (ii) Once synthesized, dopamine is immediately sequestered into vesicles by the vesicular monoamine transporter 2 (VMAT2). Several lines of evidence suggest that α -synuclein is involved in regulating synaptic vesicle function and dopamine release into the synaptic cleft [51–53]. (iii) Dopaminergic signaling at the synapse is terminated by the reuptake of dopamine via the dopamine transporter (DAT), with co-transport of two Na^+ and one Cl^- ions. Studies in cell culture systems have shown that α -synuclein is necessary for the trafficking of DAT to the cell surface [61–64].

Figure 3.

Schematic model of α -synuclein's proposed roles in regulating presynaptic vesicle cycling in situations of different α -synuclein levels. **(a)** When α -synuclein levels are reduced the availability of vesicles in the reserve pool is decreased [49,50] and more vesicles are readily available to be released [52], and this can lead to an increase in dopamine release. **(b)** Under normal conditions α -synuclein is thought to play a physiological role in regulating vesicle availability in the different pools and vesicle docking and fusion. **(c)** By contrast, elevated α -synuclein levels or mutated E46K or A53T α -synuclein lead to a reduction in dopamine release [56,57] possibly by affecting a late step in exocytosis [57] or by decreasing vesicle availability in the recycling pool due to impaired vesicle endocytosis [51].

Figure 4.

A schematic working model illustrating various proposed cellular mechanisms for how preferential neurodegeneration of substantia nigra pars compacta (SNc) dopaminergic neurons could take place. Neurons in the SNc display characteristics which make them highly susceptible to cell death, for example a higher dopamine transporter (DAT)/vesicular monoamine transporter 2 (VMAT2) ratio [82,83] and the use of CaV1.3 calcium (Ca^{2+}) channels for autonomous pacemaking [78]. Subtle increases in Ca^{2+} levels in the cytoplasm activate dopamine (DA) synthesis [90]. Impairment of vesicle docking and recycling due to α -synuclein dysfunction could prevent incorporation of newly synthesized and newly taken up DA into vesicles. This could lead to an increase in cytosolic DA concentration (DA_{cyt}), which causes increased generation of toxic reactive species and ultimately contributes to dopaminergic neurodegeneration. Thus, the combined action of α -synuclein dysfunction and increased dopamine in the cytosol of SNc neurons could drive cell death in Parkinson's disease (PD).