Published in final edited form as:
Nat Rev Neurosci. 2011 June ; 12(6): 359–366. doi:10.1038/nrn3039.

Ageing as a primary risk factor for Parkinson's disease: evidence from studies of non-human primates

Timothy J. Collier,
Division of Translational Science and Molecular Medicine, Michigan State University, Grand Rapids, Michigan 49503, USA. Center of Excellence in Parkinson's Disease Research, Michigan State University, Grand Rapids, Michigan 49503, USA

Nicholas M. Kanaan, and
Division of Translational Science and Molecular Medicine, Michigan State University, Grand Rapids, Michigan 49503, USA

Jeffrey H. Kordower
Department of Neurological Sciences, Rush University Medical Center, Chicago, Illinois 60612, USA

Abstract

Ageing is the greatest risk factor for the development of Parkinson's disease. However, the current dogma holds that cellular mechanisms that are associated with ageing of midbrain dopamine neurons and those that are related to dopamine neuron degeneration in Parkinson's disease are unrelated. We propose, based on evidence from studies of non-human primates, that normal ageing and the degeneration of dopamine neurons in Parkinson's disease are linked by the same cellular mechanisms and, therefore, that markers of cellular risk factors accumulate with age in a pattern that mimics the pattern of degeneration observed in Parkinson's disease. We contend that ageing induces a pre-parkinsonian state, and that the cellular mechanisms of dopamine neuron demise during normal ageing are accelerated or exaggerated in Parkinson's disease through a combination of genetic and environmental factors.

Based on epidemiological studies, ageing is acknowledged to be the greatest risk factor for developing Parkinson's disease[1–3]. However, early studies that compared the ageing-associated pattern of neurochemical and morphological changes in dopamine neurons with the pattern associated with Parkinson's disease led to the conclusion that the underlying mechanisms of the two are distinct and unrelated[4–7]. These seminal studies were based on histological and neurochemical techniques available at the time, which lacked methods for accurately quantifying cell numbers and expression of immunocytochemical markers, and were not informed by current notions of the factors that are likely to have a role in dopamine neuron degeneration in Parkinson's disease.

Correspondence to: T.J.C. timothy.collier@hc.msu.edu.
T.J.C. and N.M.K. contributed equally to this work.

Competing interests statement
J.H.K. declares competing financial interests: see web version for details. The remaining authors declare no competing financial interests.

FURTHER INFORMATION
Timothy J. Collier's homepage: http://translationalscience.msu.edu/index.html
ALL LINKS ARE ACTIVE IN THE ONLINE PDF

In recent studies we have revisited the relationship between ageing and Parkinson's disease, and have focused on an important characteristic of the disease: the fact that some midbrain dopamine neurons are more vulnerable to degeneration than other midbrain dopamine neurons. In this regard, the loss of dopamine neurons occurs with a similar pattern of vulnerability and resistance in patients with Parkinson's disease[4,5,8], non-human primate 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP) models of Parkinson's disease[9–12] and rodent toxin models of Parkinson's disease[13–15]. Dopamine neurons of the ventral tier of the substantia nigra (vtSN) are the most vulnerable, neurons of the dorsal tier of the substantia nigra (dtSN) are more resistant and dopamine neurons of the ventral tegmental area (VTA) are the most resistant to degeneration (FIG. 1). This difference is apparent even though these cells form a continuous sheet of dopamine neurons that occupy the ventral midbrain and merge seamlessly with one another.

In this Perspective, we review a series of studies from our laboratories that support the hypothesis that ageing and Parkinson's disease are related at the level of cellular mechanisms, and that markers of likely contributors to dopamine neuron demise in Parkinson's disease accumulate with age in a region-specific pattern. These include α-synuclein, markers of the lysosome and proteasome systems, markers of oxidative and nitrative stress, dopamine transporters and glia. Levels of these markers were measured in midbrain tissue from a cohort of rhesus monkeys ranging in age from 2 to 34 years.

Dopamine neuron degeneration patterns

The rhesus monkey is an important model of human biology and has been used extensively in studies of ageing and neurodegenerative diseases. Previous studies of non-human primates have documented ageing-related changes in midbrain dopamine neurons that correlate with declining motor function, similar to those seen in ageing humans[16,17]. However, little attention has been paid to regional differences in these changes among midbrain dopamine neurons.

In a series of studies[18–22], two cohorts of rhesus monkeys were used. Monkeys in the first cohort ranged in age from 9 to 29 years and were categorized into young adult (9 to 10 years of age), middle-aged (14 to 17 years of age) and aged (22 to 29 years of age) subjects. Anatomical studies indicate that these monkeys age approximately three times faster than humans[23]. Thus, these studies model humans aged from 27 to 87 years. Monkeys in the second cohort were aged 2 to 34 years (equivalent to humans aged 6 to 102 years).

To determine whether midbrain dopamine neurons in ageing non-human primates exhibit changes in neuron number and/or phenotype that are analogous to the regional differences in susceptibility to degeneration that is seen in Parkinson's disease, tyrosine hydroxylase was used as a marker for the dopamine neuron phenotype. Stereological counts of tyrosine hydroxylase immunoreactive (THir) neurons in vtSN, dtSN and VTA at different ages[19] revealed that the number of THir neurons was negatively correlated with age only in the vtSN (FIG. 2a–d). In addition, the intensity of THir staining decreased with age in all three midbrain dopamine regions, and the resulting tyrosine hydroxylase intensity followed the pattern vtSN < dtSN < VTA. It should be acknowledged that identification of dopamine neurons by staining for tyrosine hydroxylase does not distinguish a change in phenotype from overt cell loss. In fact, other studies[24–27] using different immunostaining protocols or additional markers have not found loss of dopamine neurons in the ageing non-human primate midbrain. However, decreased expression of a primary marker of dopamine neuron phenotype (and, by extension, of dopamine neuron function) during ageing follows a regional pattern identical to that seen for overt loss of dopamine neurons in Parkinson's disease[4,5,8].

Distribution pattern of cellular changes

Selected histological markers of cellular and molecular processes that have been implicated in dopamine neuron degeneration in Parkinson's disease also show age-related and region-specific changes in distribution in rhesus monkeys. These markers include α-synuclein, ubiquitin as a marker of the proteasome system, lipofuscin as a marker of the lysosome system, 3-nitrotyrosine (3NT) as a marker of oxidative and nitrative stress (along with its relationship to the sodium-dependent dopamine transporter (DAT) and the synaptic vesicular amine transporter (VMAT2)), and glial fibrillary acidic protein (GFAP) and HLA class II histocompatibility antigen, DR α-chain (HLA-DRA) as markers of resident astrocytes and microglia, respectively.

α-synuclein

α-synuclein is a major constituent of Lewy bodies, the cytoplasmic ubiquitin-positive inclusions in the midbrain that are diagnostic for most forms of Parkinson's disease and that occur in all individuals with the sporadic form of the disease[28,29]. Associations between mutations in the gene encoding α-synuclein (*PARK1*) and Parkinson's disease provided the first evidence for the existence of genetic forms of Parkinson's disease[29,30]. Duplication or triplication of wild-type *PARK1* is also associated with Parkinson's disease[31]. α-synuclein increases in the substantia nigra during normal human ageing, but is rarely present in inclusions typical of Parkinson's disease[18,32,33].

A comparison of the pattern of α-synuclein immunoreactivity in THir neurons in the substantia nigra and VTA in ageing rhesus monkeys[18] revealed that both the number of THir substantia nigra neurons showing α-synuclein staining and the intensity of α-synuclein staining in these neurons increased dramatically with age (FIG. 2e,f). Moreover, there was an ageing-related shift in staining for α-synuclein, from primarily punctate staining in the neuropil of young adult subjects, to light cytoplasmic staining in middle-aged subjects, to intense cytoplasmic staining in aged subjects[18]. In addition, substantia nigra neurons showing α-synuclein staining exhibited a substantial decrease in tyrosine hydroxylase immuno-reactivity in ageing monkeys[18], suggesting that accumulation of α-synuclein may contribute to ageing-related declines in dopamine neuron phenotype. These effects were specific for α-synuclein as there were no ageing-related changes in β-synuclein staining in the substantia nigra. By contrast, there was negligible staining for α-synuclein in VTA neurons[18]. Indeed, converging lines of evidence indicate that excess α-synuclein may be associated with selective dopamine neuron degeneration, as viral overexpression of α-synuclein in the substantia nigra induces neuron degeneration in this region, whereas overexpression in the adjacent VTA does not[34]. Thus, the accumulation of cytoplasmic α-synuclein in ageing monkeys was specific for dopamine neurons in the substantia nigra.

Proteasome and lysosome systems—One genetic form of Parkinson's disease involves mutation of a gene associated with the ubiquitin–proteasome system: parkinson protein 2, E3 ubiquitin protein ligase (*PARK2*; also known as *PARKIN*)[35]. The ubiquitylation of α-synuclein within Lewy bodies suggests a connection with the proteasome, and α-synuclein is a known substrate of chaperone-mediated autophagy involving the lysosome[36–38]. Furthermore, viral overexpression of α-synuclein in the rat substantia nigra is associated with reductions in structural and functional markers of the lysosome and proteosome systems specifically in neurons that have been successfully transfected. A reduction in similar markers is also seen in patients with Parkinson's disease[39]. Thus, the synucleinopathy associated with Parkinson's disease may be a product of impaired processing of abnormal forms of α-synuclein and/or abnormal levels of α-synuclein by the intracellular proteasome and lysosome systems.

Abundant evidence also implicates oxidative stress in Parkinson's disease pathology[40,41]. Dysfunctional mitochondria are the primary intracellular source of damaging reactive oxygen species and lysosome-mediated autophagy is the primary cellular mechanism responsible for removing defective mitochondria[42,43]. The autofluorescent pigment lipofuscin accumulates during ageing in many brain regions, leading to its characterization as 'age pigment'. This accumulation is thought to reflect increasing age-related mitochondrial damage and subsequent degradation of mitochondria by the lysosome system[44,45].

Ageing non-human primates do not accumulate ubiquitin-positive cytoplasmic inclusions like the Lewy bodies that are found in most forms of Parkinson's disease. However, ageing monkeys[46] and humans[47,48] do accumulate intranuclear ubiquitin-positive inclusions known as Marinesco bodies. Marinesco bodies are also present in Lewy body-containing dopamine neurons in Parkinson's disease[47]. In rhesus monkeys, Marinesco bodies accumulated with advancing age specifically in THir neurons of the vtSN[19] (FIG. 2g,h), so that the percentage of THir neurons in vtSN of aged monkeys that contained Marinesco bodies was higher than that in the dtSN and VTA[19]. Moreover, vtSN neurons containing Marinesco bodies in middle-aged monkeys exhibited a premature decline in the intensity of tyrosine hydroxylase immunoreactivity, equivalent to that seen in aged monkeys. Indeed, aged vtSN neurons exhibiting multiple, or large, Marinesco bodies often had a complete absence of tyrosine hydroxylase immunoreactivity, although they remained identifiable by their normal expression of neuromelanin[19]. These findings are consistent with the view that the nuclear ubiquitin–proteasome system becomes progressively less efficient, or dysfunctional, with advancing age. This is reflected at least in part by the accumulation of Marinesco bodies, is associated with decreased expression of tyrosine hydroxylase and occurs with the highest frequency in neurons of the vtSN.

By contrast, the distribution of lipofuscin followed an opposite pattern. Contrary to the historical characterization of lipofuscin as age pigment[49], it is expressed in midbrain dopamine neuron regions even in young adult monkeys, and the number of lipofuscin-containing dopamine neurons does not increase with age. The highest percentage of THir neurons containing lipofuscin was in the degeneration-resistant neurons of the dtSN and VTA, and lipofuscin was relatively absent in dopamine neurons of the vtSN[19] (FIG. 2i,j). This finding was surprising, and inconsistent with the view that accumulation of lipofuscin is a precursor to cell death[42,43]. Whether lipofuscin is neuroprotective or neurodestructive remains to be conclusively determined, but there is evidence that increased lysosome activity and lipofuscin accumulation are associated with neurons that survive in Parkinson's disease and animal models of the disease. Surviving neurons in the nucleus basalis of Meynert in patients with Parkinson's disease have greater amounts of lipofuscin compared with age-matched controls[50], and surviving neurons in the substantia nigra of MPTP-treated monkeys[51] and in MPTP and probenicid treated mice[52] show increased levels of lipofuscin compared with controls. Analysis also indicated that, in middle-aged monkeys, dtSN neurons that contained lipofuscin had THir intensity similar to that of young adult dtSN neurons, and these neurons were seemingly resistant to the ageing-related decrease in expression of this phenotypic marker[19]. Taken together, higher expression of lipofuscin in midbrain dopamine neuron regions that are resistant to degeneration, and its association with youthful THir intensity, suggest that active autophagic removal of defective organelles and proteins from cells has a neuroprotective function during ageing.

Oxidative stress, nitrative stress and dopamine transporters

In addition to the oxidative damage caused by mitochondrial dysfunction, dopamine neurons are exposed throughout their lifespan to reactive oxygen and nitrogen species from the metabolism of cytosolic dopamine[53,54]. The main cellular defence against such metabolic

by-products is the recapture of dopamine released into the synapse by DAT, followed by sequestration into synaptic vesicles by VMAT2. However, if this process is not efficient, cytosolic dopamine can produce H_2O_2 and OH^- that can damage cells[55,56]. Accordingly, triple-label immunocytochemistry was performed to examine the relationship between the expression of DAT and VMAT2 and the presence of 3NT — a marker for oxidative and nitrative damage — in midbrain dopamine neuron regions of ageing monkeys[20].

The number of 3NT immunoreactive (3NTir) neurons, expressed as a percentage of THir neurons, in the three midbrain subregions increased with age and was greatest in the vtSN, and the intensity of 3NTir increased with age specifically in the vtSN[20] (FIG. 2k,l). The age-related increase in the number of 3NT-expressing vtSN neurons was equivalent to the age-related decrease in the number of tyrosine hydroxylase- expressing vtSN neurons, and 3NT was expressed before there was loss of tyrosine hydroxylase-expressing cells. Thus, the increase in 3NT expression precedes the decrease in tyrosine hydroxylase expression. This suggests that vtSN dopamine neurons experience the greatest levels of oxidative stress, even early in the lifespan before tyrosine hydroxylase loss, and are particularly vulnerable to oxidative and nitrative stress.

Staining for markers of the dopamine transporters varied less between regions and with age. The intensity of staining for DAT was greatest in the vtSN and this intensity did not change with ageing. By contrast, an ageing-related decline in DAT staining was detected in dtSN neurons and in VTA neurons (although this DAT decline in VTA neurons did not reach statistical significance). VMAT2 staining was also most intense in vtSN neurons and did not change with age in any of the midbrain regions. Triple labelling for 3NT, DAT and VMAT2 revealed that the intensity of staining for both transporters correlated with the intensity of staining for 3NT in individual neurons of all three regions[20].

Taken together, these findings support the view that the capacity for accumulation of cytosolic dopamine (that is, the level of DAT) in vtSN neurons — which is higher than that in dtSN and VTA neurons, and does not diminish with age — is correlated with accumulation of oxidative and nitrative damage as reflected by 3NT staining in aged subjects. It remains to be determined whether the expression of VMAT2 in vtSN neurons is not fully efficient in protecting against metabolism of intracellular dopamine, or whether additional sources of oxidative stress, including defective mitochondria, account for the higher levels of oxidative damage in these cells.

Glial environment

In addition to intrinsic cellular contributors to dopamine neuron degeneration, it has been postulated that extrinsic factors in the environment of dopamine neurons also contribute to the pathogenesis of Parkinson's disease. Primary among these are the resident glial cells. Evidence for changes in the number or reactivity of astrocytes (as measured by GFAP immunoreactivity (GFAPir)) in post-mortem brains of Parkinson's disease patients is inconsistent[57,58]. However, the ventral midbrain of patients with Parkinson's disease consistently shows increased microglial reactivity and the presence of markers of inflammation[59–61], and in animal models of Parkinson's disease this microglial response has been shown to contribute to dopamine neuron degeneration[62,63]. To examine this component of the environment, an analysis was carried out of the number, staining intensity and morphology of astrocytes (based on GFAPir) and microglia (based on HLA-DRA immunoreactivity (HLA-DRAir)) in the vtSN, dtSN and VTA of ageing monkeys[21,22].

There were no regional changes in GFAPir cell numbers with advancing age. Characteristics of astrocyte activation — such as increased staining for GFAP and increased number and thickness of astrocyte processes — revealed an inverted U-shaped relationship with age:

activation increased from young adult to middle-age and declined again in advanced age in all regions. HLA-DRAir microglia also showed no change in cell number with advancing age in any of the regions. However, characteristics of activation of these cells — increased HLA-DRA staining intensity, decreased process ramification, increased thickness of processes and clustering of macrophage-like cells — preferentially occurred in the vtSN and increased with advancing age (FIG. 2m,n). This is consistent with the view that the vtSN enters into a chronic and increasing state of inflammation that probably contributes to the vulnerability of the dopamine neurons to degeneration with advancing age.

Caveats and technical issues

The conclusions drawn from our observations must be evaluated in the context of the source material: tissue from non-human primates. Monkeys are not humans, and Parkinson's disease is a human condition. What cannot be disputed is that monkeys represent the closest phylogenetic model to humans when compared with other models commonly used in CNS research. Although in our opinion the analogy of non-human primate biology to human biology is a model that has yielded valuable insights, the limitations of the model must be acknowledged, and opinions on the value of the comparison vary widely. In the present context, several recent studies indicate that ageing humans, like ageing monkeys, exhibit little or no overt loss of substantia nigra dopamine neurons[64–66]. Accordingly, this disparity between ageing and Parkinson's disease is shared by monkeys and humans. Furthermore, reports characterizing the motor behaviours of ageing monkeys indicate that aged monkeys exhibit behavioural signs that are consistent with the early stages of parkinsonism[16,17,67].

It should be emphasized that markers of cellular and molecular processes that have been implicated in dopamine neuron degeneration in Parkinson's disease were present in all three midbrain dopamine subregions of ageing monkeys. It is the relative differences in expression of these markers in these regions as a function of ageing that lead to our conclusions regarding the possible role of these cellular and molecular processes in the selective vulnerability of midbrain dopamine neurons.

Regionally selective dopamine neuron dysfunction and degeneration is a multi-faceted process, and the studies reviewed above did not examine all of the factors involved. Indeed, evidence exists for the involvement of disturbed calcium homeostasis[68–70], impaired mitochondrial function[71,72], axonal transport dysfunction[73,74], reduced growth factor availability and/or efficacy[75–77] and other factors[78–85] in the degeneration of dopamine neurons. The majority of these factors remain to be examined in the non-human primate model of human ageing.

Another caveat is that the tissue used in these studies was paraformaldehyde fixed and therefore could not be subjected to many common biochemical and molecular analyses. Future studies of these mechanisms are warranted and will be of great interest.

The inclusion of middle-aged subjects in the studies reviewed here has provided important information, and this supports the contention that inclusion of middle-aged subjects is appropriate and necessary in the design of studies on mechanisms of ageing[86]. It has been postulated that middle age represents a transitional stage between young and old age, and that including samples from middle-aged subjects allows one to determine whether changes associated with ageing occur early or late. Indeed, a study examining the effects of MPTP in young, middle-aged and aged monkeys showed that a failure to exhibit compensatory increases in dopamine turnover following MPTP injection begins to occur in some middle-aged animals, whereas all aged monkeys fail to compensate[24]. In the studies reviewed here, the number of dopamine neurons accumulating Marinesco bodies increased dramatically between middle age and old age in the vtSN, and neurons that accumulated Marinesco

bodies in middle age showed premature declines in tyrosine hydroxylase immunoreactivity. Neurons of the dtSN that accumulated lipofuscin in middle age maintained youthful expression of tyrosine hydroxylase. Midbrain astrocytes exhibited increased activation in middle age that receded in old age. These changes would not have been appreciated if middle-aged subjects had not been included in our studies.

Finally, the fact that the studies reviewed here used tissue from the same subjects in multiple analyses has increased our understanding of changes that occur simultaneously. For example, in neurons of the aged vtSN, which are vulnerable to degeneration, the findings indicate that reduced tyrosine hydroxylase immunoreactivity is associated with accumulation of Marinesco bodies, diminished lysosome activity, a higher capacity to accumulate cytosolic dopamine, excessive oxidative and nitrative damage, and increased inflammation. By contrast, dopamine neurons in midbrain regions that are resistant to degeneration have fewer ubiquitin-positive inclusions, higher lysosome activity, a lower capacity to accumulate cytosolic dopamine, lower levels of oxidative and nitrative damage, and experience less inflammation. This convergence of multiple markers in vulnerable versus degeneration-resistant dopamine neurons presents an interesting foundation for future studies that seek to determine whether dopamine neuron dysfunction occurring in ageing and disease is a product of multiple, parallel processes that may combine in unpredictable ways, or is a cascade emanating from a single trigger.

Rate of progression of Parkinson's disease

Although these studies provide information regarding the link between ageing of the nigrostriatal system and the pathogenesis of Parkinson's disease, they also have implications for the differences in disease progression between patients that experience early-onset Parkinson's disease (EOPD) and late-onset Parkinson's disease (LOPD). EOPD is typically defined by onset before age 45–50, and is characterized by a tremor-predominant syndrome and more rapid development of L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesias[87]. By contrast, LOPD is typically defined as having onset later than 70 years of age. Patients with LOPD tend to have a postural instability gait disorder and a lower susceptibility to develop L-DOPA-induced dyskinesias[87]. Importantly, EOPD patients typically exhibit a slower rate of disease progression, whereas LOPD patients have more rapid progression and more severe motor impairments. The data discussed above support the view that the accumulation of harmful changes during normal ageing precipitates the accelerated progression of dopamine neuron degeneration in LOPD[88–90]. In addition, we have proposed that the faster progression of LOPD may be the result of the failure of older nigral neurons to generate meaningful compensatory mechanisms[24].

A stochastic acceleration hypothesis

The clinically silent, presymptomatic progression of Parkinson's disease makes it difficult to study pathogenic mechanisms of dopamine neuron degeneration in the disease. Ageing is the strongest risk factor for developing Parkinson's disease, and by inference this suggests that changes in nigrostriatal dopamine neurons during normal ageing are related to those contributing to the pathogenesis of Parkinson's disease[91,92]. However, the association between ageing and Parkinson's disease has largely been based on increased incidence of Parkinson's disease diagnosis with advancing age, a finding that is consistent across geographic, cultural and ethnic boundaries[1–3]. Now, an increasing number of commonalities, ranging from molecular to functional, have been identified for changes in dopamine neurons that occur in normal ageing and those that occur in Parkinson's disease (TABLE 1). It is our contention that the combined analysis of changes occurring in dopamine systems during normal ageing — specifically those that vary between midbrain

dopamine neurons that are vulnerable versus those that are resistant to degeneration — in a species closely related to humans can provide meaningful insights into the pathogenesis of Parkinson's disease.

Despite the earlier assumptions that ageing and Parkinson's disease are not related by cellular mechanisms[4–7], conceptual models of Parkinson's disease commonly incorporate a connection between ageing and Parkinson's disease pathogenesis[93–95]. For example, Calne and Langston proposed that Parkinson's disease develops from an insult that results in degeneration of dopamine neurons to such an extent that it alters the normal course of age-related dopamine neuron dysfunction to reach a threshold for Parkinson's disease symptoms[93]. The 'multiple hit hypothesis' proposes that several life events (for example, environmental insults, prenatal infections and genetic predispositions) acting in sequence, perhaps separated by years, accelerate the ageing-related decline in dopamine neuron function, resulting in Parkinson's disease[94,95].

Although these models postulate that one or more biological insults alter the trajectory of the natural ageing-related decline in dopamine neuron function, they do not provide evidence implicating specific cellular mechanisms whose markers are simultaneously present and increase with ageing in dopamine neurons with the greatest vulnerability to degeneration. Furthermore, the models do not propose how these mechanisms may combine to produce Parkinson's disease. Here, we propose a revised hypothesis that largely agrees with prior hypotheses, but incorporates two findings: first, that the cellular mechanisms underlying vulnerability to decreased dopamine neuron function in ageing and the overt degeneration of these neurons in Parkinson's disease are fundamentally the same; and second, that the sequence, combination and magnitude of these cellular changes varies between patients. We term this hypothesis the 'stochastic acceleration hypothesis' (FIG. 3). According to this hypothesis, age-related changes in the nigrostriatal dopamine system are the biological foundation for Parkinson's disease. Thus, the cellular events that contribute to normal ageing of substantia nigra dopamine neurons are fundamentally the same as those underlying the development and progression of Parkinson's disease. Indeed, these processes are identical regardless of whether they are precipitated by ageing, environmental toxins, prenatal infections or genetic predispositions. Our hypothesis suggests that these cellular changes exist along a continuum in which ageing actively produces a vulnerable pre-parkinsonian state that, when exaggerated or accelerated by a combination of genetic and environmental factors (which can differ between individuals), results in the Parkinson's disease phenotype. Furthermore, the altered cellular mechanisms can interconnect in multiple ways and in patient-specific patterns, fulfilling the basic definition of 'stochastic'; thus, this model incorporates elements of randomness that produce the same outcome: dopamine neuron dysfunction and, eventually, degeneration.

One corollary of this model is the question of whether it is inevitable that every individual would eventually develop Parkinson's disease. Here, the model predicts both outcomes. It suggests that individuals who outlive the normal functioning of their dopamine system will inevitably develop Parkinson's disease. But the model also suggests that elements of lifestyle and genetics that promote successful ageing will decrease the incidence of Parkinson's disease in the general population. In addition, the model suggests that there is no single target to cure Parkinson's disease. The most effective treatments will target the multiple mechanisms contributing to dopamine neuron dysfunction, tailored to each patient's specific form of the disease.

Acknowledgments

We are grateful for the dedication and effort of all members of our investigative teams and the generous support provided by the US National Institutes of Health (NIH) awards AG10851 and NS58830 (to the Udall Center of Excellence in Parkinson's Disease Research at Michigan State University, USA).

Glossary

Lipofuscin	Autofluorescent lipid-containing residues of lysosomal digestion that accumulate in many tissues of the body with advancing age and have been termed 'age pigment'
Neuromelanin	A modified form of melanin pigment found in dopamine neurons of the substantia nigra
Probenicid	An adjuvant that, when co-administered with MPTP, blocks rapid clearance of the toxin and its metabolites, producing a progressive rodent model of parkinsonism
Synucleinopathy	An abnormal structure or quantity of α-synuclein that disrupts the function of cells

References

1. Bennett DA, et al. Prevalence of parkinsonian signs and associated mortality in a community population of older people. N Engl J Med. 1996; 334:71–76. [PubMed: 8531961]
2. Morens DM, et al. Epidemiologic observations on Parkinson's disease: incidence and mortality in a prospective study of middle aged men. Neurology. 1996; 46:1044–1050. [PubMed: 8780088]
3. Tanner CM, Goldman SM. Epidemiology of Parkinson's disease. Neurol Clin. 1996; 14:317–335. [PubMed: 8827174]
4. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain. 1991; 114:2283–2301. [PubMed: 1933245]
5. Gibb WR, Lees AJ. Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatr. 1991; 54:388–396. [PubMed: 1865199]
6. Hornykiewicz O. Ageing and neurotoxins as causative factors in idiopathic Parkinson's disease – a critical analysis of the neurochemical evidence. Prog Neuropsychopharmacol Biol Psychiatry. 1989; 13:319–328. [PubMed: 2664888]
7. Kish SJ, Shannak K, Rajput A, Deck JH, Hornykiewicz O. Aging produces a specific pattern of striatal dopamine loss: implications for the etiology of idiopathic Parkinson's disease. J Neurochem. 1992; 58:642–648. [PubMed: 1729408]
8. Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain. 1999; 122:1437–1448. [PubMed: 10430830]
9. Chiueh CC, Burns RS, Markey SP, Jacobowitz DM, Kopin IJ. Primate model of parkinsonism: selective lesion of nigrostriatal neurons by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine produces an extrapyramidal syndrome in rhesus monkeys. Life Sci. 1985; 36:213–218. [PubMed: 3871241]
10. German DC, Dubach M, Askari S, Speciale SG, Bowden DM. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonian syndrome in *Macaca fascicularis*: which midbrain dopaminergic neurons are lost? Neuroscience. 1988; 24:161–174. [PubMed: 3259295]
11. Kitt CA, Cork LC, Eidelberg F, Joh TH, Price DL. Injury of nigral neurons exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: a tyrosine hydroxylase immuunocytochemical study in monkey. Neuroscience. 1986; 17:1089–1103. [PubMed: 2872615]
12. Schnieder JS, Yuwiler A, Markham CH. Selective loss of subpopulations of ventral mesencephalic dopaminergic neurons in the monkey following exposure to MPTP. Brain Res. 1987; 411:144–150. [PubMed: 2886180]

13. Betarbet R, et al. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nature Neurosci. 2000; 3:1301–1306. [PubMed: 11100151]
14. German DC, et al. The neurotoxin MPTP causes degeneration of specific nucleus A8, A9 and A10 dopaminergic neurons in the mouse. Neurodegeneration. 1996; 5:299–312. [PubMed: 9117541]
15. Grant RJ, Clarke PB. Susceptibility of ascending dopamine projections to 6-hydroxydopamine in rats: effect of hypothermia. Neuroscience. 2002; 115:1281–1294. [PubMed: 12453497]
16. Emborg ME, et al. Age-related declines in nigral neuronal function correlate with motor impairments in rhesus monkeys. J Comp Neurol. 1998; 401:253–265. [PubMed: 9822152]
17. Zhang Z, et al. Motor slowing and parkinsonian signs in aging rhesus monkeys mirror human aging. J Gerontol A Biol Sci Med Sci. 2000; 55:B473–B480. [PubMed: 11034220]
18. Chu Y, Kordower JH. Age-associated increases of α-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: is this the target for Parkinson's disease? Neurobiol Dis. 2007; 25:134–149. [PubMed: 17055279]
19. Kanaan NM, Kordower JH, Collier TJ. Age-related accumulation of Marinesco bodies and lipofuscin in rhesus monkey midbrain dopamine neurons: relevance to selective neuronal vulnerability. J Comp Neurol. 2007; 502:683–700. [PubMed: 17436290]
20. Kanaan NM, Kordower JH, Collier TJ. Age-related changes in dopamine transporters and accumulation of 3-nitrotyrosine in rhesus monkey midbrain dopamine neurons: relevance in selective neuronal vulnerability to degeneration. Eur J Neurosci. 2008; 27:3205–3215. [PubMed: 18598263]
21. Kanaan NM, Kordower JH, Collier TJ. Age-related changes in glial cells of dopamine midbrain subregions in rhesus monkeys. Neurobiol Aging. 2008; 31:937–952. [PubMed: 18715678]
22. Kanaan NM, Kordower JH, Collier TJ. Age and region-specific responses of microglia, but not astrocytes, suggest a role in selective vulnerability of dopamine neurons after 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine exposure in monkeys. Glia. 2008; 56:1199–1214. [PubMed: 18484101]
23. Andersen AH, Zhang Z, Zhang M, Gash DM, Avison MJ. Age-associated changes in rhesus CNS composition identified by MRI. Brain Res. 1999; 829:90–98. [PubMed: 10350533]
24. Collier TJ, et al. Aging-related changes in the nigrostriatal dopamine system and the response to MPTP in nonhuman primates: diminished compensatory mechanisms as a prelude to parkinsonism. Neurobiol Dis. 2007; 26:56–65. [PubMed: 17254792]
25. Irwin I, et al. Aging and the nigrostriatal dopamine system: a non-human primate study. Neurodegeneration. 1994; 3:251–265. [PubMed: 7531106]
26. McCormack AL, et al. Aging of the nigrostriatal system in the squirrel monkey. J Comp Neurol. 2004; 471:387–395. [PubMed: 15022260]
27. Pakkenberg H, Andersen BB, Burns RS, Pakkenberg B. A stereological study of substantia nigra in young and old rhesus monkeys. Brain Res. 1995; 693:201–206. [PubMed: 8653409]
28. Irizarry MC, et al. Nigral and cortical Lewy bodies and dystrophic nigral neurites in Parkinson's disease and cortical Lewy body disease contain α-synuclein immunoreactivity. J Neuropathol Exp Neurol. 1998; 57:334–337. [PubMed: 9600226]
29. Spillantini MG, et al. α-synuclein in Lewy bodies. Nature. 1997; 388:839–840. [PubMed: 9278044]
30. Polymeropoulos MH, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science. 1997; 276:2045–2047. [PubMed: 9197268]
31. Ross OA, et al. Genomic investigation of α-synuclein multiplication and parkinsonism. Ann Neurol. 2008; 63:743–750. [PubMed: 18571778]
32. Jellinger KA. Lewy body-related α-synucleinopathy in the aged human brain. J Neural Transm. 2004; 111:1219–1235. [PubMed: 15480835]
33. Li W, et al. Stabilization of α-synuclein protein with ageing and familial Parkinson's disease-linked A53T mutation. J Neurosci. 2004; 24:7400–7409. [PubMed: 15317865]
34. Maingay M, Romero-Ramos M, Carta M, Kirik D. Ventral tegmental area dopamine neurons are resistant to human mutant α-synuclein overexpression. Neurobiol Dis. 2006; 23:522–532. [PubMed: 16806952]

35. Shimura H, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nature Genet. 2000; 25:302–305. [PubMed: 10888878]
36. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. Science. 2004; 305:1292–1295. [PubMed: 15333840]
37. Lee HJ, Khoshaghideh F, Patel S, Lee SJ. Clearance of α-synuclein oligomeric intermediates via the lysosomal degradation pathway. J Neurosci. 2004; 24:1888–1896. [PubMed: 14985429]
38. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. α-synuclein is degraded by both autophagy and the proteasome. J Biol Chem. 2003; 278:25009–25013. [PubMed: 12719433]
39. Chu Y, Dodiya H, Aebischer P, Olanow CW, Kordower JH. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to α-synuclein inclusions. Neurobiol Dis. 2009; 35:385–398. [PubMed: 19505575]
40. Hald A, Lotharius J. Oxidative stress and inflammation in Parkinson's disease: is there a causal link? Exp Neurol. 2005; 193:279–290. [PubMed: 15869932]
41. Jenner P. Oxidative stress in Parkinson's disease. Ann Neurol. 2003; 53:S26–S36. [PubMed: 12666096]
42. Brunk UT, Terman A. The mitochondrial–lysosomal axis theory of aging: accumulation of damaged mitochondria as a result of imperfect autophagocytosis. Eur J Biochem. 2002; 269:1996–2002. [PubMed: 11985575]
43. Brunk UT, Terman A. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. Free Radic Biol Med. 2002; 33:611–619. [PubMed: 12208347]
44. Terman A, Brunk UT. Lipofuscin: mechanisms of formation and increase with age. APMIS. 1998; 106:265–276. [PubMed: 9531959]
45. Terman A, Gustafsson B, Brunk UK. Mitochondrial damage and intralysosomal degradation in cellular aging. Mol Aspects Med. 2006; 27:471–482. [PubMed: 16973208]
46. Siddiqi ZA, Peters A. The effect of aging on pars compacta of the substantia nigra in rhesus monkey. J Neuropathol Exp Neurol. 1999; 58:903–920. [PubMed: 10499434]
47. Beach TG, et al. Substantia nigra Marinesco bodies are associated with decreased striatal expression of dopaminergic markers. J Neuropathol Exp Neurol. 2004; 63:329–337. [PubMed: 15099023]
48. Yuen P, Baxter DW. The morphology of Marinesco bodies (paranucleolar corpuscles) in the melanin-pigmented nuclei of the brainstem. J Neurol Neurosurg Psychiatr. 1963; 26:178–183. [PubMed: 14002893]
49. Strehler BL. On the histochemistry and ultrastructure of age pigment. Adv Gerontol Res. 1964; 18:343–384. [PubMed: 14279520]
50. Ulfig N. Altered lipofuscin pigmentation in the basal nucleus (Meynert) in Parkinson's disease. Neurosci Res. 1989; 6:456–462. [PubMed: 2771203]
51. Elsworth JD, Deutch AY, Redmond DE Jr, Sladek JR Jr, Roth RH. Differential responsiveness to 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine toxicity in sub-regions of the primate substantia nigra and striatum. Life Sci. 1987; 40:193–202. [PubMed: 3491946]
52. Meredith GE, et al. Lysosomal malfunction accompanies α-synuclein aggregation in a progressive mouse model of Parkinson's disease. Brain Res. 2002; 956:156–165. [PubMed: 12426058]
53. Asanuma M, Miyazaki I, Diaz-Corrales FJ, Ogawa N. Quinone formation as dopaminergic neuron-specific oxidative stress in the pathogenesis of sporadic Parkinson's disease and neurotoxin-induced parkinsonism. Acta Med Okayama. 2004; 58:221–233. [PubMed: 15666991]
54. Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA. Dopamine neurotoxicity: age-dependent behavioral and histological effects. Neurobiol Aging. 2003; 24:697–706. [PubMed: 12885577]
55. Caudle WM, et al. Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. J Neurosci. 2007; 27:8138–8148. [PubMed: 17652604]
56. Gonzalez-Hernandez T, Barroso-Chinea P, De La Cruz Muros I, Del Mar Perez-Delgado M, Rodriguez M. Expression of dopamine and vesicular monoamine transporters and differential vulnerability of mesostriatal dopaminergic neurons. J Comp Neurol. 2004; 479:198–215. [PubMed: 15452855]

57. Banati RB, Daniel SE, Blunt SB. Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. Mov Disord. 1998; 13:221–227. [PubMed: 9539333]
58. Mirza B, Hadberg H, Thomsen P, Moos T. The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease. Neuroscience. 2000; 95:425–432. [PubMed: 10658622]
59. Imamura K, et al. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. Acta Neuropathol. 2003; 106:518–526. [PubMed: 14513261]
60. 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]
61. Mogi M, et al. Caspase activites and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. J Neural Transm. 2000; 107:335–341. [PubMed: 10821442]
62. Castano A, Herrera AJ, Cano J, Machado A. Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. J Neurochem. 1998; 70:1584–1592. [PubMed: 9580157]
63. Wu DC, et al. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine mouse model of Parkinson's disease. J Neurosci. 2002; 22:1763–1771. [PubMed: 11880505]
64. Alladi PA, et al. Absence of age-related changes in nigral dopaminergic neurons of Asian Indians: relevance to lower incidence of Parkinson's disease. Neuroscience. 2009; 159:236–245. [PubMed: 19135503]
65. Chu Y, Kompliti K, Cochran EJ, Mufson EJ, Kordower JH. Age-related decrease in Nurr1 immunoreactivity in the human substantia nigra. J Comp Neurol. 2002; 450:203–214. [PubMed: 12209851]
66. Kubis N, et al. Preservation of midbrain catecholaminergic neurons in very old human subjects. Brain. 2000; 123:366–373. [PubMed: 10648443]
67. Gerhardt GA, Cass WA, Yi A, Zhang Z, Gash DM. Changes in somatodendritic but not terminal dopamine regulation in aged rhesus monkeys. J Neurochem. 2002; 80:168–177. [PubMed: 11796755]
68. Chan CS, Gertler TS, Surmeier DJ. Calcium homeostasis, selective vulnerability and Parkinson's disease. Trends Neurosci. 2009; 32:249–256. [PubMed: 19307031]
69. Liang CL, Sinton CM, Sonsalla PK, German DC. Midbrain dopaminergic neurons in the mouse that contain calbindin-D28k exhibit reduced vulnerability to MPTP-induced neurodegeneration. Neurodegeneration. 1996; 5:313–318. [PubMed: 9117542]
70. Mosharov EV, et al. Interplay between cytosolic dopamine, calcium, and α-synuclein causes selective death of substantia nigra neurons. Neuron. 2009; 62:218–229. [PubMed: 19409267]
71. Bender A, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson's disease. Nature Genet. 2006; 38:515–517. [PubMed: 16604074]
72. Kraytsberg Y, et al. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. Nature Genet. 2006; 38:518–520. [PubMed: 16604072]
73. Morfini GA, et al. Axonal transport defects in neurodegenerative diseases. J Neurosci. 2009; 29:12776–12786. [PubMed: 19828789]
74. Ren Y, Liu W, Jiang H, Jiang Q, Feng J. Selective vulnerability of dopaminergic neurons to microtubule depolymerization. J Biol Chem. 2005; 280:34105–34112. [PubMed: 16091364]
75. Chauhan NB, Siegel GJ, Lee JM. Depletion of glial cell line-derived neurotrophic factor in substantia nigra neurons of Parkinson's disease brain. J Chem Neuroanat. 2001; 21:277–288. [PubMed: 11429269]
76. Howells DW, et al. Reduced *BDNF* mRNA expression in the Parkinson's disease substantia nigra. Exp Neurol. 2000; 166:127–135. [PubMed: 11031089]
77. Parain K, et al. Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. Neuroreport. 1999; 10:557–561. [PubMed: 10208589]

78. Chung CY, et al. Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. Hum Mol Genet. 2005; 14:1709–1725. [PubMed: 15888489]

79. Greene JG, Dingledine R, Greenamyre JT. Gene expression profiling of rat midbrain dopamine neurons: implications for selective vulnerability in parkinsonism. Neurobiol Dis. 2005; 18:19–31. [PubMed: 15649693]

80. Ji KA, et al. Differential neutrophil infiltration contributes to regional differences in brain inflammation in the substantia nigra pars compacta and cortex. Glia. 2008; 56:1039–1047. [PubMed: 18381656]

81. Lewers JC, et al. Consequences of impaired purine recycling in dopaminergic neurons. Neuroscience. 2008; 152:761–772. [PubMed: 18313225]

82. Liss B, et al. K-ATP channels promote the differential degeneration of dopaminergic midbrain neurons. Nature Neurosci. 2005; 8:1742–1751. [PubMed: 16299504]

83. Nafia I, et al. Preferential vulnerability of mesencephalic dopamine neurons to glutamate transporter dysfunction. J Neurochem. 2008; 105:484–496. [PubMed: 18042178]

84. Villar-Cheda B, et al. Nigral and striatal expression of angiotensin receptor expression by dopamine and angiotensin in rodents: implications for progression of Parkinson's disease. Eur J Neurosci. 2010; 32:1695–1706. [PubMed: 20964730]

85. Wang HL, Morales M. Corticotropin-releasing factor binding protein within the ventral tegmental area is expressed in a subset of dopaminergic neurons. J Comp Neurol. 2008; 509:302–318. [PubMed: 18478589]

86. Coleman P, Finch C, Joseph J. The need for multiple time points in aging studies. Neurobiol Aging. 1994; 25:3–4. [PubMed: 14675723]

87. Eshius, SA.; Leenders, KL. Parkinson's Disease: Symptoms and Age Dependency. In: Hof, PR.; Mobbs, CV., editors. Functional Neurobiology of Aging. Academic Press; San Diego: 2001. p. 675-688.

88. Diedrich NJH, Moore CG, Leurgans SE, Chmura TA, Goetz CG. Parkinson disease with old-age onset: a comparative study with subjects with middle-age onset. Arch Neurol. 2003; 60:529–533. [PubMed: 12707066]

89. Hely MA, et al. Age at onset: the major determinant of outcome in Parkinson's disease. Acta Neurol Scand. 1995; 92:455–463. [PubMed: 8750110]

90. Jankovic J, Kapadia AS. Functional decline in Parkinson's disease. Arch Neurol. 2001; 58:1611–1615. [PubMed: 11594919]

91. Muller WE, Pedigo NW Jr. Brain aging: a risk factor of neurodegenerative disorders and a target for therapeutic intervention. Life Sci. 1994; 55:1975–1976. [PubMed: 7997055]

92. Thal DR, Del Tredici K, Braak H. Neurodegeneration in normal brain aging and disease. Sci Aging Knowl Environ. 2004; 2004:pe26.

93. Calne DB, Langston JW. Aetiology of Parkinson's disease. Lancet. 1983; 2:1457–1479. [PubMed: 6140548]

94. Carvey PM, Punati A, Newman MB. Progressive dopamine neuron loss in Parkinson's disease: the multiple hit hypothesis. Cell Transplant. 2006; 15:239–250. [PubMed: 16719059]

95. Sulzer D. Multiple hit hypothesis for dopamine neuron loss in Parkinson's disease. Trends Neurosci. 2007; 30:244–250. [PubMed: 17418429]

96. Chu Y, et al. Nurr1 in Parkinson's disease and related disorders. J Comp Neurol. 2006; 494:495–514. [PubMed: 16320253]

97. Kastner A, Hirsch EC, Herrero MT, Javoy-Agid F, Agid Y. Immunocytochemical quantification of tyrosine hydroxylase at a cellular level in the mesencephalon of control subjects and patients with Parkinson's and Alzheimer's disease. J Neurochem. 1993; 61:1024–1034. [PubMed: 8103078]

98. Miller GW, et al. Immunochemical analysis of vesicular monoamine transporter (VMAT2) protein in Parkinson's disease. Exp Neurol. 1999; 156:138–148. [PubMed: 10192785]

99. Muthane U, Yasha TC, Shankar SK. Low numbers and no loss of melanized nigral neurons with increasing age in normal human brains from India. Ann Neurol. 1998; 43:283–287. [PubMed: 9506543]

100. Haycock JW, et al. Marked disparity between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum. J Neurochem. 2003; 87:574–585. [PubMed: 14535941]

101. Kish SJ, Shannak K, Hornykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. N Engl J Med. 1988; 318:876–880. [PubMed: 3352672]

102. Joyce, JN. The Basal Ganglia Dopaminergic Systems in Normal Aging and Parkinson's Disease. In: Hof, PR.; Mobbs, CV., editors. Functional Neurobiology of Aging. Academic Press; San Diego: 2001. p. 689-709.

103. Braak H, et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003; 24:197–211. [PubMed: 12498954]

104. Zeng BY, Medhurst AD, Jackson M, Rose S, Jenner P. Proteasomal activity in brain differs between species and brain regions and changes with age. Mech Ageing Dev. 2005; 126:760–766. [PubMed: 15888331]

105. Goldman JE, Yen SH, Chiu FC, Peress NS. Lewy bodies of Parkinson's disease contain neurofilament antigens. Science. 1983; 221:1082–1084. [PubMed: 6308771]

106. McNaught KS, Belizaire R, Isacson O, Jenner P, Olanow CW. Altered proteasomal function in sporadic Parkinson's disease. Exp Neurol. 2003; 179:38–46. [PubMed: 12504866]

107. McNaught KS, Belizaire R, Jenner P, Olanow CW, Isacson O. Selective loss of 20S proteasome α-subunits in the substantia nigra pars compacta in Parkinson's disease. Neurosci Lett. 2002; 326:155–158. [PubMed: 12095645]

108. Zhu JH, Kulich SM, Oury TD, Chu CT. Cytoplasmic aggregates of phosphorylated extracellular signal-regulated protein kinases in Lewy body diseases. Am J Pathol. 2002; 161:2087–2098. [PubMed: 12466125]

109. Zhu JH, Guo F, Shelburne J, Watkins S, Chu CT. Localization of phosphorylated ERK/MAP kinases to mitochondria and autophagosomes in Lewy body diseases. Brain Pathol. 2003; 13:473–481. [PubMed: 14655753]

110. Corral-Debrinski M, et al. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. Nature Genet. 1992; 2:324–329. [PubMed: 1303288]

111. Soong NW, Hinton DR, Cortopassi G, Arnheim N. Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. Nature Genet. 1992; 2:318–323. [PubMed: 1303287]

112. Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. Science. 2003; 302:819–822. [PubMed: 14593166]

113. Gu M, et al. Mitochondrial function, GSH and iron in neurodegeneration and Lewy body diseases. J Neurol Sci. 1998; 158:24–29. [PubMed: 9667773]

114. Mizuno Y, et al. Mitochondrial energy crisis in Parkinson's disease. Adv Neurol. 1993; 60:282–287. [PubMed: 8420144]

115. Squier TC. Oxidative stress and protein aggregation during biological aging. Exp Gerontol. 2001; 36:1539–1550. [PubMed: 11525876]

116. Alam ZI, et al. A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. J Neurochem. 1997; 69:1326–1329. [PubMed: 9282961]

117. Alam ZI, et al. Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. J Neurochem. 1997; 69:1196–1203. [PubMed: 9282943]

118. Dexter DT, et al. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem. 1989; 52:381–389. [PubMed: 2911023]

119. Beach TG, Walker R, McGeer EG. Patterns of gliosis in Alzheimer's disease and aging cerebrum. Glia. 1989; 2:420–436. [PubMed: 2531723]

120. Damier P, Hirsch EC, Zhang P, Agid Y, Javoy-Agid F. Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience. 1993; 52:1–6. [PubMed: 8433802]

121. Forno LS, DeLanney LE, Irwin I, Di MD, Langston JW. Astrocytes and Parkinson's disease. Prog Brain Res. 1992; 94:429–436. [PubMed: 1287728]

122. Sheffield LG, Berman NE. Microglial expression of MHC class II increases in normal aging of nonhuman primates. Neurobiol Aging. 1998; 19:47–55. [PubMed: 9562503]

123. McGeer PL, Itagaki S, McGeer EG. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. Acta Neuropathol. 1988; 76:550–557. [PubMed: 2974227]

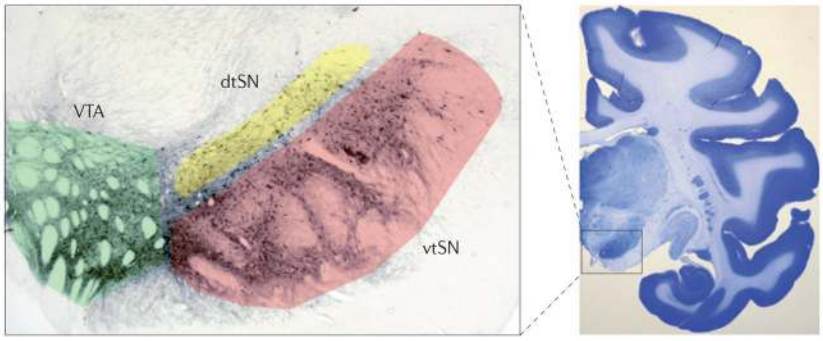

Figure 1. Regional differences in vulnerability to degeneration in Parkinson's disease and models of parkinsonism

Dopamine neurons of the ventral midbrain vary in their susceptibility to degeneration, with neurons of the ventral tier of substantia nigra (vtSN, shown in red) being the most vulnerable, the dorsal tier of substantia nigra (dtSN, shown in yellow) being less vulnerable, and the adjacent cells of the ventral tegmental area (VTA, shown in green) being the least vulnerable. The low magnification image is a coronal hemisection through the ventral midbrain of a rhesus monkey stained with cresyl violet. The expanded view is stained for tyrosine hydroxylase as a marker for dopamine neurons. The red, yellow and green areas correspond to the areas compared for cell counts and expression of immunocytochemical markers in the studies that are reviewed in this article.

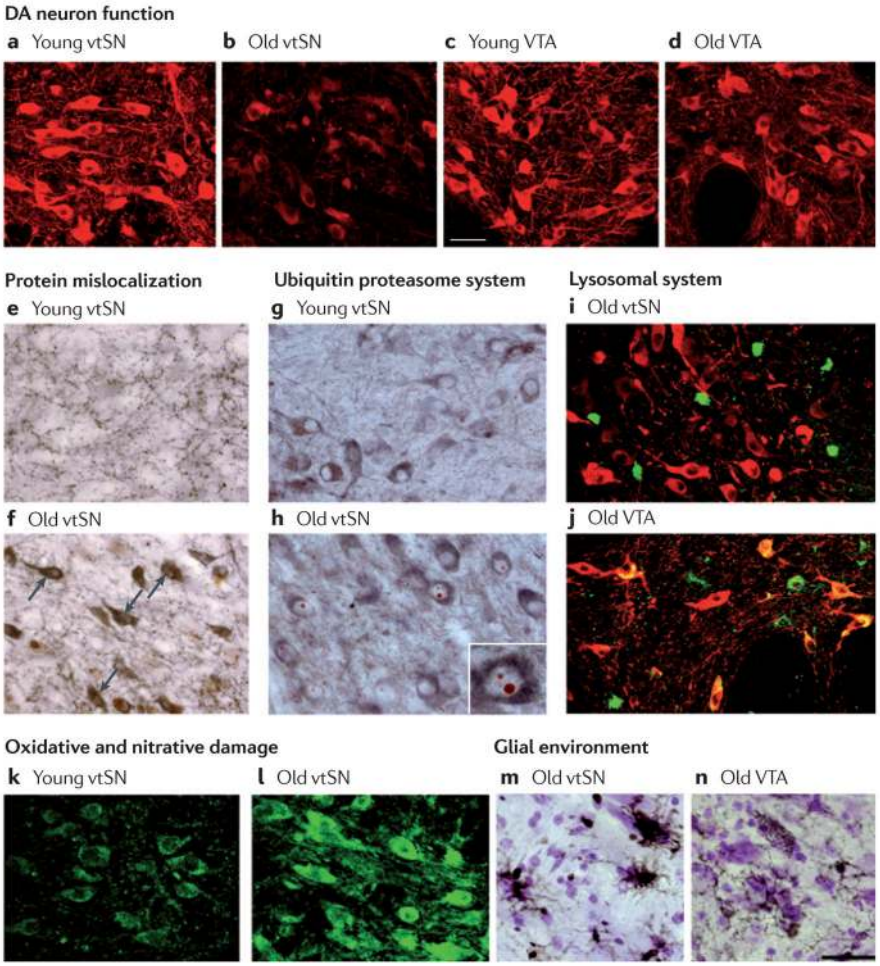

Figure 2. The pattern of ageing-related changes in markers of cellular mechanisms

With advancing age, dopamine (DA) neurons in the ventral tier of the substantia nigra (vtSN) — the population that is most vulnerable to degeneration in Parkinson's disease — show changes with ageing. **a**,**b**,**c**,**d** | Age-related decline in tyrosine hydroxylase staining (shown in red) in vtSN neurons but not in vental tegmental area (VTA) neurons. **e**,**f** | Accumulation of cytoplasmic α-synuclein (shown in brown). Tyrosine hydroxylase staining is shown in grey. Arrows show examples of cytoplasmic α-synuclein in aged vtSN. **g**,**h** | Increased numbers of Marinesco bodies, characterized by cytoplasmic inclusions of ubiquitin (shown in red). Tyrosine hydroxylase staining is shown in grey. The inset (in part **h**) is a higher magnification view of a tyrosine hydroxylase immunoreactive neuron of the vtSN exhibiting multiple Marinesco bodies. **i**,**j** | No accumulation of lipofuscin (shown in green). Tyrosine hydroxylase staining is shown in red, colocalization of lipofuscin and tyrosine hydroxylase is shown in yellow. Note that virtually all lipofuscin staining in the vtSN is not in dopamine neurons, whereas colocalization is apparent in aged VTA neurons. **k**,**l** | Accumulation of 3-nitrotyrosine (shown in green). **m**,**n** | Greater microglial reactivity in aged vtSN neurons than in aged VTA neurons, shown by greater staining for HLA class II histocompatibility antigen, DR α-chain (HLA-DRA; a marker for microglia), shown in brown. UPS, ubiquitin–proteasome system. Parts **e** and **f** are reproduced, with permission, from REF. 18 © (2007) Elsevier. Parts **k** and **l** are reproduced, with permission, from REF. 20 © (2008) Blackwell Publishing.

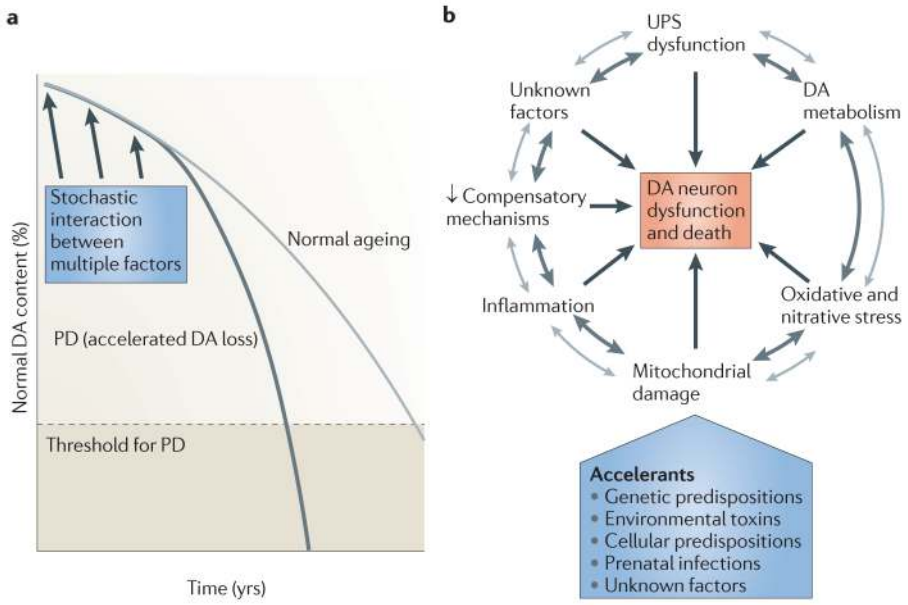

Figure 3. The stochastic acceleration hypothesis

A revised hypothesis of the relationship between ageing and Parkinson's disease (PD) as they affect the biology of midbrain dopamine (DA) neurons. The hypothesis incorporates evidence that supports the involvement of common cellular mechanisms involved in dopamine neuron dysfunction in ageing, and degeneration in Parkinson's disease. **a** | The effects of these altered cellular mechanisms as they accumulate during normal ageing result in parkinsonian dopamine neuron dysfunction, either very late in life or not at all (shown by the light grey line). However, when these same cellular mechanisms are accelerated by specific, individually determined factors, parkinsonism emerges earlier in the lifespan (shown by the dark grey line). **b** | The hypothesis contends that the cellular mechanisms that threaten dopamine neuron function are identical, but not linked in an orderly cascade of cause and effect, and instead can contribute to varying degrees and combine in patient-specific patterns, thus fulfilling the definition of a stochastic interaction: incorporating elements of randomness with directionality towards dopamine neuron dysfunction. Light grey double-ended arrows show cellular events in normal ageing. Thicker, dark grey double-ended arrows show accelerated cellular events in PD. UPS, ubiquitin–proteasome system.

Table 1

The nigrostriatal dopamine system in ageing and Parkinson's disease

Contributing factors	Ageing	PD
DA phenotype loss in the SN: regional specificity in loss of TH, DAT and others (vtSN > dtSN and VTA)	Yes[16,19,20,67,96]	Yes[96,97,98]
DA neuron loss in the SN: regional specificity (vtSN > dtSN and VTA)	No *[4,26,27,99]	Yes[4,5,8]
Striatal DA loss: regional specificity (putamen > caudate)	Yes *[7,25,26,100] (not regionally similar to PD *)	Yes[6,101]
DA metabolism: life-long exposure to cytosolic DA	Yes *[53,54]	Yes[53,54]
DA receptor: compensatory increase in DA receptors	No[102]	Yes[102]
UPS dysfunction: protein accumulation, aggregates (MBs, LBs and LNs)	Yes[18,19,46,47,103,104]	Yes[29,47,103,105–108]
Lysosome function: accumulation of lipofuscin in resistant and/or surviving neurons	Yes *[19,46]	Yes[50,109]
Mitochondrial dysfunction: oxygen radical production, reduced production of ATP	Yes[110,111]	Yes[112–114]
Oxidative and nitrative damage: to proteins, lipids, DNA and RNA	Yes[20,115]	Yes[116–118]
Astrocyte activation in midbrain	Moderate in middle age[21,119] Lost in old age	Moderate *[58,120,121]
Microglia activation in midbrain: production of pro-inflammatory cytokines	Yes[21,122] (mild)	Yes[60,123] (severe)

Based on human and nonhuman primate studies. DA, dopamine; DAT, sodium-dependent dopamine transporter; dtSN, dorsal tier of the substantia nigra; LBs, Lewy bodies; LNs, Lewy neurites; MBs, Marinesco bodies; PD, Parkinson's disease; SN, substantia nigra; TH, tyrosine hydroxylase; UPS, ubiquitin–proteasome system; VTA, ventral tegmental area; vtSN, ventral tier of the substantia nigra.

* Indicates a controversial topic and/or contradictory findings in the literature.