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α -Synuclein: A therapeutic target for Parkinson's disease?

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Abstract

Parkinson's disease is a progressive age-related neurodegenerative disease with invariant loss of substantia nigra dopamine neurons and striatal projections. This disorder is well known for the associated motoric symptoms including resting tremor and the inability to initiate movement. However, it is now apparent that Parkinson's disease is a multi-system disorder with patients exhibiting symptoms derived from peripheral nervous system and extra-nigral dysfunctions in addition to the prototypical nigrostriatal damage. Although the etiology for sporadic Parkinson's disease is unknown, information gleaned from both familial forms of the disease and animal models places misfolded α -synuclein at the forefront. The disease is currently without a cure and most therapies target the motoric symptoms relying on increasing dopamine tone. In this review, the role of α -synuclein in disease pathogenesis and as a potential therapeutic target focusing on toxic conformers of this protein is considered. The addition of protofibrillar/oligomer-directed neurotherapeutics to the existing armamentarium may extend the symptom-free stage of Parkinson's disease as well as alleviate pathogenesis.

Introduction

Parkinson's disease (PD) is an age-related progressive neurodegenerative disease with invariant loss of substantia nigra pars compacta dopaminergic neurons (DANs) resulting in a near complete deficit of the striatal presynaptic neurotransmitter dopamine. First described in 1817 by James Parkinson in his "Essay on the Shaking Palsy" [1], PD patients exhibit motoric dysfunction exemplified by resting tremors, bradykinesia, rigidity, and postural instability. However, it has now become evident that PD is a multisystem disorder that also affects the peripheral nervous system as well as central nervous system regions outside the nigrostriatal pathway [2,3]. Clinical evaluation reveals that a portion of PD patients exhibit autonomic dysfunction including dysphagia, impaired gastrointestinal motility, constipation and orthostatic hypotension while over 70% of PD patients present with olfactory dysfunction (reviewed in [3]). In addition to the invariable loss of DANs and dystrophic projections to the striatum, postmortem hallmark pathological features include the presence of activated microglia and large intracytoplasmic eosinophilic proteinaceous inclusions called Lewy bodies in the remaining substantia nigra DANs [4–8]. It was more than 150 years after Parkinson's initial description that Arvid Carlsson discovered that dopamine (DA) was a neurotransmitter and that its depletion was linked to a reserpine-induced catatonic effect in rabbits that could be relieved following treatment with the DA precursor, D,L-dopa ([9] and reviewed in [10]). By 1960, Hornykiewicz's group made the seminal discovery following postmortem analysis of human brains that the loss of DA was correlated specifically with Parkinson's disease

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[11]. Birkmayer and Hornykiewicz went on to demonstrate that L-dopa relieved akinesia in PD patients [12,13]. Nearly 40 years later, L-dopa is still the first line pharmacological agent used to treat PD symptoms.

PD exists as both a familial and sporadic disorder, however the majority of PD cases are sporadic arising from a yet unknown mechanism with age as the greatest single risk factor for developing disease. Affecting over one million people, the majority of idiopathic PD patients experience symptoms during the sixth decade of life with a significant increase in incidence with advancing age. Although the etiology of PD is unknown it is hypothesized to arise from the interplay of both environmental toxicants and genetic vulnerability [14,15]. This link has been strengthened by the identification of susceptibility genes and nigrostriatal pathology resulting from neurotoxicant exposure.

Genetic studies led to the discovery of a small percentage of familial PD cases linked directly to genetic mutations, as well as gene duplications and triplications (reviewed in [14,16]). Susceptibility genes involved with protein phosphorylation and degradation, mitochondrial function and oxidative stress responses have been identified including *parkin*, *ubiquitin carboxy-terminal-hydrolase-L1*, *PINK1*, *DJ-1* and *LRRK2 (dardarin)* [17–29]. However, the first gene associated with PD was *SNCA* (α -synuclein; SYN), which codes for a protein of unknown function. Furthermore, missense mutations within the *SNCA* coding region lead to early onset autosomal dominant PD with symptom onset between the mid-30's to 60's [30–33]. Most intriguing, duplications and triplications of wild-type *SNCA* are linked to an early onset familial PD with the triplicate disease being the most aggressive form exhibiting demonstrable symptoms by the 2nd or 3rd decade of life and often accompanied with dementia [34,35]. This critical discovery led to the hypothesis that an abundance of endogenous wild-type SYN *alone* within a susceptible cellular milieu is sufficient for the development of PD. Additionally, the genetic studies suggest that changes in SYN protein levels represent a gain of toxic function. Strengthening the link between SYN and *sporadic* PD, Ahn et al. recently demonstrated *SNCA* duplication in two sporadic PD patients with asymptomatic carriers in their families [36].

α -Synuclein: a protein in search of function

α -Synuclein (SYN) was initially isolated from *Torpedo* cholinergic synaptic vesicles [37] and later as the non-amyloid component of plaques from Alzheimer's disease brains [38]. This 14–19 kDa protein is phylogenetically conserved and abundant with wide-spread expression throughout the central nervous system [37]. SYN is localized to the cell soma and nucleus but highly enriched within presynaptic terminals. This protein lacks a secondary structure in solution and therefore exists in a random coil conformation with a propensity to fold into an α -helical configuration upon interaction with lipid membranes [39]. Further structural changes to a β -sheet conformation readily occur when SYN is exposed to conditions of molecular crowding, changes in pH and interactions with highly reactive molecules such as DA [40–52]. Formation of β -sheet SYN leads to toxic oligomers and protofibrils, which are seen in a variety of synucleinopathies including PD and are discussed in more detail below. The normal function of SYN remains elusive and no one specific role has been ascribed to this protein. However, since SYN is upregulated during song learning in zebra finches and localized preferentially to the presynaptic terminus it was first hypothesized to play an important role in synaptic plasticity and function [53].

The presynaptic terminal is uniquely designed to respond quickly and efficiently to stimuli with the release of appropriate neurotransmitters. It follows that the production and fusion of synaptic vesicles to the presynaptic membrane are essential for proper function. Recent work demonstrated that SYN rescues mice genetically deficient in cysteine-string protein α

(CSP α) a chaperone protein critical for ATP-dependent refolding of synaptic proteins [54, 55]. SYN substituted for CSP α restoring the proper folding of the SNARE complexes, which are necessary for the docking of synaptic vesicles with presynaptic membranes. SYN also acts as a molecular chaperone for the dopamine transporter (DAT) regulating the amount of DAT at the presynaptic membrane thereby controlling intracellular DA levels, the neurotransmitter deficient in PD [56,57].

A role for SYN in synaptic function is further supported by studies that employ SYN loss of function mice (SYN $^{-/-}$). While SYN $^{-/-}$ mice do not display overt behavioral or anatomical differences compared with control mice demonstrating that SYN is not essential for maintenance or survival of DANs, SYN-deficient mice, do display altered DA release following paired stimuli [58]. SYN-deficient mice also have altered fatty acid metabolism such that total brain neutral lipid mass is increased, palmitate uptake is decreased and arachidonate turnover is reduced [59–61]. Additionally, Murphy's group established that SYN's regulation of fatty acid metabolism is fatty acid-specific for example unlike arachidonate, docosahexaenoic acid is increased following SYN ablation [62]. Together these data suggest an intricate relationship between SYN and protein-membrane interactions. Further supporting its role in presynaptic function, SYN has also been shown to regulate tyrosine hydroxylase (TH) activity the rate-limiting step in the synthesis of catecholamines including DA [63,64]. Together these data portend an important function for SYN as a regulator of DA neurotransmission.

α -Synuclein's role in PD pathogenesis

Under normal expression levels, SYN is important for synaptic integrity but when overexpressed or mutated it has neuropathological effects as exhibited in PD. SYN moved to the forefront of PD research because of afore mentioned genetic studies as well as its presence in the hallmark pathological feature of PD, the Lewy body (reviewed in [3]; [4,5]). In addition, SYN is localized to the presynaptic dopaminergic neurons and terminals which degenerate in PD and it has a propensity to misfold into toxic conformations including oligomers and protofibrils with annular pore-forming structures [48,65,66]. In an effort to both model PD and elucidate SYN's role in PD pathogenesis several toxicant, transgenic animal models that overexpress wild-type or mutant SYN and SYN knock-out mice have been studied. These models have variable pathology a discussion of which is beyond the scope of this review but in some models misregulation of SYN results in neuronal cell death, dystrophic neurites, SYN aggregates and alterations in DA metabolism and release (reviewed in [67–71] and [40,41, 46,49,58,72–91]). One transgenic model with overexpression of SYN in tyrosine hydroxylase containing neurons demonstrates microglial activation and the attendant proinflammatory responses as early as one-month of age [92]. Furthermore, several studies demonstrate that cultured microglia are directly activated by SYN and capable of mounting a proinflammatory response to this protein suggesting SYN-directed inflammation may play an important role in PD pathogenesis [92–95].

Environmental agents such as pesticides, herbicides, and industrial chemicals have been indicated as susceptibility factors in PD primarily through an increase in oxidative stress, promotion of SYN misfolding and glial activation [72,95–113]. The decisive finding that exposure to the neurotoxicant MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine) leads to specific neurodegeneration of the SN DANs and loss of striatal projections resulted from inadvertent exposure in a group of drug addicts. The link between the active metabolite, MPP $^{+}$ (1-methyl-4-phenylpyridinium) and parkinsonism was made following the appearance of symptoms in a cohort of drug addicts exposed to MPTP following ingestion of contaminated synthetic heroin [114–116]. Noting the classic motoric symptoms of PD in these patients as well as postmortem analyses demonstrating the specific death of SN DANs, researchers began

utilizing MPTP in rodents and non-human primates to model PD [117–122]. It was later discovered that MPTP is converted by monoamine oxidase B to MPP⁺ in glia and taken up specifically by DANs through the dopamine transporter accounting for the vulnerability of DANs. Once MPP⁺ enters the presynaptic terminal it inhibits Complex I of the mitochondria electron transport chain resulting in an accumulation of free radicals and consequent oxidative stress and cell death [109,123–130]. The connection between neurotoxicant injury and SYN modifications was firmly established following exposure of rodents and non-human primates to MPTP. While rats are immune to MPTP toxicity exposed mice display consistent loss of SN DANs and striatal projections, motoric features similar to PD, SYN aggregates, nitrosylated SYN and in some models increased SYN expression [76,84,90,127,131–134]. Chronic infusion of MPTP in mice more faithfully recapitulates the pathological features of PD including the presence of SYN inclusion bodies compared to acute or repeated episodic injections [90]. Non-human primate MPTP models display *both* the cardinal motoric symptoms *and* pathological changes including SYN inclusions similar to those seen in PD [40,122].

Although SYN is undoubtedly linked to PD pathogenesis through human genetic data as well as genetic and toxicant non-human primate and rodent models there remains an important unanswered question-why are substantia nigral DANs more vulnerable than other neuronal populations? One hypothesis contends that the presence of the highly reactive neurotransmitter, DA and/or DA-metabolites contribute to DAN-specific vulnerability [57,100,135–137]. Dopamine directly interacts with SYN and enhances misfolding promoting the formation of toxic protofibrils [51,52,75]. Presumably under a normal cellular milieu, DA is safely sequestered by vesicular monoamine transporter (VMAT) within synaptic vesicles and unavailable to auto-oxidize and therefore interact with and promote SYN misfolding. In addition, under conditions where SYN levels are increased due to either overexpression or reduced degradation there is an augmentation of cytoplasmic DA. This free DA can be quickly auto-oxidized forming highly reactive dopamine quinone, hydrogen peroxide, and superoxide radicals leading to a vicious cycle of misfolded SYN, unregulated DA and finally cell death. In support of this, mice that express a reduced level of VMAT2 display altered DA homeostasis, nigrostriatal degeneration and SYN accumulation [138]. Therefore DA homeostasis is altered when SYN is overexpressed while in turn SYN's toxic conformation is promoted by oxidized DA [57,75,139]. Since genetic mutations and overexpression of wild-type SYN as well as toxicant models demonstrate both DA misregulation and SYN misfolding it has been hypothesized that both familial and sporadic PD despite disparate initiating events converge on a common pathway leading to SN DAN death with a common feature of misfolded SYN [15,100].

Current Parkinson's Disease Therapies

Although the etiology of PD is largely unknown, the common feature of DA deficiency due to the loss of SN DAN and striatal projections has driven therapeutic approaches (reviewed in [140–142]). Currently, PD is diagnosed upon the appearance of clinical symptoms when over 50% of SN DANs are lost. Due to the inability to diagnosis patients during the premotor/presymptomatic phase most therapies are aimed at increasing DA by directly augmenting production, extending the half-life or affecting downstream neurotransmitter systems to decrease the effect of the DA deficiency [142]. Current therapies include pharmacotherapies, surgical interventions, tissue implantation, and more recently gene therapeutic approaches. Many detailed reviews of specific PD therapies are available while only a subset are highlighted below [141–163].

The first line therapy for the last 40 years has been levodopa (L-dopa) a DA precursor capable of crossing the blood brain barrier and efficacious in a large number of PD patients relieving akinesia/bradykinesia [164]. Commonly given in conjunction with an inhibitor of extracerebral

dopa decarboxylase such as carbidopa this pharmaceutical combination is the most effective symptomatic treatment available. Levodopa therapy results in a “honeymoon period” when patients respond extremely well to therapy followed in 5–15 years by a “wearing-off” effect [165,166]. During this phase, levodopa-induced dyskinesias develop and the patient is forced to halt treatment. In addition, for levodopa to be effective a portion of SN DANs and striatal projections must be present and in fact as the neuropathology progresses, this therapy ultimately fails.

Other dopaminergic agents currently utilized effectively increase the amount of DA by extending the half-life and/or decreasing the metabolism. For example, the monoamine oxidase inhibitor deprenyl increases the half-life of DA and in one study delayed the need for levodopa therapy [149]. Another agent entacapone is an inhibitor of catechol-O-methyltransferase taken in conjunction with levodopa resulting in a more stable and constant supply of precursor effectively increasing the amount of DA available at the synapse. More recently, the use of dopamine D₂ receptor agonists such as bromocriptine have been given attention since this class of agents does not promote dyskinesias or motor fluctuations that are common with levodopa but do serve to mimic the downstream effects of DA [167]. These agents have consistent antiparkinsonian effects and are the second most utilized class of pharmaceuticals. Other classes of pharmacotherapies include anticholinergics, adenosine A_{2A} antagonists, antioxidants [168–170]. These agents have small but in some patients significant effects however they are not considered front line therapies and are often utilized following levodopa failure.

Surgical intervention with the goal of suppressing abnormal neural activity resulting from the loss of dopaminergic input is utilized in conjunction with pharmacotherapy. The most common surgical approach is deep brain stimulation (DBS) which entails MRI guided positioning of an electrode most often into the subthalamic nucleus and implantation of an impulse generator under the patient’s collarbone to produce high frequency electrical impulses which dampen the increased glutamate output from this nuclei [143,171–178]. This technology is most effective in patients that respond to levodopa. As with other therapies, as disease progresses DBS fails to be effective [176].

Tissue implantation and gene therapeutic approaches to PD are still in the investigative stages but hold promise for PD patients (reviewed in [150,158]; [152,179–191]). In its broadest definition, gene therapy is the delivery of therapeutic genes by naked or lipid encapsulated DNA, tissue transplantation or viral vector based methods. The brain represents a particularly challenging environment for gene therapy because of the difficulty in accessing particular regions, nuclei, and fiber tracts associated with disease. The invasive nature of brain-directed gene therapy is compounded by other hurdles including temporal and spatial regulation of expression. Extensive reviews of gene therapeutic approaches to PD have been published elsewhere and will only be briefly discussed here [141,142,151,154–158,192–194].

The first attempts at tissue transplantation therapy for PD were fetal brain grafts utilized in rat models of nigrostriatal destruction almost 30 years ago [190]. Since that time fetal and autologous adrenal medullary tissue grafts, cell suspension grafts, xenografts, neurospheres and embryonic stem cells have been investigated as potential therapies for PD [152,191,195–199]. The effects from long-term fetal nerve cell transplants were recently evaluated in three separate post-mortem human studies [179–182]. Interestingly, in two studies Lewy bodies were identified in the *grafted* tissue 11–16 years post transplantation suggesting that there are yet to be understood graft-host interactions [179–181]. Conceptually these data indicate a transfer of the “PD disease-state” to the exogenously grafted tissue suggesting that the local cellular environment is important for disease initiation and progression. The third analysis of five subjects that received fetal cell suspensions, not solid grafts, failed to identify graft-related Lewy body pathology suggesting that the type of cellular preparations may be critical to graft-

host interactions [182]. However, these cell suspension grafts did demonstrate an increase in the number of both DA and serotonin neurons reflecting the need for improved methodologies for the establishment of pure dopaminergic neuronal populations [182].

Neurotrophic and neuroprotective agents that support and maintain neurons have been a prime focus of PD clinical trials both via direct infusion and gene therapeutic approaches. Initial trials focused on the dopaminotrophic factor, glial cell line-derived neurotrophic factor (GDNF) [200,201]. GDNF was found to be neuroprotective in rodent and non-human primate models of PD when delivered directly into the striatum. Later viral vector mediated delivery of GDNF in animal models also demonstrated promise for this agent in PD. However, the first human trials utilizing intraventricular delivery of GDNF protein were disappointing with no demonstrable effect, likewise intraputamenal GDNF delivery lacked efficacy [202,203]. While another study utilizing unilateral intraputamenal infusion in ten patients with severe PD demonstrated some bilateral improvement of motor function [204]. One year after treatment was stopped the improvement was lost demonstrating that continuous treatment is necessary [205]. However, 7 of 10 patients exhibited anti-GDNF antibodies following treatment whether this immune response will be of any clinical relevance has not been determined [205]. Most likely, GDNF therapies have failed due to insufficient delivery of the growth factor to the target neurons. Another GDNF family member, neurturin is potentially neuroprotective for DAN and currently under investigation as a potential PD therapeutic [153,206–208]. The initial results of a phase I open-label trial of intraputamenal adeno-associated virus serotype 2-neurturin (CERE-120) delivered bilaterally to 12 patients demonstrated safety, tolerability and potential efficacy of this treatment [208]. Additional neuroprotective compounds such as coenzyme Q10 are also under examination in animal models of PD for their therapeutic potential [209].

Other gene therapeutic approaches currently under investigation are focused much like the pharmacological treatments on increasing the amount of DA. For example, lentivirus vectors carrying three genes encoding enzymes needed for DA synthesis; tyrosine hydroxylase, GTP-cyclohydrolase 1 and aromatic amino acid dopa decarboxylase and adeno-associated virus serotype 2 encoding human aromatic L-amino acid decarboxylase (AAV-hAADC-2) are currently being utilized in human clinical trials [210,211]. Although these trials hold promise, the future of neuro-gene therapy will depend on the development of gene therapy vectors capable of temporal and spatial regulated long-term expression [141,153,163,192,212]. In addition, there is a great need for novel therapeutic targets that address the initiating steps of neurodegeneration rather than symptomatic therapies.

α -Synuclein: a novel therapeutic target for PD

A shared feature of many neurodegenerative diseases is the presence of misfolded protein and as such, these toxic conformers may represent a potential common therapeutic target [213]. As reviewed above, SYN is a protein that readily misfolds and is implicated in the pathobiology of PD primarily due to human genetic studies demonstrating that overexpression of wild-type *SNCA* or the presence of mutated *SNCA* leads to familial PD and because Lewy bodies one of the hallmark pathological features of PD are replete with SYN. Structurally, SYN remains as a random coil when in solution but readily forms oligomers, protofibrils, amyloid fibrils and large aggregates both *in vivo* and *in vitro* [73,214]. The formation of fibrils or intertwined protofibrils is dependent on the individual protein and in fact for SYN the molecular mechanisms involved in this misfolding are still unclear [215]. However, for most proteins there is a change from an α -helical conformation to a β -sheet structure followed by β -sheet aggregation to form prefibrillar oligomers and subsequently transient protofibrils. Depending on the protein, protofibrils may be composed of as many as 15–40 monomers, are generally spherical in nature, and considered metastable. Spheres anneal to form chainlike protofibrils followed by a quick conversion to amyloid fibrils and in some cases annular pore-like

conformations ([216,217] and reviewed in [218]). Although the toxic moiety of SYN has not been fully established, cell culture experiments point to oligomers and protofibrils containing a β -sheet conformation as the most toxic forms of this protein however since protofibrils are unstable intermediates their connection to neurotoxicity is inferred rather than definitive [66, 219–221]. For SYN as well as A β another protein prone to fibrillization, evidence exists that the oligomeric and protofibrillar forms are the most toxic compared to mature amyloid fibrils (reviewed in [222] and [214,223–228]). However, the distinction between oligomers and protofibrils in terms of toxicity has not been made. Yet it is hypothesized that together these β -sheet intermediates which I refer to as OPSyn, for oligomeric and protofibrillar α -synuclein, are more toxic than monomers, large fibrils or aggregates.

What mechanisms underlie this toxicity? SYN has been implicated in a myriad of cellular functions making it difficult to pin point the pathogenic mechanism. Yet by virtue of its ability to interact with lipid membranes, act as a chaperone protein, interfere with the solubility of other cellular proteins, directly activate microglia and readily change conformation including the formation of annular pore-like structures, it is reasonable to posit that interference with SYN misfolding will be protective for the DAN [15,141,142,221,229,230]. Therefore, a therapy aimed at altering SYN's conformation does not require targeting the pathway activated by misfolded SYN but rather can focus on reduction of misfolded intermediates (OPSyn; oligomers/protofibrils) and as such may be successful despite our limited knowledge regarding pathogenic mechanisms [15].

The conversion of monomeric SYN to toxic OPSyn and annular pore-like structures is induced under conditions of low pH, molecular crowding, oxidative stress and interactions with other proteins and heavy metals [40,41,44–46,50,66,75,77,213,231–233]. Importantly, large insoluble SYN aggregates are posited to be less toxic than OPSyn and in fact, the intracytoplasmic proteinaceous inclusions found in PD (Lewy bodies) may represent a depository for misfolded proteins sequestered so they can no longer function as toxic moieties [234]. The overall therapeutic goal is to diminish the amount of toxic OPSyn which potentially can be accomplished by preventing the formation of OPSyn or accelerating their conversion to large less toxic mature aggregates, in both scenarios there is a resultant decrease in OPSyn. Even though SYN's role in PD pathogenesis is likely multifaceted, prevention of the resultant downstream effects of this misfolded protein may be achieved by targeting the toxic conformers. There are numerous options for therapeutic intervention including targeting formation, degradation, and/or prevention of the toxic downstream effects once OPSyn form. These novel OPSyn-directed therapies will likely focus on small soluble molecules and gene therapeutic approaches.

Attenuation of OPSyn formation can theoretically be accomplished by decreasing the pool of starting material, blocking misfolding or increasing the formation of large insoluble aggregates [235]. Several small molecule inhibitors of OPSyn formation have been tested *in vitro*. For example, arginine, lysine and guanidine regulate the chaperone activity of α B-crystallin protecting cells against SYN aggregation [236]. In contrast, catecholamines including DA inhibit fibril formation resulting in an accumulation of protofibrils [75]. *In vitro* assays capable of identifying key structural targets within the SYN sequence as well as assays for screening large numbers of molecules for inhibition of OPSyn formation are critical for the identification of novel therapeutics. Utilizing solid-state nuclear magnetic resonance (ssNMR) Madine et al. designed a N-methylated peptide inhibitor of SYN aggregation [237]. This novel method relies on the synthesis and modification of short peptides designed against self-recognition elements within the native protein sequence [238]. In this example, the modified peptide contains N-methylated backbone amide groups preventing hydrogen-bonding and inhibiting aggregation [237]. Furthermore, the size and solubility of this peptide allow for efficient diffusion across membranes portending its usefulness as a therapeutic agent. *In silico* panning for SYN-binding

peptides that modulate aggregation is another constructive method to initially interrogate a large library of peptides. Utilizing an 11-mer peptide localized to the non-amyloid component (NAC) of SYN Abe et al. employed genetic algorithms to *in silico* screen for SYN-binding peptides [239]. Surface plasmon resonance was then utilized to determine the SYN binding affinities of the screened peptides followed by a Thioflavin T-based fibril formation assay to evaluate aggregation potential [239]. In this manner, hundreds of potential binding peptides can be screened while only the most promising are later subjected to *in vitro* validation.

Other potential classes of OPSyn inhibitors are currently under investigation. Statins reduce neuronal detergent insoluble SYN suggesting that the regulation of cholesterol synthesis may be a novel therapeutic locus for PD [240]. Prolyl oligopeptidase (PO) cleaves proteins at the carboxyl side of L-proline and indeed the C-terminal fragment of SYN is a PO substrate [241]. However, full-length SYN is not cleaved by PO yet SYN aggregation is enhanced [241]. Despite this conundrum, inhibitors of PO enzymatic activity had the surprising effect of decreasing SYN aggregation suggesting a role for PO in SYN misfolding through direct interaction rather than enzymatic cleavage and for PO inhibitors as potential novel therapeutic agents. Likewise, a small molecule inhibitor of the histone deacetylase (HDACs) family member, silent information regulator 2 (sirtuin 2; SIRT2), was able to rescue SYN-mediated cell death and alter inclusion morphology [242]. HDAC inhibitors have been studied in a number of neurodegenerative diseases and as such may have broad therapeutic applications [243]. Antioxidants also represent a large class of potential pharmaceuticals for neurodegenerative diseases. These compounds are intended to prevent oxidation of other molecules reducing overall free radical levels and cellular oxidative stress. Antioxidant compounds also inhibit the formation of β -amyloid and SYN fibrils and importantly can destabilize preformed fibrils which bodes well for their usefulness in established disease [244]. Although antioxidants are commonly used as a dietary supplement, they have thus far unproven therapeutic efficacy. Therefore, the development of novel antioxidants with targeted anti-OPSyn effects and improved delivery is required for this class of neurotherapeutics. Proteins that interact with SYN to alter conformation represent an additional viable therapeutic. Another member of the synuclein family of proteins, β -synuclein is abundant in the brain and capable of inhibiting α -SYN fibril formation [245]. Utilizing a transgenic model of α -SYN overexpression and β -synuclein delivered by a lentivirus vector the formation of SYN inclusions was reduced supporting a role for β -synuclein in future gene therapeutic approaches to PD [246].

A novel method to interfere with misfolded SYN utilizes single chain fragment variable antibodies (ScFvs) directed at specific SYN protein sequences and/or conformations [141, 247]. ScFvs are composed of the minimal antigen binding site, the V_H and V_L variable domains, joined by a flexible polypeptide linker [248,249]. These antibodies are powerful since they are encoded on one contiguous gene sequence and therefore amenable to renewable large-scale production from bacterial and mammalian gene expression systems. In addition, ScFvs can be expressed intracellularly (intrabodies) or designed for secretion making them compatible with gene therapeutic delivery approaches and a broad range of applications. ScFvs have been identified which bind to specific regions of SYN [51,250–253]. Recently an ScFv directed against the non-amyloid component of SYN (NAC), a region important for aggregation, reduced the amount of intracellular SYN aggregates and associated toxicity in a cell culture model [252,254]. Furthermore, an ScFv that specifically recognizes DA-modified proteins has been identified which may prove to be important in PD since SYN misfolding has been linked to direct interaction with DA [51,75]. These conformation and sequence specific ScFvs may also provide protection against preformed OPSyn. For example, a recombinant adeno-associated vector encoding secreted ScFvs directed against the normal cellular prion protein was able to decrease the amount of proteinase K resistant scrapie prion protein suggesting that binding to the cellular protein prevented the scrapie-directed misfolding [255]. One can

imagine utilizing a similar approach in PD since *in vitro* and *in vivo* SYN overexpression models demonstrate that misfolded conformers exist and are toxic, SYN can be released from cells, microglial activation occurs as early as one month of age, and SYN directly activates microglia partially through the CD36 pattern recognition receptor [92,256,257]. Therefore, anti-SYN ScFv intrabodies may prevent the conversion of monomers to OPSyn and/or accelerate the conversion of OPSyn to non-toxic aggregates both would lead to the shared effect of diminished overall levels of the toxic species. Likewise, secreted anti-SYN ScFvs may prevent the binding of misfolded SYN to CD36 blocking the chemokine-directed activation of microglia.

This review attempts to outline the current state of PD therapy and provide a glimpse into a new arena with therapeutic potential. The future success of PD neurotherapeutics will depend on continued advancements in early diagnosis through novel biomarker discoveries and improved brain imaging technologies as well as sustained efforts toward the discovery of novel restorative agents. In addition, there is a pressing requirement for the expansion of innovative regulated delivery techniques for therapeutic molecules and viral vector encoded genes [142]. Since PD presents as a multifaceted disorder combinatorial therapies may have the most benefit. For example, one can imagine that with improved diagnostics patients could begin treatment at an earlier stage of disease preceding the devastating DAN loss. During this phase prior to frank motoric symptoms, a combination of neurotrophic factors and OPSyn-directed therapies may prove useful in extending the symptom-free stage. Even now, the addition of OPSyn-directed therapy to the current PD armamentarium augurs well for delayed pathogenesis increasing the number of years that patients remain symptom-free via elimination of toxic protein moieties.

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References

1. Parkinson, J. An essay on the shaking palsy. London: Sherwood, Neely, and Jones, Paternoster Row; 1817.
2. Braak H, Del Tredici K. Invited article: Nervous system pathology in sporadic parkinson disease. *Neurology* 2008;70:1916–1925. [PubMed: 18474848]
3. Jellinger KA. A critical reappraisal of current staging of lewy-related pathology in human brain. *Acta Neuropathol* 2008;116:1–16. [PubMed: 18592254]
4. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. Alpha-synuclein in filamentous inclusions of lewy bodies from parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci U S A* 1998;95:6469–6473. [PubMed: 9600990]
5. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in lewy bodies. *Nature* 1997;388:839–840. [PubMed: 9278044]
6. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for hla-dr in the substantia nigra of parkinson's and alzheimer's disease brains. *Neurology* 1988;38:1285–1291. [PubMed: 3399080]
7. Knott C, Stern G, Wilkin GP. Inflammatory regulators in parkinson's disease: Inos, lipocortin-1, and cyclooxygenases-1 and -2. *Mol Cell Neurosci* 2000;16:724–739. [PubMed: 11124893]
8. Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, Torizuka T. Microglial activation and dopamine terminal loss in early parkinson's disease. *Ann Neurol* 2005;57:168–175. [PubMed: 15668962]
9. Carlsson A, Lindqvist M, Magnusson T. 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* 1957;180:1200. [PubMed: 13483658]

10. Abbott A. Neuroscience: The molecular wake-up call. *Nature* 2007;447:368–370. [PubMed: 17522649]
11. Ehringer H, Hornykiewicz O. distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. *Klin Wochenschr* 1960;38:1236–1239. [PubMed: 13726012]
12. Birkmayer W, Hornykiewicz O. the l-3,4-dioxyphenylalanine (dopa)-effect in parkinson-akinesia. *Wien Klin Wochenschr* 1961;73:787–788. [PubMed: 13869404]
13. Birkmayer W, Hornykiewicz O. The effect of l-3,4-dihydroxyphenylalanine (dopa) on akinesia in parkinsonism. English Translation 1961. *Wien Klin Wochenschr* 2001;113:851–854. [PubMed: 11763859]
14. Klein C, Schlossmacher MG. Parkinson disease, 10 years after its genetic revolution: Multiple clues to a complex disorder. *Neurology* 2007;69:2093–2104. [PubMed: 17761553]
15. Maguire-Zeiss KA, Federoff HJ. Convergent pathobiologic model of parkinson's disease. *Ann N Y Acad Sci* 2003;991:152–166. [PubMed: 12846984]
16. Schiesling C, Kieper N, Seidel K, Kruger R. Review: Familial parkinson's disease--genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. *Neuropathol Appl Neurobiol* 2008;34:255–271. [PubMed: 18447897]
17. Abbas N, Lucking CB, Ricard S, Durr A, Bonifati V, De Michele G, Bouley S, Vaughan JR, Gasser T, Marconi R, Broussolle E, Brefel-Courbon C, Harhangi BS, Oostra BA, Fabrizio E, Bohme GA, Pradier L, Wood NW, Filla A, Meco G, Denefle P, Agid Y, Brice A. A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in europe. French parkinson's disease genetics study group and the european consortium on genetic susceptibility in parkinson's disease. *Hum Mol Genet* 1999;8:567–574. [PubMed: 10072423]
18. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998;392:605–608. [PubMed: 9560156]
19. Lucking CB, Durr A, Bonifati V, Vaughan J, De Michele G, Gasser T, Harhangi BS, Meco G, Denefle P, Wood NW, Agid Y, Brice A. Association between early-onset parkinson's disease and mutations in the parkin gene. French parkinson's disease genetics study group. *N Engl J Med* 2000;342:1560–1567. [PubMed: 10824074]
20. Benbunan BR, Korczyn AD, Giladi N. Parkin mutation associated parkinsonism and cognitive decline, comparison to early onset parkinson's disease. *J Neural Transm* 2004;111:47–57. [PubMed: 14714215]
21. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW. Hereditary early-onset parkinson's disease caused by mutations in pink1. *Science* 2004;304:1158–1160. [PubMed: 15087508]
22. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P. Mutations in the dj-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* 2003;299:256–259. [PubMed: 12446870]
23. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Muller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T. Mutations in lrrk2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 2004;44:601–607. [PubMed: 15541309]
24. Facheris M, Strain KJ, Lesnick TG, de Andrade M, Bower JH, Ahlskog JE, Cunningham JM, Lincoln S, Farrer MJ, Rocca WA, Maraganore DM. Uchl1 is associated with parkinson's disease: A case-unaffected sibling and case-unrelated control study. *Neurosci Lett* 2005;381:131–134. [PubMed: 15882803]
25. Maraganore DM, Lesnick TG, Elbaz A, Chartier-Harlin MC, Gasser T, Kruger R, Hattori N, Mellick GD, Quattrone A, Satoh J, Toda T, Wang J, Ioannidis JP, de Andrade M, Rocca WA. Uchl1 is a parkinson's disease susceptibility gene. *Ann Neurol* 2004;55:512–521. [PubMed: 15048890]

26. Toda T, Momose Y, Murata M, Tamiya G, Yamamoto M, Hattori N, Inoko H. Toward identification of susceptibility genes for sporadic parkinson's disease. *J Neurol* 2003;250(Suppl 3):III40–43. [PubMed: 14579123]
27. Cho JW, Kim SY, Park SS, Kim HJ, Ahn TB, Kim JM, Jeon BS. The g2019s lrrk2 mutation is rare in korean patients with parkinson's disease. *Can J Neurol Sci* 2007;34:53–55. [PubMed: 17352347]
28. Wintermeyer P, Kruger R, Kuhn W, Muller T, Woitalla D, Berg D, Becker G, Leroy E, Polymeropoulos M, Berger K, Przuntek H, Schols L, Epplen JT, Riess O. Mutation analysis and association studies of the uchl1 gene in german parkinson's disease patients. *Neuroreport* 2000;11:2079–2082. [PubMed: 10923647]
29. Wood-Kaczmar A, Gandhi S, Wood NW. Understanding the molecular causes of parkinson's disease. *Trends Mol Med* 2006;12:521–528. [PubMed: 17027339]
30. Polymeropoulos MH. Genetics of parkinson's disease. *Ann N Y Acad Sci* 2000;920:28–32. [PubMed: 11193165]
31. Polymeropoulos MH, Higgins JJ, Golbe LI, Johnson WG, Ide SE, Di Iorio G, Sanges G, Stenroos ES, Pho LT, Schaffer AA, Lazzarini AM, Nussbaum RL, Duvoisin RC. Mapping of a gene for parkinson's disease to chromosome 4q21-q23. *Science* 1996;274:1197–1199. [PubMed: 8895469]
32. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with parkinson's disease. *Science* 1997;276:2045–2047. [PubMed: 9197268]
33. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O. Ala30pro mutation in the gene encoding alpha-synuclein in parkinson's disease. *Nat Genet* 1998;18:106–108. [PubMed: 9462735]
34. Singleton A, Gwinn-Hardy K, Sharabi Y, Li ST, Holmes C, Dendi R, Hardy J, Crawley A, Goldstein DS. Association between cardiac denervation and parkinsonism caused by alpha-synuclein gene triplication. *Brain* 2004;127:768–772. [PubMed: 14736756]
35. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. Alpha-synuclein locus triplication causes parkinson's disease. *Science* 2003;302:841. [PubMed: 14593171]
36. Ahn TB, Kim SY, Kim JY, Park SS, Lee DS, Min HJ, Kim YK, Kim SE, Kim JM, Kim HJ, Cho J, Jeon BS. Alpha-synuclein gene duplication is present in sporadic parkinson disease. *Neurology* 2008;70:43–49. [PubMed: 17625105]
37. Maroteaux L, Campanelli JT, Scheller RH. Synuclein: A neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J Neurosci* 1988;8:2804–2815. [PubMed: 3411354]
38. Ueda K, Fukushima H, Masliah E, Xia Y, Iwai A, Yoshimoto M, Otero DA, Kondo J, Ihara Y, Saitoh T. Molecular cloning of cDNA encoding an unrecognized component of amyloid in alzheimer disease. *Proc Natl Acad Sci U S A* 1993;90:11282–11286. [PubMed: 8248242]
39. Chandra S, Chen X, Rizo J, Jahn R, Sudhof TC. A broken alpha-helix in folded alpha-synuclein. *J Biol Chem* 2003;278:15313–15318. [PubMed: 12586824]
40. Kowall NW, Hantraye P, Brouillet E, Beal MF, McKee AC, Ferrante RJ. Mptp induces alpha-synuclein aggregation in the substantia nigra of baboons. *Neuroreport* 2000;11:211–213. [PubMed: 10683860]
41. Paxinou E, Chen Q, Weisse M, Giasson BI, Norris EH, Rueter SM, Trojanowski JQ, Lee VM, Ischiropoulos H. Induction of alpha-synuclein aggregation by intracellular nitrative insult. *J Neurosci* 2001;21:8053–8061. [PubMed: 11588178]
42. Souza JM, Giasson BI, Chen Q, Lee VM, Ischiropoulos H. Dityrosine cross-linking promotes formation of stable alpha-synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. *J Biol Chem* 2000;275:18344–18349. [PubMed: 10747881]
43. Uversky VN, Lee HJ, Li J, Fink AL, Lee SJ. Stabilization of partially folded conformation during alpha-synuclein oligomerization in both purified and cytosolic preparations. *J Biol Chem* 2001;276:43495–43498. [PubMed: 11590163]

44. Uversky VN, Li J, Fink AL. Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular link between parkinson's disease and heavy metal exposure. *J Biol Chem* 2001;276:44284–44296. [PubMed: 11553618]
45. Uversky VN, Li J, Fink AL. Evidence for a partially folded intermediate in alpha-synuclein fibril formation. *J Biol Chem* 2001;276:10737–10744. [PubMed: 11152691]
46. Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: Paraquat and alpha-synuclein. *J Biol Chem* 2002;277:1641–1644. [PubMed: 11707429]
47. Li J, Zhu M, Manning-Bog AB, Di Monte DA, Fink AL. Dopamine and l-dopa disaggregate amyloid fibrils: Implications for parkinson's and alzheimer's disease. *Faseb J* 2004;18:962–964. [PubMed: 15059976]
48. Rochet JC, Outeiro TF, Conway KA, Ding TT, Volles MJ, Lashuel HA, Bieganski RM, Lindquist SL, Lansbury PT. Interactions among alpha-synuclein, dopamine, and biomembranes: Some clues for understanding neurodegeneration in parkinson's disease. *J Mol Neurosci* 2004;23:23–34. [PubMed: 15126689]
49. Fink AL. The aggregation and fibrillation of alpha-synuclein. *Acc Chem Res* 2006;39:628–634. [PubMed: 16981679]
50. Cappai R, Leck SL, Tew DJ, Williamson NA, Smith DP, Galatis D, Sharples RA, Curtain CC, Ali FE, Cherny RA, Culvenor JG, Bottomley SP, Masters CL, Barnham KJ, Hill AF. Dopamine promotes alpha-synuclein aggregation into sds-resistant soluble oligomers via a distinct folding pathway. *Faseb J* 2005;19:1377–1379. [PubMed: 15946991]
51. Maguire-Zeiss KA, Wang CI, Yehling E, Sullivan MA, Short DW, Su X, Gouzer G, Henricksen LA, Wuertzer CA, Federoff HJ. Identification of human alpha-synuclein specific single chain antibodies. *Biochem Biophys Res Commun* 2006;349:1198–1205. [PubMed: 16973126]
52. Moussa CE, Mahmoodian F, Tomita Y, Sidhu A. Dopamine differentially induces aggregation of a53t mutant and wild type alpha-synuclein: Insights into the protein chemistry of parkinson's disease. *Biochem Biophys Res Commun* 2008;365:833–839. [PubMed: 18039462]
53. George JM, Jin H, Woods WS, Clayton DF. Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron* 1995;15:361–372. [PubMed: 7646890]
54. Bonini NM, Giasson BI. Snaring the function of alpha-synuclein. *Cell* 2005;123:359–361. [PubMed: 16269324]
55. Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC. Alpha-synuclein cooperates with cspalpha in preventing neurodegeneration. *Cell* 2005;123:383–396. [PubMed: 16269331]
56. Sidhu A, Wersinger C, Vernier P. Alpha-synuclein regulation of the dopaminergic transporter: A possible role in the pathogenesis of parkinson's disease. *FEBS Lett* 2004;565:1–5. [PubMed: 15135042]
57. Sidhu A, Wersinger C, Vernier P. Does alpha-synuclein modulate dopaminergic synaptic content and tone at the synapse? *Faseb J* 2004;18:637–647. [PubMed: 15054086]
58. Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* 2000;25:239–252. [PubMed: 10707987]
59. Barcelo-Coblijn G, Golovko MY, Weinhofer I, Berger J, Murphy EJ. Brain neutral lipids mass is increased in alpha-synuclein gene-ablated mice. *J Neurochem* 2007;101:132–141. [PubMed: 17250686]
60. Golovko MY, Faergeman NJ, Cole NB, Castagnet PI, Nussbaum RL, Murphy EJ. Alpha-synuclein gene deletion decreases brain palmitate uptake and alters the palmitate metabolism in the absence of alpha-synuclein palmitate binding. *Biochemistry* 2005;44:8251–8259. [PubMed: 15938614]
61. Golovko MY, Rosenberger TA, Faergeman NJ, Feddersen S, Cole NB, Pribill I, Berger J, Nussbaum RL, Murphy EJ. Acyl-coa synthetase activity links wild-type but not mutant alpha-synuclein to brain arachidonate metabolism. *Biochemistry* 2006;45:6956–6966. [PubMed: 16734431]
62. Golovko MY, Rosenberger TA, Feddersen S, Faergeman NJ, Murphy EJ. Alpha-synuclein gene ablation increases docosahexaenoic acid incorporation and turnover in brain phospholipids. *J Neurochem* 2007;101:201–211. [PubMed: 17250657]

63. Perez RG, Hastings TG. Could a loss of alpha-synuclein function put dopaminergic neurons at risk? *J Neurochem* 2004;89:1318–1324. [PubMed: 15189334]
64. Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ. A role for alpha-synuclein in the regulation of dopamine biosynthesis. *J Neurosci* 2002;22:3090–3099. [PubMed: 11943812]
65. Volles MJ, Lee SJ, Rochet JC, Shtilerman MD, Ding TT, Kessler JC, Lansbury PT Jr. Vesicle permeabilization by protofibrillar alpha-synuclein: Implications for the pathogenesis and treatment of parkinson's disease. *Biochemistry* 2001;40:7812–7819. [PubMed: 11425308]
66. Danzer KM, Haasen D, Karow AR, Moussaud S, Habeck M, Giese A, Kretschmar H, Hengerer B, Kostka M. Different species of alpha-synuclein oligomers induce calcium influx and seeding. *J Neurosci* 2007;27:9220–9232. [PubMed: 17715357]
67. Chesselet MF. In vivo alpha-synuclein overexpression in rodents: A useful model of parkinson's disease? *Exp Neurol* 2008;209:22–27. [PubMed: 17949715]
68. Emborg ME. Nonhuman primate models of parkinson's disease. *Ilar J* 2007;48:339–355. [PubMed: 17712221]
69. Kahle PJ. Alpha-synucleinopathy models and human neuropathology: Similarities and differences. *Acta Neuropathol* 2008;115:87–95. [PubMed: 17932682]
70. Meredith GE, Sonsalla PK, Chesselet MF. Animal models of parkinson's disease progression. *Acta Neuropathol* 2008;115:385–398. [PubMed: 18273623]
71. Terzioglu M, Galter D. Parkinson's disease: Genetic versus toxin-induced rodent models. *Febs J* 2008;275:1384–1391. [PubMed: 18279376]
72. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of parkinson's disease. *Nat Neurosci* 2000;3:1301–1306. [PubMed: 11100151]
73. Conway KA, Harper JD, Lansbury PT Jr. Fibrils formed in vitro from alpha-synuclein and two mutant forms linked to parkinson's disease are typical amyloid. *Biochemistry* 2000;39:2552–2563. [PubMed: 10704204]
74. Feany MB, Bender WW. A drosophila model of parkinson's disease. *Nature* 2000;404:394–398. [PubMed: 10746727]
75. Conway KA, Rochet JC, Bieganski RM, Lansbury PT Jr. Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science* 2001;294:1346–1349. [PubMed: 11701929]
76. Gomez-Santos C, Ferrer I, Reiriz J, Vinals F, Barrachina M, Ambrosio S. Mpp+ increases alpha-synuclein expression and erk/map-kinase phosphorylation in human neuroblastoma sh-sy5y cells. *Brain Res* 2002;935:32–39. [PubMed: 12062470]
77. Hashimoto M, Hsu LJ, Xia Y, Takeda A, Sisk A, Sundsmo M, Masliah E. Oxidative stress induces amyloid-like aggregate formation of nacp/alpha-synuclein in vitro. *Neuroreport* 1999;10:717–721. [PubMed: 10208537]
78. Lee M, Hyun D, Halliwell B, Jenner P. Effect of the overexpression of wild-type or mutant alpha-synuclein on cell susceptibility to insult. *J Neurochem* 2001;76:998–1009. [PubMed: 11181819]
79. Tabrizi SJ, Orth M, Wilkinson JM, Taanman JW, Warner TT, Cooper JM, Schapira AH. Expression of mutant alpha-synuclein causes increased susceptibility to dopamine toxicity. *Hum Mol Genet* 2000;9:2683–2689. [PubMed: 11063727]
80. Takenouchi T, Hashimoto M, Hsu LJ, Mackowski B, Rockenstein E, Mallory M, Masliah E. Reduced neuritic outgrowth and cell adhesion in neuronal cells transfected with human alpha-synuclein. *Mol Cell Neurosci* 2001;17:141–150. [PubMed: 11161475]
81. Beal MF. Experimental models of parkinson's disease. *Nat Rev Neurosci* 2001;2:325–334. [PubMed: 11331916]
82. Betarbet R, Sherer TB, Greenamyre JT. Animal models of parkinson's disease. *Bioessays* 2002;24:308–318. [PubMed: 11948617]
83. Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L. Dopaminergic loss and inclusion body formation in alpha-synuclein mice: Implications for neurodegenerative disorders. *Science* 2000;287:1265–1269. [PubMed: 10678833]

84. Richfield EK, Thiruchelvam MJ, Cory-Slechta DA, Wuertzer C, Gainetdinov RR, Caron MG, Di Monte DA, Federoff HJ. Behavioral and neurochemical effects of wild-type and mutated human alpha-synuclein in transgenic mice. *Exp Neurol* 2002;175:35–48. [PubMed: 12009758]
85. van der Putten H, Wiederhold KH, Probst A, Barbieri S, Mistl C, Danner S, Kauffmann S, Hofele K, Spooren WP, Ruegg MA, Lin S, Caroni P, Sommer B, Tolnay M, Bilbe G. Neuropathology in mice expressing human alpha-synuclein. *J Neurosci* 2000;20:6021–6029. [PubMed: 10934251]
86. Yang F, Ueda K, Chen P, Ashe KH, Cole GM. Plaque-associated alpha-synuclein (nACP) pathology in aged transgenic mice expressing amyloid precursor protein. *Brain Res* 2000;853:381–383. [PubMed: 10640638]
87. Narhi L, Wood SJ, Steavenson S, Jiang Y, Wu GM, Anafi D, Kaufman SA, Martin F, Sitney K, Denis P, Louis JC, Wypych J, Biere AL, Citron M. Both familial parkinson's disease mutations accelerate alpha-synuclein aggregation. *J Biol Chem* 1999;274:9843–9846. [PubMed: 10092675]
88. Ostrerova-Golts N, Petrucelli L, Hardy J, Lee JM, Farer M, Wolozin B. The A53T alpha-synuclein mutation increases iron-dependent aggregation and toxicity. *J Neurosci* 2000;20:6048–6054. [PubMed: 10934254]
89. Giasson BI, Lee VM. A new link between pesticides and parkinson's disease. *Nat Neurosci* 2000;3:1227–1228. [PubMed: 11100135]
90. Fornai F, Schluter OM, Lenzi P, Gesi M, Ruffoli R, Ferrucci M, Lazzeri G, Busceti CL, Pontarelli F, Battaglia G, Pellegrini A, Nicoletti F, Ruggieri S, Paparelli A, Sudhof TC. Parkinson-like syndrome induced by continuous MPTP infusion: Convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proc Natl Acad Sci U S A* 2005;102:3413–3418. [PubMed: 15716361]
91. Recchia A, Rota D, DeBetto P, Peroni D, Guidolin D, Negro A, Skaper SD, Giusti P. Generation of an alpha-synuclein-based rat model of parkinson's disease. *Neurobiol Dis* 2008;30:8–18. [PubMed: 18313315]
92. Su X, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, Federoff HJ. Synuclein activates microglia in a model of parkinson's disease. *Neurobiol Aging*. 2007[Epub ahead of print]; PMID: 17537546
93. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhang W, Zhou Y, Hong JS, Zhang J. Aggregated alpha-synuclein activates microglia: A process leading to disease progression in parkinson's disease. *FASEB J* 2005;19:533–542. [PubMed: 15791003]
94. Reynolds AD, Kadiu I, Garg SK, Glanzer JG, Nordgren T, Ciborowski P, Banerjee R, Gendelman HE. Nitrated alpha-synuclein and microglial neuroregulatory activities. *J Neuroimmune Pharmacol* 2008;3:59–74. [PubMed: 18202920]
95. McGeer PL, McGeer EG. Glial reactions in parkinson's disease. *Mov Disord* 2008;23:474–483. [PubMed: 18044695]
96. Mizuno Y, Shimoda-Matsubayashi S, Matsumine H, Morikawa N, Hattori N, Kondo T. Genetic and environmental factors in the pathogenesis of parkinson's disease. *Adv Neurol* 1999;80:171–179. [PubMed: 10410718]
97. Riess O, Krüger R. Parkinson's disease--a multifactorial neurodegenerative disorder. *J Neural Transm Suppl* 1999;56:113–125. [PubMed: 10370906]
98. Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nitsch R, Joerg J, Oertel WH, Ulm G, Schneider E. Possible environmental, occupational, and other etiologic factors for parkinson's disease: A case-control study in Germany. *Neurology* 1996;46:1275–1284. [PubMed: 8628466]
99. McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, Di Monte DA. Environmental risk factors and parkinson's disease: Selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. *Neurobiol Dis* 2002;10:119–127. [PubMed: 12127150]
100. Maguire-Zeiss KA, Short DW, Federoff HJ. Synuclein, dopamine and oxidative stress: Co-conspirators in parkinson's disease? *Brain Res Mol Brain Res* 2005;134:18–23. [PubMed: 15790526]
101. Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, Richardson RJ. Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of parkinson's disease. *Neurotoxicology* 1999;20:239–247. [PubMed: 10385887]

102. Coon S, Stark A, Peterson E, Gloi A, Kortsha G, Pounds J, Chettle D, Gorell J. Whole-body lifetime occupational lead exposure and risk of parkinson's disease. *Environ Health Perspect* 2006;114:1872–1876. [PubMed: 17185278]
103. Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, Comyns K, Goldman S, Korell M, Langston J, Ross G, Sandler D. Pesticide exposure and self-reported parkinson's disease in the agricultural health study. *Am J Epidemiol* 2007;165:364–374. [PubMed: 17116648]
104. Priyadarshi A, Khuder SA, Schaub EA, Priyadarshi SS. Environmental risk factors and parkinson's disease: A metaanalysis. *Environ Res* 2001;86:122–127. [PubMed: 11437458]
105. Barlow BK, Cory-Slechta DA, Richfield EK, Thiruchelvam M. The gestational environment and parkinson's disease: Evidence for neurodevelopmental origins of a neurodegenerative disorder. *Reprod Toxicol* 2007;23:457–470. [PubMed: 17350799]
106. Cicchetti F, Brownell AL, Williams K, Chen YI, Livni E, Isacson O. Neuroinflammation of the nigrostriatal pathway during progressive 6-ohda dopamine degeneration in rats monitored by immunohistochemistry and pet imaging. *Eur J Neurosci* 2002;15:991–998. [PubMed: 11918659]
107. Delgado M, Ganea D. Neuroprotective effect of vasoactive intestinal peptide (vip) in a mouse model of parkinson's disease by blocking microglial activation. *Faseb J* 2003;17:944–946. [PubMed: 12626429]
108. Gao HM, Liu B, Zhang W, Hong JS. Synergistic dopaminergic neurotoxicity of mptp and inflammogen lipopolysaccharide: Relevance to the etiology of parkinson's disease. *Faseb J* 2003;17:1957–1959. [PubMed: 12923073]
109. Wu DC, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, Przedborski S. NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of parkinson's disease. *Proc Natl Acad Sci U S A* 2003;100:6145–6150. [PubMed: 12721370]
110. Barcia C, Sanchez Bahillo A, Fernandez-Villalba E, Bautista V, Poza YPM, Fernandez-Barreiro A, Hirsch EC, Herrero MT. Evidence of active microglia in substantia nigra pars compacta of parkinsonian monkeys 1 year after mptp exposure. *Glia* 2004;46:402–409. [PubMed: 15095370]
111. Saint-Pierre M, Tremblay ME, Sik A, Gross RE, Cicchetti F. Temporal effects of paraquat/maneb on microglial activation and dopamine neuronal loss in older rats. *J Neurochem* 2006;98:760–772. [PubMed: 16893418]
112. Gash DM, Rutland K, Hudson NL, Sullivan PG, Bing G, Cass WA, Pandya JD, Liu M, Choi DY, Hunter RL, Gerhardt GA, Smith CD, Slevin JT, Prince TS. Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity. *Ann Neurol* 2008;63:184–192. [PubMed: 18157908]
113. Sugeno N, Takeda A, Hasegawa T, Kobayashi M, Kikuchi A, Mori F, Wakabayashi K, Itoyama Y. Serine 129 phosphorylation of alpha-synuclein induces unfolded protein response-mediated cell death. *J Biol Chem* 2008;283:23179–23188. [PubMed: 18562315]
114. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 1983;219:979–980. [PubMed: 6823561]
115. Langston JW, Irwin I, Langston EB, Forno LS. 1-methyl-4-phenylpyridinium ion (mpp+): Identification of a metabolite of mptp, a toxin selective to the substantia nigra. *Neurosci Lett* 1984;48:87–92. [PubMed: 6332288]
116. Langston JW, Langston EB, Irwin I. Mptp-induced parkinsonism in human and non-human primates--clinical and experimental aspects. *Acta Neurol Scand Suppl* 1984;100:49–54. [PubMed: 6333134]
117. Ali SF, David SN, Newport GD. Age-related susceptibility to mptp-induced neurotoxicity in mice. *Neurotoxicology* 1993;14:29–34. [PubMed: 8361676]
118. Chiba K, Trevor AJ, Castagnoli N Jr. Active uptake of mpp+, a metabolite of mptp, by brain synaptosomes. *Biochem Biophys Res Commun* 1985;128:1228–1232. [PubMed: 3873939]
119. Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D. Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. *Ann Neurol* 1999;46:598–605. [PubMed: 10514096]
120. Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M. The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (mptp): A technical review of its utility and safety. *J Neurochem* 2001;76:1265–1274. [PubMed: 11238711]

121. Moratalla R, Quinn B, DeLanney LE, Irwin I, Langston JW, Graybiel AM. Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci U S A* 1992;89:3859–3863. [PubMed: 1570304]
122. Varastet M, Riche D, Maziere M, Hantraye P. Chronic mptp treatment reproduces in baboons the differential vulnerability of mesencephalic dopaminergic neurons observed in parkinson's disease. *Neuroscience* 1994;63:47–56. [PubMed: 7898660]
123. Hoppel CL, Grinblatt D, Kwok HC, Arora PK, Singh MP, Sayre LM, Greenblatt D. Inhibition of mitochondrial respiration by analogs of 4-phenylpyridine and 1-methyl-4-phenylpyridinium cation (mpp+), the neurotoxic metabolite of mptp. *Biochem Biophys Res Commun* 1987;148:684–693. [PubMed: 3500717]
124. Mochizuki H, Nakamura N, Nishi K, Mizuno Y. Apoptosis is induced by 1-methyl-4-phenylpyridinium ion (mpp+) in ventral mesencephalic-striatal co-culture in rat. *Neurosci Lett* 1994;170:191–194. [PubMed: 8041505]
125. Ramsay RR, Dadgar J, Trevor A, Singer TP. Energy-driven uptake of n-methyl-4-phenylpyridine by brain mitochondria mediates the neurotoxicity of mptp. *Life Sci* 1986;39:581–588. [PubMed: 3488484]
126. Ramsay RR, Salach JI, Singer TP. Uptake of the neurotoxin 1-methyl-4-phenylpyridine (mpp+) by mitochondria and its relation to the inhibition of the mitochondrial oxidation of nad+-linked substrates by mpp+ *Biochem Biophys Res Commun* 1986;134:743–748. [PubMed: 2868716]
127. Song DD, Shults CW, Sisk A, Rockenstein E, Masliah E. Enhanced substantia nigra mitochondrial pathology in human alpha-synuclein transgenic mice after treatment with mptp. *Exp Neurol* 2004;186:158–172. [PubMed: 15026254]
128. Tatton NA, Kish SJ. In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. *Neuroscience* 1997;77:1037–1048. [PubMed: 9130785]
129. Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. Mitochondrial complex i deficiency in parkinson's disease. *J Neurochem* 1990;54:823–827. [PubMed: 2154550]
130. Gao HM, Liu B, Zhang W, Hong JS. Critical role of microglial nadph oxidase-derived free radicals in the in vitro mptp model of parkinson's disease. *Faseb J* 2003;17:1954–1956. [PubMed: 12897068]
131. Dong Z, Ferger B, Feldon J, Bueler H. Overexpression of parkinson's disease-associated alpha-synucleina53t by recombinant adeno-associated virus in mice does not increase the vulnerability of dopaminergic neurons to mptp. *J Neurobiol* 2002;53:1–10. [PubMed: 12360578]
132. Przedborski S, Chen Q, Vila M, Giasson BI, Djaldatti R, Vukosavic S, Souza JM, Jackson-Lewis VV, Lee VM, Ischiropoulos H. Oxidative post-translational modifications of alpha-synuclein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (mptp) mouse model of parkinson's disease. *J Neurochem* 2001;76:637–640. [PubMed: 11208927]
133. Vila M, Vukosavic S, Jackson-Lewis V, Neystat M, Jakowec M, Przedborski S. Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin mptp. *J Neurochem* 2000;74:721–729. [PubMed: 10646524]
134. Meredith GE, Totterdell S, Petroske E, Santa Cruz K, Callison RC Jr, Lau YS. Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of parkinson's disease. *Brain Res* 2002;956:156–165. [PubMed: 12426058]
135. Periquet M, Fulga T, Myllykangas L, Schlossmacher MG, Feany MB. Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. *J Neurosci* 2007;27:3338–3346. [PubMed: 17376994]
136. Mori F, Nishie M, Kakita A, Yoshimoto M, Takahashi H, Wakabayashi K. Relationship among alpha-synuclein accumulation, dopamine synthesis, and neurodegeneration in parkinson disease substantia nigra. *J Neuropathol Exp Neurol* 2006;65:808–815. [PubMed: 16896314]
137. Galvin JE. Interaction of alpha-synuclein and dopamine metabolites in the pathogenesis of parkinson's disease: A case for the selective vulnerability of the substantia nigra. *Acta Neuropathol* 2006;112:115–126. [PubMed: 16791599]

138. Caudle WM, Colebrooke RE, Emson PC, Miller GW. Altered vesicular dopamine storage in parkinson's disease: A premature demise. *Trends Neurosci* 2008;31:303–308. [PubMed: 18471904]
139. Lee FJ, Liu F, Pristupa ZB, Niznik HB. Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *Faseb J* 2001;15:916–926. [PubMed: 11292651]
140. Fahn S. Description of parkinson's disease as a clinical syndrome. *Ann N Y Acad Sci* 2003;991:1–14. [PubMed: 12846969]
141. Maguire-Zeiss KA, Federoff HJ. Novel gene therapeutic strategies for neurodegenerative diseases. Ernst Schering Res Found Workshop 2005:147–171. [PubMed: 16315613]
142. Maguire-Zeiss KA, Mhyre TR, Federoff HJ. Gazing into the future: Parkinson's disease gene therapeutics to modify natural history. *Exp Neurol* 2008;209:101–113. [PubMed: 18035353]
143. Benabid AL. Deep brain stimulation for parkinson's disease. *Curr Opin Neurobiol* 2003;13:696–706. [PubMed: 14662371]
144. Hsich G, Sena-Estevés M, Breakefield XO. Critical issues in gene therapy for neurologic disease. *Hum Gene Ther* 2002;13:579–604. [PubMed: 11916483]
145. Mochizuki H, Mizuno Y. Gene therapy for parkinson's disease. *J Neural Transm Suppl* 2003:205–213. [PubMed: 12946058]
146. Schapira AH, Olanow CW. Rationale for the use of dopamine agonists as neuroprotective agents in parkinson's disease. *Ann Neurol* 2003;53(Suppl 3):S149–157. [PubMed: 12666106]discussion S157-149
147. Klegeris A, McGeer EG, McGeer PL. Therapeutic approaches to inflammation in neurodegenerative disease. *Curr Opin Neurol* 2007;20:351–357. [PubMed: 17495632]
148. Gao HM, Liu B, Zhang W, Hong JS. Novel anti-inflammatory therapy for parkinson's disease. *Trends Pharmacol Sci* 2003;24:395–401. [PubMed: 12915048]
149. Shoulson I. Datatop: A decade of neuroprotective inquiry. Parkinson study group. Deprenyl and tocopherol antioxidative therapy of parkinsonism. *Ann Neurol* 1998;44:S160–166. [PubMed: 9749589]
150. Morizane A, Li JY, Brundin P. From bench to bed: The potential of stem cells for the treatment of parkinson's disease. *Cell Tissue Res* 2008;331:323–336. [PubMed: 18034267]
151. Wu SS, Frucht SJ. Treatment of parkinson's disease: What's on the horizon? *CNS Drugs* 2005;19:723–743. [PubMed: 16142989]
152. Takahashi, J. Ernst Schering Res Found Workshop. 2006. Stem cell therapy for parkinson's disease; p. 229-244.
153. Peterson AL, Nutt JG. Treatment of parkinson's disease with trophic factors. *Neurotherapeutics* 2008;5:270–280. [PubMed: 18394569]
154. Mochizuki H, Yasuda T, Mouradian MM. Advances in gene therapy for movement disorders. *Neurotherapeutics* 2008;5:260–269. [PubMed: 18394568]
155. Wang X, Liang XB, Li FQ, Zhou HF, Liu XY, Wang JJ, Wang XM. Therapeutic strategies for parkinson's disease: The ancient meets the future-traditional chinese herbal medicine, electroacupuncture, gene therapy and stem cells. *Neurochem Res*. 2008[Epub ahead of print];PMID: 18404373
156. Palfi S. Towards gene therapy for parkinson's disease. *Lancet Neurol* 2008;7:375–376. [PubMed: 18387851]
157. Fiske BK, Frasier MA, Sherer TB. Special focus section: Gene therapy for parkinson's disease. *Exp Neurol* 2008;209:28–29. [PubMed: 17980867]
158. Fiandaca M, Forsayeth J, Bankiewicz K. Current status of gene therapy trials for parkinson's disease. *Exp Neurol* 2008;209:51–57. [PubMed: 17920059]
159. Prasad KN, Cole WC, Kumar B. Multiple antioxidants in the prevention and treatment of parkinson's disease. *J Am Coll Nutr* 1999;18:413–423. [PubMed: 10511322]
160. Neff F, Wei X, Nolker C, Bacher M, Du Y, Dodel R. Immunotherapy and naturally occurring autoantibodies in neurodegenerative disorders. *Autoimmun Rev* 2008;7:501–507. [PubMed: 18558370]

161. Evans JR, Barker RA. Neurotrophic factors as a therapeutic target for parkinson's disease. *Expert Opin Ther Targets* 2008;12:437–447. [PubMed: 18348680]
162. Factor SA. Current status of symptomatic medical therapy in parkinson's disease. *Neurotherapeutics* 2008;5:164–180. [PubMed: 18394561]
163. LeWitt PA, Taylor DC. Protection against parkinson's disease progression: Clinical experience. *Neurotherapeutics* 2008;5:210–225. [PubMed: 18394564]
164. Anden NE, Carlsson A, Kerstell J, Magnusson T, Olsson R, Roos BE, Steen B, Steg G, Svanborg A, Thieme G, Werdinius B. Oral l-dopa treatment of parkinsonism. *Acta Med Scand* 1970;187:247–255. [PubMed: 4911257]
165. Fabbrini G, Mouradian MM, Juncos JL, Schlegel J, Mohr E, Chase TN. Motor fluctuations in parkinson's disease: Central pathophysiological mechanisms, part i. *Ann Neurol* 1988;24:366–371. [PubMed: 3228270]
166. Mouradian MM, Juncos JL, Fabbrini G, Schlegel J, Bartko JJ, Chase TN. Motor fluctuations in parkinson's disease: Central pathophysiological mechanisms, part ii. *Ann Neurol* 1988;24:372–378. [PubMed: 3228271]
167. Ramaker C, van Hilten JJ. Bromocriptine versus levodopa in early parkinson's disease. *Cochrane Database Syst Rev* 2000;CD002258. [PubMed: 10908538]
168. Asanuma M, Miyazaki I. Nonsteroidal anti-inflammatory drugs in experimental parkinsonian models and parkinson's disease. *Curr Pharm Des* 2008;14:1428–1434. [PubMed: 18537665]
169. Simola N, Morelli M, Pinna A. Adenosine a2a receptor antagonists and parkinson's disease: State of the art and future directions. *Curr Pharm Des* 2008;14:1475–1489. [PubMed: 18537671]
170. LeWitt PA, Guttman M, Tetrud JW, Tuite PJ, Mori A, Chaikin P, Sussman NM. Adenosine a2a receptor antagonist istradefylline (kw-6002) reduces "Off" Time in parkinson's disease: A double-blind, randomized, multicenter clinical trial (6002-us-005). *Ann Neurol* 2008;63:295–302. [PubMed: 18306243]
171. Guridi J, Obeso JA, Rodriguez-Oroz MC, Lozano AA, Manrique M. L-dopa-induced dyskinesia and stereotactic surgery for parkinson's disease. *Neurosurgery* 2008;62:311–323. [PubMed: 18382309]discussion 323-315
172. Herzog J, Pogarell O, Pinsker MO, Kupsch A, Oertel WH, Lindvall O, Deuschl G, Volkmann J. Deep brain stimulation in parkinson's disease following fetal nigral transplantation. *Mov Disord* 2008;23:1293–1296. [PubMed: 18536037]
173. Limousin P, Martinez-Torres I. Deep brain stimulation for parkinson's disease. *Neurotherapeutics* 2008;5:309–319. [PubMed: 18394572]
174. Montgomery EB Jr, Gale JT. Mechanisms of action of deep brain stimulation(dbs). *Neurosci Biobehav Rev* 2008;32:388–407. [PubMed: 17706780]
175. Rempel MS, Sarpong Y, Neimat JS. Frontiers in the surgical treatment of parkinson's disease. *Expert Rev Neurother* 2008;8:897–906. [PubMed: 18505355]
176. Wider C, Pollo C, Bloch J, Burkhard PR, Vingerhoets FJ. Long-term outcome of 50 consecutive parkinson's disease patients treated with subthalamic deep brain stimulation. *Parkinsonism Relat Disord* 2008;14:114–119. [PubMed: 17822940]
177. Yamada K, Goto S, Hamasaki T, Kuratsu JI. Effect of bilateral subthalamic nucleus stimulation on levodopa-unresponsive axial symptoms in parkinson's disease. *Acta Neurochir (Wien)* 2008;150(15):22.discussion 22
178. Yu H, Neimat JS. The treatment of movement disorders by deep brain stimulation. *Neurotherapeutics* 2008;5:26–36. [PubMed: 18164481]
179. Braak H, Del Tredici K. Assessing fetal nerve cell grafts in parkinson's disease. *Nat Med* 2008;14:483–485. [PubMed: 18463652]
180. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in parkinson's disease. *Nat Med* 2008;14:504–506. [PubMed: 18391962]
181. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehnacrona S, Bjorklund A, Widner H, Revesz T, Lindvall O, Brundin P. Lewy bodies in grafted neurons in subjects with parkinson's disease suggest host-to-graft disease propagation. *Nat Med* 2008;14:501–503. [PubMed: 18391963]

182. Mendez I, Vinuela A, Astradsson A, Mukhida K, Hallett P, Robertson H, Tierney T, Holness R, Dagher A, Trojanowski JQ, Isacson O. Dopamine neurons implanted into people with parkinson's disease survive without pathology for 14 years. *Nat Med* 2008;14:507–509. [PubMed: 18391961]
183. Bankiewicz KS, Plunkett RJ, Jacobowitz DM, Porrino L, di Porzio U, London WT, Kopin IJ, Oldfield EH. The effect of fetal mesencephalon implants on primate mptp-induced parkinsonism. Histochemical and behavioral studies. *J Neurosurg* 1990;72:231–244. [PubMed: 2295921]
184. Lindner MD, Winn SR, Baetge EE, Hammang JP, Gentile FT, Doherty E, McDermott PE, Frydel B, Ullman MD, Schallert T, et al. Implantation of encapsulated catecholamine and gdnf-producing cells in rats with unilateral dopamine depletions and parkinsonian symptoms. *Exp Neurol* 1995;132:62–76. [PubMed: 7720827]
185. Barkats M, Nakao N, Grasbon-Frodl EM, Bilang-Bleuel A, Revah F, Mallet J, Brundin P. Intrastratial grafts of embryonic mesencephalic rat neurons genetically modified using an adenovirus encoding human cu/zn superoxide dismutase. *Neuroscience* 1997;78:703–713. [PubMed: 9153652]
186. Clarke DJ, Brundin P, Strecker RE, Nilsson OG, Bjorklund A, Lindvall O. Human fetal dopamine neurons grafted in a rat model of parkinson's disease: Ultrastructural evidence for synapse formation using tyrosine hydroxylase immunocytochemistry. *Exp Brain Res* 1988;73:115–126. [PubMed: 3145209]
187. Costantini LC, Jacoby DR, Wang S, Fraefel C, Breakefield XO, Isacson O. Gene transfer to the nigrostriatal system by hybrid herpes simplex virus/adeno-associated virus amplicon vectors. *Hum Gene Ther* 1999;10:2481–2494. [PubMed: 10543613]
188. Lin Q, Cunningham LA, Epstein LG, Pechan PA, Short MP, Fleet C, Bohn MC. Human fetal astrocytes as an ex vivo gene therapy vehicle for delivering biologically active nerve growth factor. *Hum Gene Ther* 1997;8:331–339. [PubMed: 9048200]
189. Redmond DE, Sladek JR Jr, Roth RH, Collier TJ, Elsworth JD, Deutch AY, Haber S. Fetal neuronal grafts in monkeys given methylphenyltetrahydropyridine. *Lancet* 1986;1:1125–1127. [PubMed: 2871381]
190. Perlow MJ, Freed WJ, Hoffer BJ, Seiger A, Olson L, Wyatt RJ. Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* 1979;204:643–647. [PubMed: 571147]
191. Moses D, Drago J, Teper Y, Gantois I, Finkelstein DI, Horne MK. Fetal striatum- and ventral mesencephalon-derived expanded neurospheres rescue dopaminergic neurons in vitro and the nigrostriatal system in vivo. *Neuroscience* 2008;154:606–620. [PubMed: 18472226]
192. Mandel RJ, Burger C, Snyder RO. Viral vectors for in vivo gene transfer in parkinson's disease: Properties and clinical grade production. *Exp Neurol* 2008;209:58–71. [PubMed: 17916354]
193. Porras G, Bezard E. Preclinical development of gene therapy for parkinson's disease. *Exp Neurol* 2008;209:72–81. [PubMed: 17904121]
194. Kordower JH, Olanow CW. Regulatable promoters and gene therapy for parkinson's disease: Is the only thing to fear, fear itself? *Exp Neurol* 2008;209:34–40. [PubMed: 17888424]
195. Ostenfeld T, Tai YT, Martin P, Deglon N, Aebischer P, Svendsen CN. Neurospheres modified to produce glial cell line-derived neurotrophic factor increase the survival of transplanted dopamine neurons. *J Neurosci Res* 2002;69:955–965. [PubMed: 12205689]
196. Sun J, Gao Y, Yang L, Li Z, Lu G, Yew D. Neural-tube-derived neuroepithelial stem cells: A new transplant resource for parkinson's disease. *Neuroreport* 2007;18:543–547. [PubMed: 17413654]
197. Takagi Y, Takahashi J, Saiki H, Morizane A, Hayashi T, Kishi Y, Fukuda H, Okamoto Y, Koyanagi M, Ideguchi M, Hayashi H, Imazato T, Kawasaki H, Suemori H, Omachi S, Iida H, Itoh N, Nakatsuji N, Sasai Y, Hashimoto N. Dopaminergic neurons generated from monkey embryonic stem cells function in a parkinson primate model. *J Clin Invest* 2005;115:102–109. [PubMed: 15630449]
198. Lindvall O, Backlund EO, Farde L, Sedvall G, Freedman R, Hoffer B, Nobin A, Seiger A, Olson L. Transplantation in parkinson's disease: Two cases of adrenal medullary grafts to the putamen. *Ann Neurol* 1987;22:457–468. [PubMed: 3435067]
199. Madrazo I, Drucker-Colin R, Diaz V, Martinez-Mata J, Torres C, Beceril JJ. Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable parkinson's disease. *N Engl J Med* 1987;316:831–834. [PubMed: 3821826]

200. Kordower JH. In vivo gene delivery of glial cell line--derived neurotrophic factor for parkinson's disease. *Ann Neurol* 2003;53(Suppl 3):S120–132. [PubMed: 12666104]discussion S132-124
201. Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, Brooks DJ, Svendsen CN, Heywood P. Direct brain infusion of glial cell line-derived neurotrophic factor in parkinson disease. *Nat Med* 2003;9:589–595. [PubMed: 12669033]
202. Lang AE, Gill S, Patel NK, Lozano A, Nutt JG, Penn R, Brooks DJ, Hotton G, Moro E, Heywood P, Brodsky MA, Burchiel K, Kelly P, Dalvi A, Scott B, Stacy M, Turner D, Wooten VG, Elias WJ, Laws ER, Dhawan V, Stoessl AJ, Matcham J, Coffey RJ, Traub M. Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in parkinson disease. *Ann Neurol* 2006;59:459–466. [PubMed: 16429411]
203. Nutt JG, Burchiel KJ, Comella CL, Jankovic J, Lang AE, Laws ER, Lozano A, Penn R, Simpson RK, Stacy M, Wooten GF. Icv gdnf study group: Implanted intracerebroventricular glial cell line-derived neurotrophic factor. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (gdnf) in pd. *Neurology* 2003;60:69–73. [PubMed: 12525720]
204. Slevin JT, Gerhardt GA, Smith CD, Gash DM, Kryscio R, Young B. Improvement of bilateral motor functions in patients with parkinson disease through the unilateral intraputamenal infusion of glial cell line-derived neurotrophic factor. *J Neurosurg* 2005;102:216–222. [PubMed: 15739547]
205. Slevin JT, Gash DM, Smith CD, Gerhardt GA, Kryscio R, Chebrolu H, Walton A, Wagner R, Young AB. Unilateral intraputamenal glial cell line-derived neurotrophic factor in patients with parkinson disease: Response to 1 year of treatment and 1 year of withdrawal. *J Neurosurg* 2007;106:614–620. [PubMed: 17432712]
206. Horger BA, Nishimura MC, Armanini MP, Wang LC, Poulsen KT, Rosenblad C, Kirik D, Moffat B, Simmons L, Johnson E Jr, Milbrandt J, Rosenthal A, Bjorklund A, Vandlen RA, Hynes MA, Phillips HS. Neurturin exerts potent actions on survival and function of midbrain dopaminergic neurons. *J Neurosci* 1998;18:4929–4937. [PubMed: 9634558]
207. Grondin R, Zhang Z, Ai Y, Ding F, Walton AA, Surgener SP, Gerhardt GA, Gash DM. Intraputamenal infusion of exogenous neurturin protein restores motor and dopaminergic function in the globus pallidus of mptp-lesioned rhesus monkeys. *Cell Transplant* 2008;17:373–381. [PubMed: 18522240]
208. Marks WJ Jr, Ostrem JL, Verhagen L, Starr PA, Larson PS, Bakay RA, Taylor R, Cahn-Weiner DA, Stoessl AJ, Olanow CW, Bartus RT. Safety and tolerability of intraputamenal delivery of cere-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic parkinson's disease: An open-label, phase i trial. *Lancet Neurol* 2008;7:400–408. [PubMed: 18387850]
209. Cleren C, Yang L, Lorenzo B, Calingasan NY, Schomer A, Sireci A, Wille EJ, Beal MF. Therapeutic effects of coenzyme q10 (coq10) and reduced coq10 in the mptp model of parkinsonism. *J Neurochem* 2008;104:1613–1621. [PubMed: 17973981]
210. Bayes M, Rabasseda X, Prous JR. Gateways to clinical trials. *Methods Find Exp Clin Pharmacol* 2007;29:303–311. [PubMed: 17609744]
211. Dass B, Olanow CW, Kordower JH. Gene transfer of trophic factors and stem cell grafting as treatments for parkinson's disease. *Neurology* 2006;66:S89–103. [PubMed: 16717256]
212. Maguire-Zeiss KA, Bowers WJ, Federoff HJ. Hsv vector-mediated gene delivery to the central nervous system. *Curr Opin Mol Ther* 2001;3:482–490. [PubMed: 11699893]
213. Ross CA, Pickart CM. The ubiquitin-proteasome pathway in parkinson's disease and other neurodegenerative diseases. *Trends Cell Biol* 2004;14:703–711. [PubMed: 15564047]
214. Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE, Lansbury PT Jr. Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset parkinson's disease: Implications for pathogenesis and therapy. *Proc Natl Acad Sci U S A* 2000;97:571–576. [PubMed: 10639120]
215. Tashiro M, Kojima M, Kihara H, Kasai K, Kamiyoshihara T, Ueda K, Shimotakahara S. Characterization of fibrillation process of alpha-synuclein at the initial stage. *Biochem Biophys Res Commun* 2008;369:910–914. [PubMed: 18329380]
216. Kheterpal I, Lashuel HA, Hartley DM, Walz T, Lansbury PT Jr, Wetzel R. Abeta protofibrils possess a stable core structure resistant to hydrogen exchange. *Biochemistry* 2003;42:14092–14098. [PubMed: 14640676]

217. Stefani M, Dobson CM. Protein aggregation and aggregate toxicity: New insights into protein folding, misfolding diseases and biological evolution. *J Mol Med* 2003;81:678–699. [PubMed: 12942175]
218. Caughey B, Lansbury PT. Protofibrils, pores, fibrils, and neurodegeneration: Separating the responsible protein aggregates from the innocent bystanders. *Annu Rev Neurosci* 2003;26:267–298. [PubMed: 12704221]
219. Cookson MR, van der Brug M. Cell systems and the toxic mechanism(s) of alpha-synuclein. *Exp Neurol* 2008;209:5–11. [PubMed: 17603039]
220. Hasegawa T, Matsuzaki-Kobayashi M, Takeda A, Sugeno N, Kikuchi A, Furukawa K, Perry G, Smith MA, Itoyama Y. Alpha-synuclein facilitates the toxicity of oxidized catechol metabolites: Implications for selective neurodegeneration in parkinson's disease. *FEBS Lett* 2006;580:2147–2152. [PubMed: 16567160]
221. Meredith SC. Protein denaturation and aggregation: Cellular responses to denatured and aggregated proteins. *Ann N Y Acad Sci* 2005;1066:181–221. [PubMed: 16533927]
222. El-Agnaf OM, Paleologou KE, Greer B, Abogreïn AM, King JE, Salem SA, Fullwood NJ, Benson FE, Hewitt R, Ford KJ, Martin FL, Harriott P, Cookson MR, Allsop D. A strategy for designing inhibitors of alpha-synuclein aggregation and toxicity as a novel treatment for parkinson's disease and related disorders. *Faseb J* 2004;18:1315–1317. [PubMed: 15180968]
223. Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* 2002;416:507–511. [PubMed: 11932737]
224. El-Agnaf OM, Nagala S, Patel BP, Austen BM. Non-fibrillar oligomeric species of the amyloid abri peptide, implicated in familial british dementia, are more potent at inducing apoptotic cell death than protofibrils or mature fibrils. *J Mol Biol* 2001;310:157–168. [PubMed: 11419943]
225. El-Agnaf OM, Walsh DM, Allsop D. Soluble oligomers for the diagnosis of neurodegenerative diseases. *Lancet Neurol* 2003;2:461–462. [PubMed: 12878433]
226. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL. Diffusible, nonfibrillar ligands derived from abeta1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A* 1998;95:6448–6453. [PubMed: 9600986]
227. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 2002;416:535–539. [PubMed: 11932745]
228. Tetzlaff JE, Putcha P, Outeiro TF, Ivanov A, Berezovska O, Hyman BT, McLean PJ. Chip targets toxic alpha-synuclein oligomers for degradation. *J Biol Chem* 2008;283:17962–17968. [PubMed: 18436529]
229. Kawahara K, Hashimoto M, Bar-On P, Ho GJ, Crews L, Mizuno H, Rockenstein E, Imam SZ, Masliah E. Alpha-synuclein aggregates interfere with parkin solubility and distribution: Role in the pathogenesis of parkinson disease. *J Biol Chem* 2008;283:6979–6987. [PubMed: 18195004]
230. Windisch M, Wolf H, Hutter-Paier B, Wronski R. The role of alpha-synuclein in neurodegenerative diseases: A potential target for new treatment strategies? *Neurodegener Dis* 2008;5:218–221. [PubMed: 18322395]
231. Ischiropoulos H. Oxidative modifications of alpha-synuclein. *Ann N Y Acad Sci* 2003;991:93–100. [PubMed: 12846977]
232. Lee HJ, Shin SY, Choi C, Lee YH, Lee SJ. Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. *J Biol Chem* 2002;277:5411–5417. [PubMed: 11724769]
233. Li J, Uversky VN, Fink AL. Conformational behavior of human alpha-synuclein is modulated by familial parkinson's disease point mutations a30p and a53t. *Neurotoxicology* 2002;23:553–567. [PubMed: 12428728]
234. Tanaka M, Kim YM, Lee G, Junn E, Iwatsubo T, Mouradian MM. Aggresomes formed by alpha-synuclein and synphilin-1 are cytoprotective. *J Biol Chem* 2004;279:4625–4631. [PubMed: 14627698]

235. Murphy RM. Peptide aggregation in neurodegenerative disease. *Annu Rev Biomed Eng* 2002;4:155–174. [PubMed: 12117755]
236. Ecroyd H, Carver JA. The effect of small molecules in modulating the chaperone activity of alphas-crystallin against ordered and disordered protein aggregation. *Febs J* 2008;275:935–947. [PubMed: 18218039]
237. Madine J, Doig AJ, Middleton DA. Design of an n-methylated peptide inhibitor of alpha-synuclein aggregation guided by solid-state nmr. *J Am Chem Soc* 2008;130:7873–7881. [PubMed: 18510319]
238. Sciarretta KL, Gordon DJ, Meredith SC. Peptide-based inhibitors of amyloid assembly. *Methods Enzymol* 2006;413:273–312. [PubMed: 17046402]
239. Abe K, Kobayashi N, Sode K, Ikebukuro K. Peptide ligand screening of alpha-synuclein aggregation modulators by in silico panning. *BMC Bioinformatics* 2007;8:451. [PubMed: 18005454]
240. Bar-On P, Crews L, Koob AO, Mizuno H, Adame A, Spencer B, Masliah E. Statins reduce neuronal alpha-synuclein aggregation in in vitro models of parkinson's disease. *J Neurochem* 2008;105:1656–1667. [PubMed: 18248604]
241. Brandt I, Gerard M, Sergeant K, Devreese B, Baekelandt V, Augustyns K, Scharpe S, Engelborghs Y, Lambey AM. Prolyl oligopeptidase stimulates the aggregation of alpha-synuclein. *Peptides* 2008;29:1472–1478. [PubMed: 18571285]
242. Outeiro TF, Kontopoulos E, Altmann SM, Kufareva I, Strathearn KE, Amore AM, Volk CB, Maxwell MM, Rochet JC, McLean PJ, Young AB, Abagyan R, Feany MB, Hyman BT, Kazantsev AG. Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of parkinson's disease. *Science* 2007;317:516–519. [PubMed: 17588900]
243. Morrison BE, Majdzadeh N, D'Mello SR. Histone deacetylases: Focus on the nervous system. *Cell Mol Life Sci* 2007;64:2258–2269. [PubMed: 17530170]
244. Ono K, Yamada M. Antioxidant compounds have potent anti-fibrillogenic and fibril-destabilizing effects for alpha-synuclein fibrils in vitro. *J Neurochem* 2006;97:105–115. [PubMed: 16524383]
245. Uversky VN, Li J, Souillac P, Millett IS, Doniach S, Jakes R, Goedert M, Fink AL. Biophysical properties of the synucleins and their propensities to fibrillate: Inhibition of alpha-synuclein assembly by beta- and gamma-synucleins. *J Biol Chem* 2002;277:11970–11978. [PubMed: 11812782]
246. Hashimoto M, Rockenstein E, Mante M, Crews L, Bar-On P, Gage FH, Marr R, Masliah E. An antiaggregation gene therapy strategy for lewy body disease utilizing beta-synuclein lentivirus in a transgenic model. *Gene Ther* 2004;11:1713–1723. [PubMed: 15483670]
247. Messer A, McLearn J. The therapeutic potential of intrabodies in neurologic disorders: Focus on huntington and parkinson diseases. *BioDrugs* 2006;20:327–333. [PubMed: 17176119]
248. Haidaris CG, Malone J, Sherrill LA, Bliss JM, Gaspari AA, Insel RA, Sullivan MA. Recombinant human antibody single chain variable fragments reactive with candida albicans surface antigens. *J Immunol Methods* 2001;257:185–202. [PubMed: 11687252]
249. Malone J, Sullivan MA. Analysis of antibody selection by phage display utilizing anti-phenobarbital antibodies. *J Mol Recognit* 1996;9:738–745. [PubMed: 9174966]
250. Emadi S, Barkhordarian H, Wang MS, Schulz P, Sierks MR. Isolation of a human single chain antibody fragment against oligomeric alpha-synuclein that inhibits aggregation and prevents alpha-synuclein-induced toxicity. *J Mol Biol* 2007;368:1132–1144. [PubMed: 17391701]
251. Emadi S, Liu R, Yuan B, Schulz P, McAllister C, Lyubchenko Y, Messer A, Sierks MR. Inhibiting aggregation of alpha-synuclein with human single chain antibody fragments. *Biochemistry* 2004;43:2871–2878. [PubMed: 15005622]
252. Lynch SM, Zhou C, Messer A. An scfv intrabody against the nonamyloid component of alpha-synuclein reduces intracellular aggregation and toxicity. *J Mol Biol* 2008;377:136–147. [PubMed: 18237741]
253. Zhou C, Emadi S, Sierks MR, Messer A. A human single-chain fv intrabody blocks aberrant cellular effects of overexpressed alpha-synuclein. *Mol Ther* 2004;10:1023–1031. [PubMed: 15564134]
254. Giasson BI, Murray IV, Trojanowski JQ, Lee VM. A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. *J Biol Chem* 2001;276:2380–2386. [PubMed: 11060312]

255. Wuertzer CA, Sullivan MA, Qiu X, Federoff HJ. CNS delivery of vectored prion-specific single-chain antibodies delays disease onset. *Mol Ther* 2008;16:481–486. [PubMed: 18180775]
256. Fortin DL, Nemani VM, Voglmaier SM, Anthony MD, Ryan TA, Edwards RH. Neural activity controls the synaptic accumulation of alpha-synuclein. *J Neurosci* 2005;25:10913–10921. [PubMed: 16306404]
257. Lee HJ, Patel S, Lee SJ. Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *J Neurosci* 2005;25:6016–6024. [PubMed: 15976091]