THERAPEUTICS of PARKINSON'S DISEASE and OTHER MOVEMENT DISORDERS

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Preface

Over the past few decades the field of neurology has seen spectacular developments in diagnostic techniques, most vividly exemplified by modern neuroimaging and molecular genetics. Although not always at the same speed this evolution has gone hand in hand with an enlarging armentarium of effective therapies to treat neurological disease. This is particularly true for the field of movement disorders, where one of the most exciting success stories of modern translational research in neuroscience unfolded more than 40 years ago: the discovery of dopamine deficiency in the striatum of patients with Parkinson's disease and the subsequent introduction of levodopa as a dramatically effective therapy of this hitherto devastating illness. Since then the therapeutic options for Parkinson's disease have grown exponentially, often making treatment decisions difficult. Moreover, there are now numerous therapies for other movement disorders with substantial impact on patients. While many therapies remain symptomatic, a number normalize the condition such as de-coppering in Wilson's disease and levodopa in dopa-responsive dystonia.

While there are a number of textbooks on movement disorders, none so far has emphasized treatment, and this current work attempts to fill this gap. Practitioners want and need practical detailed advice on how to treat patients. We have recruited a team of experts who have attempted to deal with most situations. Wherever available, chapter authors have used evidence from randomized controlled clinical trials to develop practical recommendations for every day clinical practice. As is the case for all of medicine there are many situations in the treatment of movement disorders where evidence from controlled trials is either insufficient or open to interpretation. We have therefore deliberately encouraged the expert authors to share with the reader their personal clinical acumen and therapeutic wisdom. Summary tables and algorithms are part of many chapters and will hopefully serve as a quick reference guide for practical treatment decisions in many different circumstances. Of course, each patient presents unique circumstances, so physicians will need to use their judgement every step of the way, but having expert guidance should at least set the general direction.

We are grateful to the movement disorder experts whom we have recruited from all over the world to bring their knowledge to this textbook. We appreciate their expertise and patience with our compulsive editing, as we have tried to give a uniform style to the recommendations, and occasionally added our own opinions.

We have tried to be up to date, but medications and other treatment options may change. New agents appear and some may even be withdrawn because new adverse effects surface. So, we hope that this book and its advice will be a helpful guide, but physicians must continue to be alert to any changes in practice that might arise.

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Part I

PARKINSON'S DISEASE AND PARKINSONISM

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The Etiopathogenesis of Parkinson's Disease: Basic Mechanisms of Neurodegeneration

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INTRODUCTION

Parkinson's disease (PD) is a slowly progressive, neurodegenerative movement disorder characterized clinically by bradykinesia, rigidity, tremor and postural instability (Lang and Lozano, 1998; Lang and Lozano, 1998). PD is the second most common neurodegenerative illness (after Alzheimer's disease), and both incidence and prevalence rates increase with aging. As life expectancy of the general population rises, both the occurrence and prevalence of PD are likely to increase dramatically (Dorsey *et al.*, 2007). Levodopa is the mainstay of current treatment, but long-term therapy is associated with motor complications and advanced disease is associated with non-dopaminergic features such as falling and dementia, which are not controlled with current therapies and are the major source of disability. These trends underscore the urgent need to move beyond the present time of symptomatic treatment to an era where neuroprotective therapies are available that prevent or impede the natural course of the disorder (Schapira and Olanow, 2004). The achievement of this goal would be facilitated by deciphering the factors that underlie the initiation, development and progression of the neurodegenerative process.

The primary pathology of PD is degeneration of dopaminergic neurons with protein accumulation and the formation of inclusions (Lewy bodies) in the substantia nigra pars compacta (SNc) (Forno, 1996). However, it is now appreciated that neurodegeneration with Lewy bodies or Lewy neurites is widespread and can be seen in noradrenergic neurons in the locus coeruleus, cholinergic neurons in the nucleus basalis of Meynert, and serotonin neurons in the median raphe, as well as in nerve cells in the dorsal motor nucleus of the vagus, olfactory regions, pedunculopontine nucleus, cerebral hemisphere, brain stem, and peripheral autonomic nervous system (Forno, 1996; Braak *et al.*, 2003; Zarow *et al.*, 2003). Indeed, non-dopaminergic pathology may even predate the classic dopaminergic pathology (Braak *et al.*, 2003). Pathology in PD is thus widespread and progressive, but still specific in that some areas, such as the cerebellum and specific brain stem nuclei are unaffected by the disease process.

It now appears that there are many different causes of PD (Table 1.1). Approximately 5–10% of all cases of the illness are familial and likely genetic in origin, but most cases occur sporadically and are of unknown cause. Most recent attention has focused on genetic causes of PD based on linkage of familial patients to a variety of different chromosomal loci (PARK 1-11). Mutations in six specific proteins (α-synuclein, parkin, UCH-L1, DJ-1, PINK1 and LRRK2) have now been identified (Hardy *et al.*, 2006). Further, mutations in LRRK2 have now been identified to be present in some late-onset PD patients with typical clinical and pathological features of PD and no family history (Gilks *et al.*, 2005). Indeed, as many as 40% of North African and Ashkenazy Jewish PD patients carry this mutation (Ozelius *et al.*, 2006; Lesage *et al.*, 2006). However, a genetic basis for the vast majority of sporadic cases is far from established. In sporadic PD, epidemiologic studies suggest that environmental factors play an important role in development of the illness (Tanner, 2003). Further, two large genome-wide screens have failed to identify any specific genetic abnormality (Elbaz *et al.*, 2006; Fung *et al.*, 2006). The cause of PD thus remains a mystery. A widely held view is that environmental toxins might cause PD in patients who are susceptible because of

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Table 1.1 Genetic and sporadic forms of Parkinson's disease.

Locus	Chromosome location	Gene product and properties	Mutations	Age of Onset (yr)	Clinical spectrum	Pathological features
Autosomal Dominant PD						
PARK 1&4	4q21–q23	α-Synuclein	Point mutations (A53T, A30P and E46K)	Range: 30–60	Levodopa-responsive; rapid progression; prominent dementia	Neuronal loss in the SNc, LC and DMN
		140 amino acids/ 14 kDa protein	Duplication	Mean: 45	E46K and multiplication cases demonstrate overlap with dementia with Lewy bodies	Lewy bodies are rare and tau accumulation occur in some A53T cases. Extensive Lewy bodies in E46K and multiplication cases
		Localized to synaptic terminals	Triplication			Triplication cases demonstrate degeneration in the hippocampus, vacuolation in the cortex and glial cytoplasmic inclusions
		Function: Unknown. Possibly play a role in synaptic activity				
PARK 8	12p11.2–12q31.1	Dardarin/LRRK2	Missense	Range: 35–79	Typical PD features; slow progression;	SNc degeneration
		2482/2527 amino acids		Mean: 57.4	dementia present; features of motor neuron disease reported	Some cases show extensive Lewy bodies; some do not have Lewy bodies
		Function: Unknown. May be a protein kinase				Also, intranuclear inclusions, tau-immunoreactive inclusions and neurofibriallry tangles are present

PARK 5	4p14	Ubiquitin C-terminal hydrolase L1 230 amino acids/ 26 kDa protein Neuron specific protein Function: De-ubiquitinating enzyme (possible E3 activity also)	Missense mutation (I93M)	49 and 50	Typical PD	Lewy bodies reported in a single case
Autosomal Recesive PD						
PARK 2	6q25.2–q27	Parkin	Deletions	Range: 7–58	Levodopa-responsive and severe dyskinesias; foot dystonia; diurnal fluctuations; hyperreflexia; slow progression	Selective and severe destruction of the SNc and LC
		465 amino acids/ 52 kDa protein Expressed in cytoplasm, golgi complex, nuclei and processes Function: E3 ubiquitin ligase	Point mutations Multiplications	Mean: 26.1		Generally Lewy body-negative
PARK 6	1p35–1p36	PINK 1	Missense	Range: 32–48	Levodopa-responsive; slow progression	Neuropathology not yet determined
		581 amino acids/ 62.8 kDa protein Localized to mitochondria Function: Unknown. May be a protein kinase	Truncating			

(*Continued*)

Table 1.1 (*Continued*).

Locus	Chromosome location	Gene product and properties	Mutations	Age of Onset (yr)	Clinical spectrum	Pathological features
PARK 7	1p36	DJ-1	Deletion	Range: 20–40s	Levodopa responsive; dystonia; psychiatric disturbance; slow progression	Neuropathology not yet determined
		189 amino acids/ 20 kDa protein	Truncating	Mean: mid 30s		
		More prominent in the cytoplasm and nucleus of astrocytes compared to neurons	Missense			
		Function: Unknown. Possible antioxidant, molecular chaperone and protease				
Sporadic PD						
—	—	—	—	Mean: 59.5 yr	Insidious onset and slow progression. L-DOPA-responsive.	Neurodegeneration with Lewy bodies in the SNc, LC, DMN, NBM, etc

their genetic profile, poor ability to metabolize toxins, and/or advancing age (Hawkes, Del Tredici and Braak, 2007).

Several factors have been implicated in the pathogenesis of cell death in PD, including oxidative stress, mitochondrial dysfunction, excitotoxicity, and inflammation (Wood-Kaczmar, Gandhi and Wood, 2006; Olanow, 2007). Interest has also focused on the possibility that proteolytic stress due to excess levels of misfolded proteins might be central to each of the different etiologic and pathogenic mechanisms that could lead to cell death in PD (Olanow, 2007). Finally, there is evidence that cell death occurs by way of a signal-mediated apoptotic process. Each of these mechanisms provides candidate targets for developing putative neuroprotective therapies. However, the precise pathogenic mechanism responsible for cell death remains unknown, and to date no therapy has been established to be neuroprotective (Schapira and Olanow, 2004). Indeed, it remains uncertain if any one or more of these factors is primary and initiates cell death, or if they develop only secondary to an alternative process.

In this chapter, we consider those etiologic and pathogenic factors that have been implicated in PD, based on genetic and pathological findings, and consider how they might contribute to the various familial and sporadic forms of PD (Figure 1.1).

AUTOSOMAL DOMINANT PD

α-*Synuclein*

The first linkage discovered to be associated with PD was located at chromosome 4q21–q23 (PARK 1&4). Genetic analyses showed A53T and A30P point mutations in the gene that encodes for a 140 amino acid/14 kDa protein known as α-synuclein (Polymeropoulos *et al.*, 1996; Polymeropoulos *et al.*, 1997). Subsequently, an E46K mutation in α-synuclein was reported in another family with autosomal dominant PD (plus features of dementia with Lewy bodies) (Zarranz *et al.*, 2004), but no other point mutation has subsequently been found. In recent years, duplication (three copies) and triplication (four copies) of the normal α-synuclein gene have also been found to cause autosomal dominant PD (Chartier-Harlin *et al.*, 2004; Farrer *et al.*, 2004; Ibanez *et al.*, 2004; Miller *et al.*, 2004; Singleton *et al.*, 2003).

Familial PD caused by α-synuclein shares many features with common sporadic PD, but patients tend to have a relatively early age of onset (mean in the 40s) and high occurrence of dementia. Also, patients with duplication/triplication of the α-synuclein gene tend to present with a dementia with Lewy bodies (DLB) pattern rather than more conventional PD. Pathological studies show a marked increase in α-synuclein levels with protein aggregation in various brain regions (Singleton *et al.*, 2003; Duda *et al.*, 2002; Kotzbauer *et al.*, 2004). However, this is often in the form of Lewy neurites rather than Lewy bodies. In patients with the A53T mutation, Lewy bodies are rarely present and there is a marked accumulation of α-synuclein and tau in the cerebral cortex and striatum (Duda *et al.*, 2002; Kotzbauer *et al.*, 2004). Also, patients with triplication of the normal α-synuclein gene have vacuoles in the cortex, neuronal death in the hippocampus and inclusion bodies in glial cells (Singleton *et al.*, 2003). These findings show that there are significant differences between the pathology that occurs in the α-synuclein-linked familial PD and common sporadic PD.

α-Synuclein, so called because of its preferential localization in synapses and the region of the nuclear envelope (Jakes, Spillantini and Goedert, 1994; Maroteaux, Campanelli and Scheller, 1988), is diffusely expressed throughout the CNS (Solano *et al.*, 2000). It is a member of a family of related proteins that also include β- and γ-synucleins (Goedert, 2001). α-Synuclein is enriched in presynaptic nerve terminals and associates with lipid membranes and vesicles. The normal function of α-synuclein is unknown, but there is some evidence that it plays a role in synaptic neurotransmission, neuronal plasticity and lipid metabolism. Since the discovery of α-synuclein-linked familial PD, there has been a great deal of effort aimed at deciphering how mutations in this protein induce neurodegeneration. The dominant mode of inheritance suggests a gain of function. Wild-type α-synuclein is monomeric and intrinsically unstructured/natively unfolded at low concentrations, but in high concentrations it has a propensity to oligomerize and aggregate into β-pleated sheets (Conway, *et al.*, 1998; Weinreb *et al.*, 1996). Mutations in the protein increase this potential for misfolding, oligomerization and aggregation (Conway, Harper and Lansbury, 1998; Weinreb *et al.*, 1996; Caughey and Lansbury, 2003; Conway *et al.*, 2000; Lashuel *et al.*, 2002;Li, Uversky and Fink, 2001; Pandey, Schmidt and Galvin, 2006). Oligomerization of α-synuclein produces intermediary species (protofibrils) that form annular structures with pore-like properties that permeabilize synthetic vesicular membranes *in vitro*. It has been suggested that protofibrils are the toxic α-synuclein species that are responsible for cell death. It is also possible that protein aggregation itself can interfere with critical cell functions and promote apoptosis.

It is possible that the cytotoxicity associated with mutant/excess α-synuclein involves interference with proteolysis and autophagy. Wild-type α-synuclein is a substrate for both the 26S and 20S proteasome and is preferentially degraded in a ubiquitin-independent manner (Bennett *et al.*, 1999; Liu *et al.*, 2003;Tofaris, Layfield and Spillantini, 2001). *In vitro* and *in vivo* studies have demonstrated that mutant α-synuclein, which misfolds, oligomerizes and aggregates, is resistant to UPS-mediated degradation and

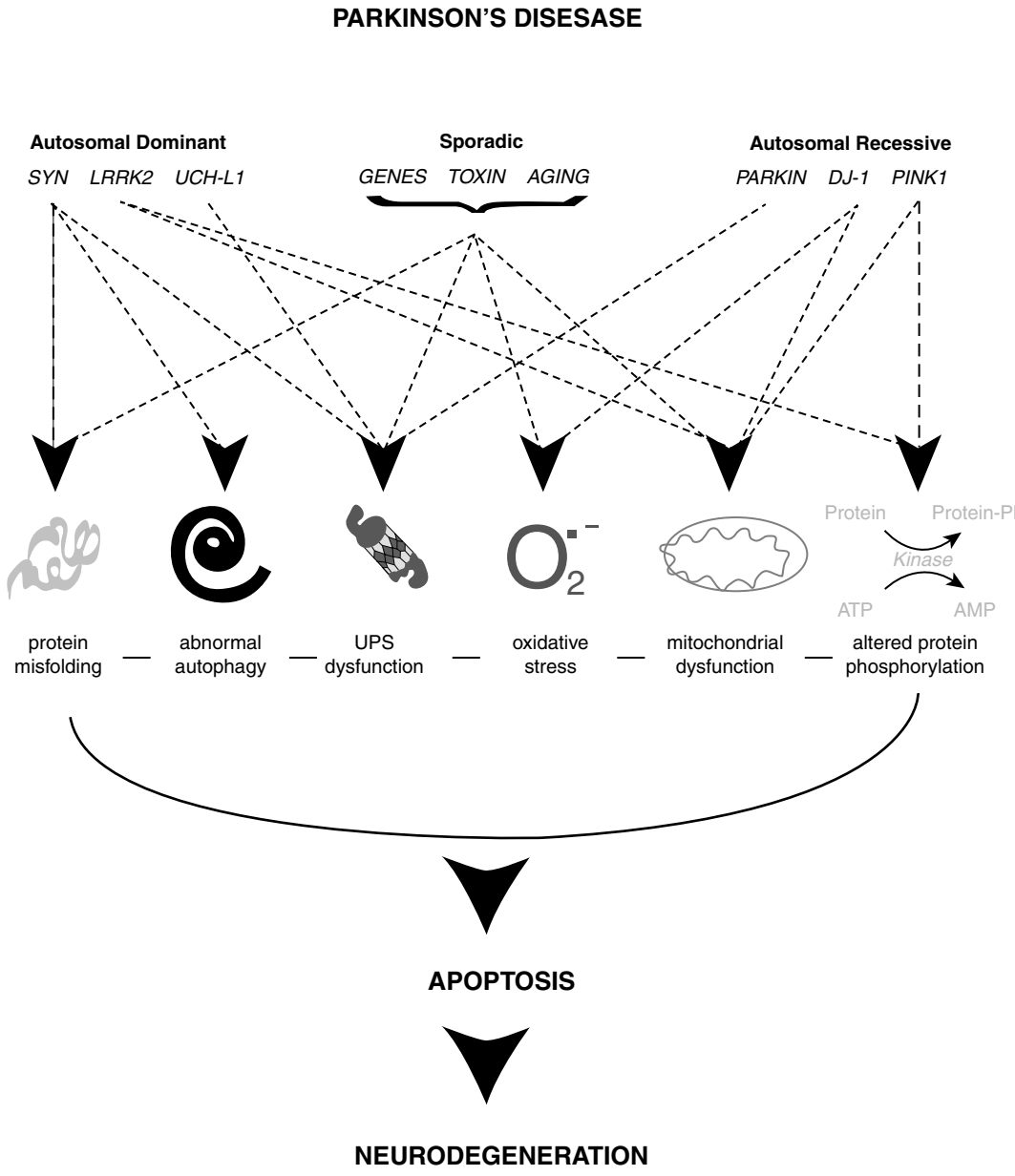

Figure 1.1 Schematic illustration of different forms of PD and factors that are thought to be associated with the development of cell death and that might be candidates for putative neuroprotective therapies.

also inhibits this pathway and its ability to clear other proteins (Snyder *et al.*, 2003; Stefanis *et al.*, 2001; Tanaka *et al.*, 2001). As a result, there is accumulation of a wide range of proteins, in addition to α-synuclein, in cells expressing mutant α-synuclein. High levels of undegraded or poorly degraded proteins have a tendency to aggregate with each other and other proteins, form inclusion bodies, disrupt intracellular processes, and cause cell death (Bence Sampat and Kopito, 2001). Recent studies indicate that α-synuclein can also be broken down by the 20S proteasome through endoproteolytic degradation that does not involve the –N or –C terminus (Liu *et al.*, 2003). This type of degradation yields truncated α-synuclein fragments, which are particularly prone to aggregate, promote aggregation of the full-length protein, as well as other proteins, and cause cytotoxicity (Liu *et al.*, 2005). Thus, it is reasonable to

consider that alterations in the α-synuclein gene can interfere with the clearance of unwanted proteins, and that this defect may underlie protein aggregation, Lewy body formation and neurodegeneration in hereditary PD (Olanow and McNaught, 2006). α-Synuclein can also be degraded by the lysosomal system, and mutations in the protein are associated with impaired chaperone-mediated clearance by autophagy which also promotes accumulation and aggregation of the protein (Cuervo *et al.*, 2004; Lee *et al.*, 2004).

Numerous studies, employing a variety of approaches, have examined the effects of expressing PD-related mutant (and wild-type) α-synuclein in transgenic animals (Fernagut and Chesselet, 2004). Expression of mutant (A53T, A30P) or wild-type α-synuclein in transgenic *Drosophila* (Feany and Bender, 2000), or the adenoviral-mediated expression of A53T mutant or wild-type α-synuclein in the SNc of adult non-human primates (common marmosets) (Kirik *et al.*, 2003), causes selective dopamine cell degeneration. Interestingly, overexpression of A53T, A30P or wild-type α-synuclein causes inclusion body formation, but does not cause neurodegeneration in transgenic mice (Fernagut and Chesselet, 2004). In addition, some species normally express the mutant form of α-synuclein with a threonine in the alanine position, yet do not show aggregation as is found in PD patients (Polymeropoulos *et al.*, 1997), possibly because α-synuclein is degraded differently in these species.

The relative roles of the UPS and lysosomal systems in the degradation of wild-type and mutant α-synuclein has not been clearly defined, and it is possible that defects in either the proteasomal or lysosomal systems could contribute to the accumulation of α-synuclein and other proteins. It is also noteworthy that not all carriers of point mutations in α-synuclein develop PD, suggesting that additional factors, such as environmental toxins, might be required to trigger the development of PD in individuals carrying mutations in α-synuclein.

It is noteworthy that α-synuclein accumulates in patients with sporadic PD (see below), suggesting that this protein might also have relevance to the cause of cell death in these cases. In support of this concept, it is noteworthy that knockdown of α-synuclein prevents dopaminergic toxicity associated with MPTP (Dauer *et al.*, 2002). Heat shock proteins act to promote protein refolding and also as chaperones to facilitate protein clearance through the proteasome or autophagal systems. Indeed, it has been found that overexpression of heat shock protein prevents dopamine neuronal degeneration in *Drosophila* that overexpress wild-type or mutant α-synuclein (Auluck *et al.*, 2002). Similarly the naturally occurring benzoquinone ansamycin, geldanamycin, prevents aggregation and protects dopamine neurons in this model (Auluck and Bonini, 2002). Geldanamycin binds to an ATP site on HSP90, blocking its normally negative regulation of heat shock transcription factor 1 (HSF1), thus promoting the synthesis of heat shock protein (Whitesell *et al.*, 1994). These studies offer promising targets for candidate neuroprotective drugs for PD. It also possible that agents that can prevent or dissolve α-synuclein aggregates such as β-synuclein or immunization with α-synuclein might be protective in PD (Hashimoto *et al.*, 2004; Masliah *et al.*, 2005), although it has not yet been shown that these strategies can provide protective effects in model systems.

UCH-L1

An I93M missense mutation in the gene (4p14; PARK 5) encoding ubiquitin *C*-terminal L1 (UCH-L1), a 230 amino acid/26 kDa de-ubiquitinating enzyme, was associated with the development of autosomal dominant PD in two siblings of a European family (Leroy *et al.*, 1998). The parents were asymptomatic, suggesting that the gene defect causes disease with incomplete penetrance. The affected individuals had clinical features that resemble sporadic PD, including a good response to levodopa, but the age (49 and 51) of onset was relatively early. Postmortem analyses on one of the siblings revealed Lewy bodies in the brain (Auberger *et al.*, 2005). Genetic screening studies have failed to detect UCH-L1 mutations in other families with PD, suggesting that this mutation is either very rare, or not a true cause of PD (Wintermeyer *et al.*, 2000). Interestingly, several studies have found that the UCH-L1 gene is a susceptibility locus in sporadic PD and that polymorphisms, such as the S18Y substitution, confers some degree of protection against developing the illness (Maraganore *et al.*, 2004). However, another study failed to find any association between UCH-L1 polymorphisms and PD (Healy *et al.*, 2006).

UCH-L1 is expressed exclusively in neurons in many areas of the CNS (Solano *et al.*, 2000), and constitutes 1–2% of the soluble proteins in the brain (Solano *et al.*, 2000; Wilkinson, Deshpande and Larsen, 1992; Wilkinson *et al.*, 1989). UCH-L1 is responsible for cleaving ubiquitin from protein adducts to enable the protein to enter the proteasome. Mutations in UCH-L1 cause a reduction in de-ubiquitinating activity *in vitro* and result in gracile axonal dystrophy (GAD) in transgenic mice (Leroy *et al.*, 1998; Nishikawa *et al.*, 2003; Osaka *et al.*, 2003). Further, toxin- or mutation-induced inhibition of UCH-L1's activity leads to a marked decrease in levels of ubiquitin in cultured cells and in the brain of GAD mice (Osaka *et al.*, 2003; McNaught *et al.*, 2002), and degeneration of dopaminergic neurons with protein accumulation and the formation of Lewy body-like inclusions in rat ventral midbrain cell cultures (McNaught *et al.*, 2002). Therefore, it is possible that a mutation in UCH-L1 alters UPS function leading to altered proteolysis and ultimately cell death. It also appears that UCH-L1 has E3 ubiquitin ligase activity, but it remains

unclear if the PD-related mutation alters this function of the protein (Liu *et al.*, 2002).

LRRK2

LRRK2 mutations are now thought to be the commonest cause of familial PD. Several missense mutations in the gene (12p11.2–q13.1, PARK 8) encoding a 2527 amino acid/ ≈250 kDa protein called dardarin or LRRK2 (leucine-rich repeat kinase 2) can cause an autosomal dominant form of PD with incomplete penetrance (Funayama *et al.*, 2002; Paisan-Ruiz *et al.*, 2004; Zimprich *et al.*, 2004). This gene defect has been found in several families from different countries, and it is estimated that the mutation could account for 5% or more of familial PD cases (Farrer, 2006), although this percentage is significantly higher in north African arabs and Ashkenazi Jews perhaps reflecting a founder effect (Ozelius *et al.*, 2006; Lesage *et al.*, 2006). Not all individuals who carry these mutations develop parkinsonism, suggesting the possible requirement of other etiological factors to act as a trigger for the illness (Di Fonzo *et al.*, 2005).

The clinical spectrum of LRRK2-linked PD can be similar to sporadic PD, with an age of onset ranging from 32 to 79 years. Pathologically, most have a PD-like picture, but there can be considerable variability even within family members who carry the same mutation (Zimprich *et al.*, 2004; Wszolek *et al.*, 2004). While all subjects with LRRK2-linked familial PD have nigrostriatal degeneration, some have nigral Lewy bodies and some do not, some have a DLB picture with extensive cortical Lewy bodies, and some have tau-immunoreactive glial and neuronal inclusions resembling tauopathies such as progressive supranuclear palsy. Interestingly, some patients with this mutation have a late-onset form of PD with no family history and clinical and pathologic features typical of sporadic PD. It has been estimated that the LRRK2 mutation might account for as many as 7% of familial cases and 1.5–3% of cases of sporadic PD (Di Fonzo *et al.*, 2005; Gilks *et al.*, 2005; Nichols *et al.*, 2005).

LRRK2 protein is expressed throughout the brain (Paisan-Ruiz *et al.*, 2004; Simon-Sanchez *et al.*, 2006), but its normal function is unknown. It is a large protein that is bound to the outer mitochondrial membrane. Based on its molecular structure, it has been suggested that LRRK2 might be a cytoplasmic kinase in the MAP kinase family (Paisan-Ruiz *et al.*, 2004; Zimprich *et al.*, 2004). It is also not known how mutations in LRRK2 alter the structure and function of the protein or how these might lead to cell death. It is now appreciated that LRRK2 has kinase (West, Moore and Biskup, 2005) and GTPase (Li *et al.*, 2007) activities, and that mutations are associated with enhanced GTP binding and kinase activities that are linked to toxicity (West *et al.*, 2007). Indeed, knockdown of kinase activity leads to reduced toxicity in model systems (Greggio *et al.*, 2006; Smith *et al.*, 2006). It is therefore possible that PD-related LRRK2 mutations might be due to an increase in kinase activity leading to altered phosphorylation of substrate proteins (West, Moore and Biskup, 2005).

AUTOSOMAL RECESSIVE PD

Parkin

A hereditary form of PD, autosomal recessive juvenile parkinsonism (AR-JP) was first described in Japanese families, and is linked to chromosome 6q25.2–q27 (PARK 2) (Matsumine *et al.*, 1997). This locus was found to host the gene that encodes for a 465 amino acid/52 kDa protein called parkin (Kitada *et al.*, 1998). It is now appreciated that many deletions, point mutations, and mutations that span the entire parkin gene can cause familial PD (Hattori and Mizuno, 2004). Some estimates suggest that parkin mutations might account for as many as 50% of early-onset (<45 years) familial cases of PD (Lucking *et al.*, 2000). It is noteworthy, though, that parkin mutations can also be associated with late-onset (≥60 years old) hereditary PD (Foroud *et al.*, 2003).

Clinically, AR-JP is similar to common sporadic PD, but there are notable differences. Patients with parkin mutations tend have a very early age of onset, ranging from 7 to 72 years (average, 30 years), and demonstrate a rather slow rate of progression. The neuropathology of patients with parkin mutations differs from sporadic PD in that neurodegeneration is restricted to the SNc and LC, and Lewy bodies are largely absent (Mori *et al.*, 1998), although a few have been noted in a few older patients with parkin-linked autosomal PD (Farrer *et al.*, 2001; Pramstaller *et al.*, 2005).

Parkin is expressed in the cytoplasm, nucleus, golgi apparatus and processes of neurons throughout the CNS (Horowitz *et al.*, 2001). Several studies have shown that parkin is an E3 ubiquitin ligase (Imai *et al.*, 2001; Imai *et al.*, 2000; Shimura *et al.*, 2000; Shimura *et al.*, 2001; Zhang *et al.*, 2000) which contains a RING finger domain (comprising two RING finger motifs separated by an in-between-RING domain) at the C-terminus. The protein also contains a central linker region and a ubiquitin-like domain (UBL) at the N-terminus. Parkin acts in conjunction with several E2 enzymes, Ubc6, UbcH7 and UbcH8, to ubiquitinate a variety of substrates. These include synphilin-1, CDCrel-1, parkin-associated endothelin receptor-like receptor (Pael-R), an O-glycosylated isoform of α-synuclein (αSp22), cyclin E α/β-tubulin, p38 subunit of the aminoacyl-tRNA synthetase complex, and synaptotagmin X1. Interestingly, parkin may polyubiquitinate proteins with linkages at lysine 48 (K48) or lysine 63 (K63) (Lim *et al.*, 2005). Parkin has been shown to interact through its UBL domain with the 26S proteasome Rpn10/S5a subunit, and along with Rpt5/S6′, plays a role in the recognition of ubiquitinated substrates by the PA700 proteasome activator

(Pickart and Cohen, 2004; Sakata *et al.*, 2003). Parkin also interacts with a protein complex containing CHIP/HSP70 which promotes parkin's activity (Cyr, Hohfeld and Patterson, 2002) and with proteasomal subunits (Dachsel *et al.*, 2005).

Precisely how parkin induces pathology in familial PD is not known, but could relate to a loss of E3 ubiquitin ligase activity with consequent impairment in the ubiquitination of its protein substrates. Levels of parkin, and its enzymatic activity, are decreased in the SNc and LC in AR-JP (Shimura *et al.*, 2000; Shimura *et al.*, 2001;Cyr, Hohfeld and Patterson, 2002; Shimura *et al.*, 1999). This defect may thus underlie the accumulation of undegraded parkin substrates, including Pael-R and αSp22, found in these brain areas in PD (Imai *et al.*, 2001; Shimura *et al.*, 2001). It has been shown that normal parkin prevents endoplasmic reticulum dysfunction and unfolded protein-induced cell death following overexpression of Pael-R in cultured cells and *Drosophila* (Imai *et al.*, 2001; Imai, Soda and Takahashi, 2000; Yang *et al.*, 2003). So, it is reasonable to consider that accumulation of undegraded substrate proteins disrupts intracellular processes leading to neurodegeneration in familial PD.

Interestingly, parkin mutations in transgenic mice do not cause nigrostriatal degeneration (Goldberg *et al.*, 2003; Itier *et al.*, 2003; Perez and Palmiter, 2005; Von Coelln *et al.*, 2004). Further, the frequency of point mutations, deletions and duplications of parkin is similar in AR-JP (3.8%) and normal controls (3.1%) (Lincoln *et al.*, 2003). Taken together, these observations raise the possibility that additional factors, for example exposure to environmental substances or other gene alterations, might be necessary to trigger the development of parkinsonism in individuals carrying parkin mutations.

DJ-1

Missense and deletion mutations in the gene (chromosome 1p36, PARK 7) that encodes for a 189 amino acid/20 kDa protein called DJ-1 is responsible for an autosomal recessive early-onset form of parkinsonism (Bonifati, Oostra and Heutink, 2004; Bonifati *et al.*, 2003; Nagakubo *et al.*, 1997; van Duijn *et al.*, 2001). Since no additional mutations in DJ-1 have been reported, it is likely that this defect accounts for only a very small percentage of early-onset cases (Lockhart *et al.*, 2004). Clinically, DJ-1-linked PD is similar to parkin-related PD, namely early onset of symptoms (age 20–40 years), slow progression, presence of dystonia, levodopa-responsiveness, and the common occurrence of psychiatric disturbance. The neuropathological features of DJ-1 are not yet known.

In the CNS, DJ-1 is more prominent in astrocytes than neurons, and is present in the cytosol, nucleus and mitochondria of cells (Bandopadhyay *et al.*, 2004; Shang *et al.*, 2004). The normal function of DJ-1 is not known, but there is evidence to suggest that it acts as a sensor of oxidative stress and proteasomal damage (Taira *et al.*, 2004; Yokota *et al.*, 2003). Additionally, the molecular structure and *in vitro* properties of DJ-1 indicate that it might act as a molecular chaperone and a protease (Lee *et al.*, 2003; Olzmann *et al.*, 2004; Wilson *et al.*, 2004). Interestingly, DJ-1 interacts with parkin, CHIP and HSP70, suggesting a link to these proteolytic systems (Moore *et al.*, 2005).

The mechanism by which mutations in DJ-1 induces pathogenesis is unknown. The recessive pattern of inheritance raises the possibility that the mutations induce a loss of function of the protein. The PD-related mutations (e.g., L166P) destabilize and inactivate the protein, impair its proteolytic activity, and promote its rapid degradation by the proteasome (Olzmann *et al.*, 2004; Moore *et al.*, 2003). In cell cultures, overexpression of DJ-1 protects cells from oxidative stress, and knockdown of DJ-1 increases susceptibility to oxidative stress, endoplasmic reticulum stress and proteasomal inhibition (Taira *et al.*, 2004; Yokota *et al.*, 2003). Further, mutations in DJ-1 reduce its ability to inhibit the aggregation of α-synuclein both *in vitro* and *in vivo* (Shendelman *et al.*, 2004). Interestingly, deletion of DJ-1 in transgenic mice does not induce neurodegeneration (Goldberg *et al.*, 2005), suggesting that other factors might be involved in the pathogenic process in PD. Thus, one may speculate that mutations in DJ-1 might lead to a loss of its putative anti-oxidant, chaperone and proteolytic activity.

PINK1

More than 20 homozygous or compound heterozygous mutations in the gene (1p35–p36, PARK 6) that codes for a 581 amino acid/62.8 kDa protein, designated PINK1 (PTEN (phosphatase and tensin homolog deleted on chromosome 10)-induced kinase 1), are known to cause autosomal recessive early-onset PD (Hatano *et al.*, 2004; Healy, Abou-Sleiman and Wood, 2004; Valente *et al.*, 2004; Valente *et al.*, 2001; Valente *et al.*, 2002). Clinically, this form of PD is characterized by early onset of symptoms (20–40 years), slow progression and a good response to levodopa (Healy, Abou-Sleiman and Wood, 2004; Valente *et al.*, 2001). Late-onset forms of the disease that resemble sporadic PD have also been described.

PINK1 is localized to mitochondria but additional studies are required to determine its precise cellular and anatomical distribution (Valente *et al.*, 2004). The normal function of PINK1 is unknown. It appears to be a serine/threonine protein kinase that phosphorylates proteins involved in signal transduction pathways. In cell culture studies, wild-type PINK1 prevents proteasome inhibitor-induced mitochondrial dysfunction and apoptosis, but this protection is lost with the mutations found in PD (Valente *et al.*, 2001). Interestingly, loss of function mutations in

PINK1 in *Drosophila* causes male sterility, muscle wasting, dopaminergic neuronal degeneration, and increased sensitivity to stressors (Clark *et al.*, 2006; Park *et al.*, 2006). These changes are associated with mitochondrial morphologic abnormalities, notably enlargement and fragmentation of christae. Thus, mitochondrial dysfunction appears to play a role in the pathogenesis of cell death associated with PINK1 mutations. Interestingly, defects in the parkin gene induced by knockout or by RNA interference also lead to alterations in mitochondrial morphology with dopamine neuronal degeneration, and enhance the degree of mitochondrial damage seen with PINK1 mutations (Park *et al.*, 2006;Yang, Gehrke and Imai, 2006). Further, overexpression of wild-type parkin restores mitochondrial morphology in the PINK1 mutant *Drosophila*, suggesting that PINK1 and parkin act in a common pathway that is critical for normal mitochondrial function (Yang, Gehrke and Imai, 2006). PINK-1 mutations have been found in normal control subjects who do not have clinical features of parkinsonism (Rogaeva *et al.*, 2004), again raising again the possibility that multiple factors may be necessary for the development of PD.

SPORADIC PD

Pathogenic Factors

The majority of PD cases occur sporadically, and are of unknown cause. It is thought that a combination of factors, acting sequentially or in parallel, and perhaps to varying degrees in each individual, might underlie the development of sporadic PD. The widely held view is that environmental toxins might cause PD in individuals who are susceptible due to their genetic profile, poor ability to metabolize toxins and/or advancing age. However, a specific infectious agent or toxin has not as yet been identified and the biological basis of possible vulnerabilities is unknown. Several pathogenic factors have been implicated in the disorder, including mitochondrial dysfunction, oxidative stress, excitotoxicity and inflammation (see reviews in reference Olanow, 2006). These defects may interact with each other and form a cascade or network of events that lead to apoptosis and cell death. It should be noted, however, that none of these pathogenetic factors have been established to be the primary source of neurodegeneration or for that matter to actually be involved in the cell death process (Olanow, 2007). It is certainly possible that as yet undiscovered pathogenic factors play a more critical role, and further that the pathogenic factors involved in cell death in an individual patient may differ.

Oxidative stress has been implicated in PD (Jenner, 2003) based on findings in the SNc of reduced levels of the major brain antioxidant reduced glutathione (GSH) (Sian *et al.*, 1994), increased levels of the pro-oxidant iron (Dexter *et al.*, 1991; Hirsch *et al.*, 1991; Sofic *et al.*, 1988), and evidence of oxidative damage to proteins, lipids and DNA (Alam *et al.*, 1997; Dexter *et al.*, 1989; Dexter *et al.*, 1994; Zhang *et al.*, 1999). It is noteworthy that oxidative stress can be linked to the various gene mutations associated with PD, and that oxidative stress can lead to mitochondrial damage and cause proteasome dysfunction (Ding and Keller, 2001; Jha *et al.*, 2002; Okada *et al.*, 1999). However, clinical trials of anti-oxidants have failed to provide benefit in PD patients (Parkinson Study Group, 1993). Mitochondrial dysfunction has been implicated in PD based on findings of reduced activity and decreased staining for complex I of the mitochondrial respiratory chain (Schapira *et al.*, 1990). Further, toxins that specifically damage complex I such as rotenone and MPTP selectively damage dopamine neurons and induce a model of PD (Langston *et al.*, 1983; Betarbet *et al.*, 2000). As mentioned above, it is also noteworthy that mutations in DJ-1 and parkin are associated with mitochondrial abnormalities. However, whether mitochondrial defects found in PD are primary or secondary is not known, and bioenergetic agents have not yet been established to have disease-modifying effects in PD. Recent interest has also focused on the possibility that calcium cytotoxicity might contribute to neurodegeneration in PD. Recent studies have also demonstrated that with maturation, SNc dopamine neurons convert from using sodium channels to 1.3 L-type calcium channels in order to maintain their pacemaker activities which could make these cells vulnerable to calcium cytotoxicity. It is noteworthy that blockage of these channels in cultured dopamine neurons causes them to revert to using sodium channels and is protective (Chan *et al.*, 2007).

Proteolytic Stress

Much of our own interest has focused on the possibility that cell death in PD results from proteolytic stress due to increased formation and/or a failure to clear misfolded proteins (McNaught *et al.*, 2001). There is abundant evidence for protein accumulation in areas that undergo neurodegeneration in PD. Quantitative western blot analyses demonstrate a marked increase in the levels of truncated, full-length, oligomeric and aggregates (of high and various molecular weights) of α-synuclein and other proteins in the SNc (Baba *et al.*, 1998; Tofaris *et al.*, 2003). These α-synuclein species have various post-translation modifications, including phosphorylation, glycosylation, nitration and ubiquitination (Tofaris *et al.*, 2003; Giasson *et al.*, 2000; Hasegawa *et al.*, 2002; Sampathu *et al.*, 2003). Accumulated α-synuclein can exist in a fibrillar form and cross-link with other proteins (e.g., by advanced glycation endproducts) and with neuromelanin (Fasano *et al.*, 2003; Munch *et al.*, 2000; Spillantini *et al.*, 1998). In addition to α-synuclein, many other proteins accumulate and are

post-translationally modified in the SNc and other brain regions in PD. There is a several-fold increase in levels of ubiquitin–protein conjugates and phosphorylated proteins in the SNc (McNaught *et al.*, 2002; Zhu *et al.*, 2002). There is also an increase in the content of oxidatively damaged proteins, as indicated by an elevation in the levels of protein carbonyls and protein adducts of 4-hydroxy-2-nonenal (derived from lipid peroxidation) (Alam *et al.*, 1997; Yoritaka *et al.*, 1996). Nuclear magnetic relaxation field-cycling relaxometry, which measures water solubility in tissues, has also been used to demonstrate a generalized increase in protein aggregates in the SNc in PD (Shimura *et al.*, 1999).

Lewy Bodies

The most striking evidence for protein dysfunction in PD is the presence of Lewy bodies, Lewy neurites and small protein aggregates in the SNc and other sites of neurodegeneration (McNaught *et al.*, 2002). The Lewy body is usually 8–30 μm in diameter, and in the SNc in PD it demonstrates an intensely stained central core with a lightly staining surrounding halo with the protein-binding dye eosin. Electron microscopy demonstrates a core comprised of dense granular material, which may contain punctate aggregates of ubiquitinated proteins, while the outer region is an arrangement of radiating filaments (7–20 nm in diameter) comprised of fibrillar α-synuclein and neurofilaments (Spillantini *et al.*, 1998; McNaught *et al.*, 2002). Immunohistochemical staining shows that Lewy bodies contain a wide range of proteins, the most prominent being α-synuclein (Spillantini *et al.*, 1998; McNaught *et al.*, 2002; Spillantini *et al.*, 1997), neurofilaments (Schmidt *et al.*, 1991), and ubiquitin/ubiquitinated proteins (McNaught *et al.*, 2002; Lennox *et al.*, 1989). Lewy bodies also contain components of the UPS (e.g., ubiquitination/de-ubiquitination enzymes, proteasomal subunits, and proteasome activators) (McNaught *et al.*, 2002; Ii *et al.*, 1997; Lowe *et al.*, 1990; Schlossmacher *et al.*, 2002), and heat shock proteins (e.g., HSP70 and HSP90) (McNaught *et al.*, 2002), but it is not known if the proteasome subunits unite to form a functioning proteasomal complex. Within Lewy bodies, proteins may be oxidized (Castellani *et al.*, 2002), nitrated (Giasson *et al.*, 2000; Good *et al.*, 1998), ubiquitinated and/or phosphorylated (Fujiwara *et al.*, 2002). It is noteworthy that not all proteins are found in Lewy bodies (e.g., synaptophysin, β-tubulin, and tau).

The consistent organization and composition of Lewy bodies suggests that they are unlikely to be formed in a random manner by the non-specific passive diffusion and coalescing of cellular proteins. Recent studies have led to the speculation that Lewy bodies could be formed and function in an aggresome-like manner (McNaught *et al.*, 2002; Ardley *et al.*, 2003; Kopito, 2000; Olanow *et al.*, 2004). Aggresomes are inclusion bodies that form at the centrosome in response to proteolytic stress. They serve to sequester, segregate and degrade excess levels of abnormal and potentially toxic proteins when these products cannot be cleared by other proteolytic systems (Kopito, 2000; Olanow *et al.*, 2004; Taylor *et al.*, 2003). In this respect, we and others have postulated that aggresomes appear to have a cytoprotective role (Olanow *et al.*, 2004; Taylor *et al.*, 2003; Kawaguchi *et al.*, 2003; Tanaka *et al.*, 2003). In support of this concept, inhibition of aggresome formation in cells undergoing proteolytic stress impairs the clearance of abnormal proteins and enhances cellular toxicity (Taylor *et al.*, 2003;Johnston, Illing and Kopito, 2002; Johnston, Ward and Kopito, 1998; Junn *et al.*, 2002). Lewy bodies resemble aggresomes and stain positively for γ-tubulin and pericentrin, specific markers of the centrosome/aggresome. These observations have led to the suggestion that Lewy bodies might be aggresome-related inclusions that are cytoprotective, and slow or halt the demise of some neurons in PD (McNaught *et al.*, 2002; Olanow *et al.*, 2004; Chen and Feany, 2005). This hypothesis is consistent with other lines of evidence indicating that Lewy bodies are not deleterious to cells (Gertz, Siegers and Kuchinke, 1994; Tompkins and Hill, 1997). Indeed, neurodegeneration can occur in the SNc without Lewy bodies in both sporadic and familial forms of PD (Mori *et al.*, 1998; Wakabayashi *et al.*, 1999), and Lewy bodies can be present without neurodegeneration (van Duinen *et al.*, 1999). Indeed, degeneration in disorders such as parkin, which lack Lewy bodies, appear to have an aggressive form of dopamine cell loss such that patients present at a very early age, perhaps because they are incapable of manufacturing these protective structures.

The ultimate fate of Lewy bodies and their host cell in PD seems to vary. Some Lewy bodies are observed in the cytoplasm of remaining neurons, while others are extruded into the extracellular space following destruction of the host neuron (Katsuse *et al.*, 2003). In addition, Lewy bodies may be internalized and destroyed by the lysosomal/autophagic system, as has been reported for aggresomes (Taylor *et al.*, 2003; Fortun *et al.*, 2003). Finally, Lewy bodies could be engulfed along with the host cell by activated microglia cells, which are observed at pathological sites in PD (Iseki *et al.*, 2000). Thus, while excess levels of abnormal proteins and aggregates can interfere with intracellular processes and alter cell viability, the formation of Lewy body inclusions might be a cytoprotective response aimed at segregating unwanted proteins to preserve cell viability.

While protein accumulation might occur as a result of increased production in genetic cases (e.g., mutant or excess production of wild-type α-synuclein), there is evidence that protein aggregation in sporadic PD might result from impaired clearance of unwanted proteins due to proteasomal dysfunction (McNaught *et al.*, 2001).

Altered Proteasomal Fuction

Proteasomes are multicatalytic enzymes primarily responsible for the degradation and clearance of unwanted proteins within eukaryocytic cells. Several studies have examined the structure and function of proteasomes in the PD. In one study comparing PD patients to controls, all three proteolytic activities of the 20S proteasome in the SNc were reduced by approximately 45%, but not in other unaffected brain areas (McNaught *et al.*, 2003; McNaught and Jenner, 2001). This defect was accompanied by a marked reduction in the levels of the 20S proteasome α-, but not β-, subunits in dopaminergic neurons of the SNc in PD. In addition, while levels of the PA700 proteasome activator are reduced in the SNc in PD, PA700 expression is increased in other brain regions, such as the frontal cortex and striatum, possibly as a compensation to a proteasomal toxin. This finding raised the possibility that the compensatory capacity of the 26S proteasome is also altered in PD. Further, levels of the PA28 proteasome activator are very low to almost undetectable in the SNc, compared to other brain areas, in both PD and normal subjects. Another study reported a 55% decrease in 20S proteasomal enzyme activity in the SNc, but not elsewhere in the brain of PD subjects (Tofaris *et al.*, 2003). Interestingly, this investigation used PD cases with relatively mild neuropathology, suggesting that proteasomal dysfunction occurs early in the pathogenic process. An additional study also demonstrated that proteasomal activity is not inhibited in extranigral areas in the brain of patients with sporadic PD (Furukawa *et al.*, 2002). Indeed, there was marked upregulation of proteasomal enzymatic activity in the striatum and cerebral cortex in PD patients compared to control subjects, consistent with our demonstration of increased expression of PA700 in these brain areas (McNaught *et al.*, 2003).

The basis of proteasomal dysfunction in sporadic PD is presently unknown, but could relate to encoding changes, oxidative damage, ATP depletion, and toxic modifications. Recently, DNA microarray analyses in the SNc in PD demonstrated a reduction in the mRNA levels of 20S proteasome α-subunits (PSAM2, PSMA3 and PSMA5), a non-ATPase subunit (PSMD8/Rpn12) and an ATPase subunit (PSMC4/Rpt3) of PA700 (Grunblatt *et al.*, 2004). Proteasomal subunits are susceptible to free radical-mediated injury and to mitochondrial damage, and this could account for secondary proteasomal damage in PD (Ding and Keller, 2001; Jha *et al.*, 2002; Okada *et al.*, 1999; Hoglinger *et al.*, 2003; Shamoto-Nagai *et al.*, 2003). Assembly/re-assembly of proteasomal components and their subsequent proteolytic activity require ATP (Hoglinger *et al.*, 2003; Eytan *et al.*, 1989; Hendil, Hartmann-Petersen and Tanaka, 2002; Imai *et al.*, 2003). Thus, primary or secondary inhibition of complex I activity could contribute to proteasomal dysfunction in PD. Interestingly, continuous administration of low doses of MPTP, which inhibits complex I through its active metabolite MPP+, was recently shown to impair proteasomal function and to cause neurodegeneration with inclusion body formation in mice (Fornai *et al.*, 2005). Abnormal proteins themselves may also interfere with proteasomal function in PD (Snyder *et al.*, 2003; Tanaka *et al.*, 2001; Bennett *et al.*, 2005; Hyun *et al.*, 2002; Lindersson *et al.*, 2004). Consistent with this possibility, recent studies have shown that incompletely or partially degraded α-synuclein directly inhibits proteasomal function (Liu *et al.*, 2005). Finally, naturally occurring environmental toxins could play a role in proteasomal dysfunction in PD (McNaught *et al.*, 2001).

The stage at which proteasomal dysfunction first occurs is not known. If this occurs early it might play a role in the initiation of the neurodegenerative processes, or if it occurs late it could contribute to the progression of the disease process. Either way, proteasomal dysfunction could be a central feature of cell death in PD and underlie the protein accumulation/aggregation and Lewy body formation that characterize PD. In support of this concept, we (McNaught *et al.*, 2002; McNaught *et al.*, 2002) and others (Fornai *et al.*, 2003; Rideout *et al.*, 2005; Rideout *et al.*, 2001; Miwa *et al.*, 2005) showed that proteasome inhibitors induced selective degeneration of dopaminergic cells in culture and nigrostriatal degeneration with motor dysfunction when injected directly into the SNc or striatum of rats. Importantly, neuronal death was associated with the accumulation of α-synuclein and ubiquitin, and the formation of intracytoplasmic Lewy body-like inclusions containing these and other proteins. Further, several studies have shown that lactacystin, PSI and other proteasome inhibitors can also induce degeneration of non-dopaminergic cells with inclusion body formation (Kisselev and Goldberg, 2001; Rideout and Stefanis, 2002). This observation has important implications for a role of proteasomal dysfunction in PD, since brain regions containing non-dopaminergic neurons also degenerate in the illness. Indeed, we and others recently demonstrated that systemic administration of proteasome inhibitors to rats induces degeneration of nigral dopaminergic neurons (McNaught *et al.*, 2004; Nair *et al.*, 2006; Schapira *et al.*, 2006; Zeng *et al.*, 2006). However, these results are somewhat controversial, as several groups have not been able to confirm these findings (Kordower *et al.*, 2006; Manning-Bog *et al.*, 2006). In addition, inhibition of proteasomal function can induce cellular, biochemical and molecular changes that are similar to those that occur in PD (Hoglinger *et al.*, 2003; Kikuchi *et al.*, 2003; Sullivan *et al.*, 2004). Further, there is a strong theoretical basis for considering that mutations in α-synuclein, UCH-L1 and parkin genes could theoretically lead to interference with UPS function and protein accumulation (Olanow and McNaught, 2006). Therefore, it is reasonable to suggest

that proteolytic stress could play a key role in the pathogenesis of PD, and that therapies designed to prevent the formation or enhance the clearance of misfolded proteins might have neuroprotective effects in PD.

Recent attention has also focused on the role of autophagy in clearing misfolded and unwanted proteins, raising the possibility that defects in this lysosomal system could also lead to protein accumulation and Lewy body formation (Martinez-Vicente and Cueve, 2007). No studies have as yet examined the status of the autophage system in PD.

Apoptosis

Regardless of the precise pathogenic mechanism, there is considerable evidence indicating that cell death in PD occurs by way of a signal-mediated apoptotic process. Numerous studies have found increased numbers of apoptotic nuclei in the SNc of PD patients in comparison to controls (Hirsch *et al.*, 1999). Further, Tatton and colleagues showed evidence of both chromatin clumping and DNA fragmentation in the same nigral neurons, virtually eliminating the possibility of false positive results (Tatton *et al.*, 1998). In addition, there is increased expression of pro-apoptotic signals such as caspase 3 and Bax and nuclear translocation of GAPDH in SNc neurons in PD (Tatton, 2000), supporting the concept that these cells have been injured and are in a pro-apoptotic state. Recent studies also demonstrate increased levels of p-p53 in PD nigral neurons compared to controls (Nair *et al.*, 2006). As a non-transcriptional increase in p53 is a key signal mediating cell death following proteasome inhibition, this may be a particularly relevant finding (Nair *et al.*, 2006).

CONCLUSIONS

The mechanism of cell death in PD remains unknown, despite many promising and even tantalizing clues. Small numbers of familial cases of PD are known to be caused by gene mutations, and mutations have now been identified in some cases with sporadic forms of PD. However, it is not at all clear that genetic factors cause the majority of sporadic cases. Environmental toxins have been implicated, but none has as yet been established to cause PD. It is possible that there are many different forms of PD, and many different causes. Post mortem studies have implicated oxidative stress, mitochondrial dysfunction, inflammation and exictotoxicity, but what role each of these play remains uncertain, and it is possible that some or even all are epiphenomena and do not directly contribute to cell death. More recently, attention has focused on the possibility that proteolytic stress due to impaired clearance of unwanted proteins is at the heart of cell death in PD. This is supported by the almost universal finding of protein accumulation and inclusion body formation in areas that undergo neurodegeneration. This concept is also supported by the observation that increased production of both mutant and wild-type α-synuclein can cause PD in humans and dopamine degeneration in animal models. Similarly, proteasome dysfunction is found in sporadic PD and proteasome inhibitors induce dopamine cell death with inclusion bodies in animal models. It is possible that many or all of these various pathogenic factors might interact in a cascade of events leading to cell death and that the precipitating factor may be different in different individuals. Many candidate targets for developing possible neuroprotective therapies have been identified, but to date no agent has been shown to have disease-modifying effects in PD. The identification of gene mutations that cause PD provide additional opportunities for identifying mechanisms that lead to cell death that hopefully will also be relevant to sporadic PD. Already, transgenic models that carry these mutations have begun to shed light on how cells might die in PD, although it is disturbing that no animal model to date fully replicates the dopaminergic and non-dopaminergic pathology of PD. Still, there is enthusiasm that with further research we will better understand why cells die in PD, develop animal models that replicate all of the features of the disease, and ultimately produce a drug which slows or stops disease progression.

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2

Physiology of Parkinson's Disease

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INTRODUCTION

Parkinson's disease (PD) is a common and disabling disorder of movement in humans. The main symptoms of PD are poverty and slowing of voluntary movements (akinesia and bradykinesia), muscle rigidity and low-frequency rest tremor. These clinical symptoms are due to dopaminergic denervation of the striatum—the input nucleus of the basal ganglia. However, how this striatal dopamine depletion perverts normal functioning to cause the clinical symptoms of PD has remained unclear. Recent work in tissue slice preparations, animal models and in humans with PD has demonstrated abnormally synchronized oscillatory activity at multiple levels of the basal ganglia-cortical loop. These excessive oscillations correlate with the motor deficits and their suppression by dopaminergic therapies; ablative surgery or deep brain stimulation ameliorate the motor symptoms of PD. Nevertheless, persistent and robust correlation between the basal ganglia oscillations and the Parkinsonian tremor has not been established in the minute-to-minute time scale.

In this chapter we will discuss the clinical physiology of PD symptoms, and will outline the hypothesis that abnormal basal ganglia oscillations and synchronization disrupt motor cortex and brain stem activity leading to akinesia and bradykinesia (core negative symptoms of the disease). The positive motor symptoms of Parkinsonism—rigidity and tremor—are probably generated by compensatory mechanisms downstream to the basal ganglia.

PARKINSON'S DISEASE: CLINICAL SYMPTOMS

In 1817 the English physician James Parkinson wrote an "Essay on the Shaking Palsy" providing the first clinical description of the motor symptoms of the disease now bearing his name (Parkinson, 1817). Today, PD is the most common basal ganglia movement disorder, and affects from 1% to as many as 5% of those in the 65 and 85 year age brackets, respectively (Van Den Eeden *et al.*, 2003).

Only 5% of PD cases can be attributed to specific genetic causes (Farrer, 2006; Benmoyal-Segal and Soreq, 2006). Most of the remaining cases cannot be attributed to metabolic or toxic causes either, and are therefore classified as idiopathic PD. The clinical manifestations of PD are the result of a neurodegenerative process that causes damage to multiple neuronal circuits (Braak *et al.*, 2003). The dopaminergic system is the most seriously damaged (Sulzer, 2007), but the noradrenergic, serotonergic and the cholinergic systems are also affected ((Jellinger, 1991), and see also Chapter 1: The Etiopathogenesis of Parkinson's Disease: Basic Mechanisms of Neurodegeneration, this volume).

Based on a very small number of clinical observations (six patients, including two whom he met on the street and a third he observed at a distance) James Parkinson pinpointed two of the most important and paradoxically related symptoms of PD—shaking and palsy. PD shaking is now characterized as a low frequency (4–7 Hz) tremor at rest. However, other non-harmonically related forms (e.g., postural/kinetic 7–12 Hz tremor) are very common in PD (Elble and Koller, 1990; Deuschl *et al.*, 2000). Palsy (or akinesia in modern terminology) is a hypokinetic disorder characterized by a poverty of voluntary goal-directed movements. Automatic (involuntary) movements, such as emotional facial expression are more severely reduced than instructed (voluntary) movements. Bradykinesia (slowness of voluntary movements) and related hypokinetic PD clinical features are also considered as akinetic symptoms. The other cardinal motor symptoms of PD include rigidity (increased muscular tone), and postural abnormalities,

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characteristically loss of postural reflexes. Cognitive and mood (emotional) deficits frequently (Ravina *et al.*, 2007) accompany the motor symptoms (see Chapter 6, Managing the Non-Motor Symptoms of Parkinson's Disease, this volume). However, in this review we focus on the pathophysiology of the three main motor symptoms of PD: akinesia, tremor and rigidity.

PD is not a homogenous disease, either across patients or even during the natural progression of a single patient. Unlike rigidity and akinesia, there is no correlation between the clinical severity of PD tremor and the severity of the dopaminergic deficit in the striatum or the clinical progression of the disease (Deuschl *et al.*, 2000). Temporally, tremor is a more episodic phenomenon, as opposed to other PD symptoms. The classic PD tremor is a rest tremor, since its amplitude decreases during voluntary action. PD tremor increases during mental and emotional stress and is absent during sleep (Elble and Koller, 1990; Deuschl *et al.*, 2000). Although our clinical impression is that akinesia and rigidity fluctuate less in the daily course of a PD patient, quantitative data is still missing. The heterogeneous nature of PD between patients is revealed by its broad spectrum and clinical sub-types. PD can present as a predominant resting tremor (T-sub-type) or primarily as marked akinesia and rigidity (AR-sub-type), sometime defined as the "postural instability gait difficulty (PIGD) sub-type" (Jankovic *et al.*, 1990; Burn *et al.*, 2006). As early as the nineteenth century, the great French neurologist Jean-Martin Charcot noted that tremor is not always present in human PD patients, and therefore suggested changing the name of the disease from *paralysis agitans* (Latin for shaking palsy) to *la maladie de Parkinson* (French for Parkinson's disease). T-sub-type PD patients have a better prognosis and slower disease progression than AR-sub-type patients (Jankovic *et al.*, 1990). Interestingly, most forms of non-idiopathic PD (as most animal models of the disease, see below) display akinesia and rigidity but not rest tremor (Rajput, 1995). Anti-cholinergic agents, which were the first drugs available for the symptomatic treatment of PD, tend to have better effects on tremor than on akinetic-rigid symptoms, whereas akinesia may show better and earlier response to dopamine replacement therapy (Tolosa and Marin, 1995). Several studies have indicated that the pathology of human T-type PD differs from the AR-type PD, with the retrorubral area (A8) more severely affected than the substantia nigra pars compacta (A9) in the tremor dominant form (Paulus and Jellinger, 1991). Taken together, it therefore seems logical to conclude that PD akinesia and tremor are not caused by a single pathophysiological process.

Physiology of PD Tremor

The frequency of tremor in a given PD patient is often remarkably similar in different muscles of the extremities and trunk (Hunker and Abbs, 1990). These observations led to the assumption that a common single central oscillator controls all tremulous muscles. Coherence analysis, however, has shown that although the *muscles* within one body part (e.g., a limb) are mostly coherent, the tremor in *different extremities*, even on the same body side, is almost never coherent (Raethjen *et al.*, 2000; Ben-Pazi *et al.*, 2001), indicating that different oscillators underlie PD tremor in the different extremities. This lack of tremor coherence may hint at mechanical or spinal reflex mechanisms rather than a single central oscillator. However, several studies have failed to demonstrate any frequency reduction of PD tremor after loading of the trembling limb (Elble and Koller, 1990; Deuschl *et al.*, 2000). Resetting experiments have been less conclusive. Initial studies indicated that resetting of tremor by mechanical perturbation is much more easily achieved in essential tremor than in PD tremor (Lee and Stein, 1981). However, later studies have shown that the resetting index varies significantly with the magnitude of the mechanical perturbation and with the tremor amplitude (Britton *et al.*, 1992). No significant difference was found in mean resetting indexes between PD tremor, essential tremor and normal subjects mimicking tremor when these mechanical factors were equalized. Resetting experiments with electrical stimulation of the median nerve or transcranial magnetic stimulation of the motor cortex did not show consistent resetting of the tremor rhythm when the periphery (median nerve) was stimulated, but depicted complete tremor resetting when the cortex was stimulated (see review in (Deuschl *et al.*, 2000)). Thus, PD tremor seems to be generated in the central nervous system (CNS) by more than one single (coupled) oscillator; however, the central oscillators and the tremor can be modulated by peripheral inputs.

In line with the CNS hypothesis on the origin of PD tremor, it has long been known that different lesions within the CNS can suppress PD tremor. Early attempts to remove parts of the motor cortex or its downstream projections were successful in suppressing tremor, but produced unacceptable side effects. The cerebellar receiving nuclei of the thalamus (e.g., the ventralis-intermedius, Vim) have traditionally been considered the optimal target for stereotaxic procedures for amelioration of PD and other tremors. Recently it has been demonstrated that chronic high-frequency stimulation of these same thalamic targets, as well as the subthalamic nucleus and the pallidum are all able to efficiently suppress Parkinsonian tremor and other motor symptoms ((Machado *et al.*, 2006), and see Chapter 7: Surgery for Parkinson's Disease, this volume).

Physiology of PD Rigidity

PD rigidity is often characterized as a uniform resistance to passive movements, but it may also have a cogwheel

intermittency due to superposition of tremor on the rigidity. Rigidity is evident throughout the full amplitude velocity and range of movements, in both flexor and extensor muscles (although more evident in flexor muscles). Early in the disease, rigidity is more evident in the axial (trunk) muscles, and later it extends to the distal limb muscles.

Increased passive mechanical stiffness of the muscles may play some role in PD rigidity (Dietz, 1987; Watts, Wiegner and Young, 1986), but its contribution to PD rigidity is probably minimal. Early studies (Pollock and Davis, 1930) showing that PD rigidity can be abolished by section of the dorsal root indicate that PD rigidity is maintained by spinal reflexes. Muscle spindle afferent activity of PD patients seems to be normally correlated with the degree of their muscular activation (Hagbarth *et al.*, 1975). Similarly, many spinal cord reflexes, including the tendon jerk, H-reflex and the tonic vibration reflex, and the excitability of the alpha motor neurons appear normal in Parkinsonian patients (Delwaide, Sabbatino and Delwaide, 1986). Thus, spinal cord responses to stretch seem to be close to normal in PD patients. However, passive stretch of muscle can evoke long-latency reflexes (Lee and Tatton, 1975; Tatton *et al.*, 1975), which are exaggerated in PD patients (Tatton *et al.*, 1984). Several studies indicate that the tonic muscle responses, initiated by slow and sustained stretch, and probably involving secondary muscle afferents, contribute more to PD rigidity than muscle reflexes triggered by brisk stretches. The long-latency reflexes are in many cases due to activation of long, for example, transcranial loops; although, as for the secondary muscle afferent reflex, they might be dependent on spinal loops.

In summary, most clinical human studies indicate that PD tremor, rigidity and akinesia, although sharing many common origins and similarities, have significantly distinct characteristics. The role of striatal dopamine depletion and the central generators seem to be much more important in akinesia. PD tremor and rigidity may be modulated by peripheral manipulation and by the activity of other central neuronal systems. It is highly possible that transmitter systems other than dopamine (e.g., cholinergic, serotonergic) or neural circuits other than the basal ganglia (e.g., cerebellum, red nucleus) play a critical additive role in these positive symptoms of PD.

ANIMAL MODELS OF PARKINSON'S DISEASE AND PARKINSONISM

Early animal models of PD were based on lesions of midbrain areas in monkeys (Poirier *et al.*, 1975). These anatomical lesions mainly produce akinesia and rigidity, but only rarely result in a spontaneous sustained tremor. Careful analysis of the correlation between the clinical symptoms and the extent of the lesion led to the conclusion that experimental rest tremor is the result of damage to the nigro-striatal dopaminergic projections as well as to the cerebellar outflow (to the red nucleus and thalamus). Damage to only one of these neuronal systems was not sufficient for reliable generation of tremor (Jenner and Marsden, 1984).

More modern animal models of PD have shifted from anatomical to chemical lesions. Early chemical—for example, the 6-hydroxydopamine (6-OHDA)—rodent models of PD were limited to dopaminergic damage, and mainly reproduced the main negative symptoms of PD; namely, akinesia (Wilms, Sievers and Deuschl, 1999). The more recently introduced primate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD (Burns *et al.*, 1983; Langston, Irwin and Langston, 1984) better mimics the clinical and the pathological picture of PD. Post-mortem examination of the brains of MPTP-treated primates reveals that the primary damage is to the dopaminergic system. However, as in human PD, other neuromodulators are also affected (Pifl, Schingnitz and Hornykiewicz, 1991). Rhesus monkeys treated with MPTP mainly exhibit the akinetic-rigid symptoms of PD (Burns *et al.*, 1983). Low-frequency (4–7 Hz) resting tremor is not readily replicated in MPTP-treated macaque monkeys; but some primate species, notably the vervet (African green) monkey often develop a prominent low-frequency tremor following MPTP injections (Bergman *et al.*, 1994; Raz, Vaadia and Bergman, 2000). It is important to note that the tremor usually appears several days after the development of clinical akinesia and rigidity (Bergman *et al.*, 1994; Heimer *et al.*, 2006). This order of presentation of clinical symptoms is reversed compared to human reports. It may be due to the fast induction of dopamine depletion in the MPTP model that impedes the development of compensatory processes found in the slowly evolving human disease. On the other hand, tremor is a much more overt phenomenon than akinesia and rigidity. A human patient or his/her family may first be aware of the slow development of PD by the more easily recognizable tremor. As in human studies there is a low coherence level between the tremor of the limbs of MPTP-treated vervet monkeys following dopamine replacement therapy (Heimer *et al.*, 2006).

THE CLINICAL ANATOMY OF THE BASAL GANGLIA AND THEIR CONNECTIONS

The major pathological event leading to the motor symptoms of PD, and especially to akinesia, is the death of midbrain dopaminergic neurons and their striatal projections. The striatum (composed of caudate, putamen and ventral striatum) is the main input stage of the basal ganglia, receiving inputs from all cortical areas as well as from many thalamic nuclei and even from the cerebellum (Hoshi *et al.*, 2005). Therefore, a good grasp of the pathophysiology of

PD depends on understanding the anatomy and physiology of the basal ganglia and dopamine networks.

The motor system has been classically described as consisting of two parts: the pyramidal and the extra-pyramidal sub-systems. The pyramidal system starts at the motor cortices, and, through the brain-stem pyramids, projects to spinal interneurons and alpha-motoneurons, innervating the distal parts of the limbs, and controls the execution of accurate, voluntary movements. In contrast, it was assumed that the extra-pyramidal system originates at the basal ganglia and the cerebellum, descends parallel to the pyramidal system, and innervates the spinal circuits involved with more axial (postural), automatic non-voluntary movements.

The revolution in anatomical methods during the second half of the twentieth century led to the conclusion that the basal ganglia are the feed-forward part of a closed loop connecting all cortical areas sequentially through the striatum, pallidum and thalamus with the frontal cortex. The frontal cortex projects downstream to the spinal level. This view of the basal ganglia networks assumes that there are two segregated internal pathways that start in the striatum and converge on the output structures of the basal ganglia (the internal segment of the globus pallidus (GPi), and the substantia nigra pars reticulata (SNr)). The "direct pathway" is a direct GABAeregic inhibitory pathway, whereas the "indirect pathway" is a polysynaptic disinhibitory pathway through the external segment of the globus pallidus (GPe) and the subthalamic nucleus (STN). The projection striatal neurons in the direct pathway express D1 dopamine receptors, whereas those in the indirect pathway express D2 dopamine receptors (Gerfen *et al.*, 1990). Dopamine has differential effects on the two striato-pallidal pathways: it facilitates transmission along the direct pathway via the D1 receptors and inhibits transmission along the indirect pathway via the D2 receptors (Gerfen *et al.*, 1990; Albin, Young and Penney, 1989). Since the output structures of the basal ganglia inhibit their thalamic targets, the direct pathway (cortex, striatum, GPi, thalamus, frontal cortex) contain two inhibitory paths (striatum to GPi and GPi to thalamus) and can be regarded as a positive feedback—facilitatory—loop and the indirect pathway, containing three inhibitory paths (striatum to GPe, GPe to STN and GPi to thalamus) connected by an excitatory path from the STN to the GPi is regarded as a negative feedback—inhibitory—loop. The dopamine facilitation of movements is therefore the combined results of its excitatory effects on the direct positive feedback loop and the inhibition of the indirect negative feedback loops.

Recently, single axon tracing anatomical studies have revealed an even more complex map of basal ganglia connectivity. Striatal neurons projecting to the GPi and SNr have been shown to send collaterals to the GPe (Levesque and Parent, 2005). The physiological evidence for the importance of the direct projections from the motor cortex to the STN (the "hyper-direct" pathway", (Nambu, 2004)) indicates that, like the striatum, the STN is an input stage of the basal ganglia. Moreover, the recently described feedback projections from the GPe to the striatum, as well as the GPe to GPi projection (Bolam *et al.*, 2000), strongly suggest that the GPe is a central nucleus in the basal ganglia circuitry rather than a simple relay station in the indirect pathway. The last twist in our understanding of the basal ganglia anatomy came with the re-discovery of basal ganglia outputs to brain-stem motor centers, such as the pedunculopontine nucleus and the superior colliculus (Delwaide *et al.*, 2000). Figure 2.1 summarizes the current view of the complex connectivity among the basal ganglia nuclei.

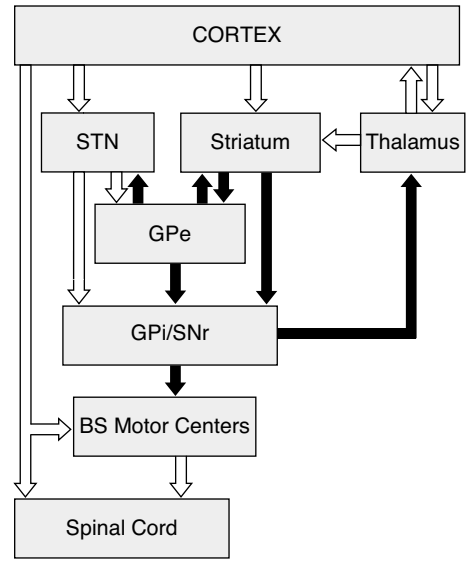

Figure 2.1 Schematic view of the connectivity of the cortex–basal ganglia–muscle networks. White arrows indicate excitatory connections, and black arrows denote inhibitory connections. The dopamine projections to the striatum are not shown. Abbreviations: STN, subthalamic nucleus; GPe, GPi, external, internal segment of the globus pallidus; SNc, SNr, substantia nigra pars compacta and reticulata; BS motor centers, brain stem motor centers, for example, superior colliculus and the pedunculopontine nucleus.

PHYSIOLOGICAL STUDIES OF THE BASAL GANGLIA IN NORMAL PRIMATES

Analysis of neuronal activity at the level of single spikes of single cells is probably the best way to study the computational physiology of a neuronal network. The background (during a quiet awake state) spiking activity of the basal

ganglia nuclei is very characteristic. The low-frequency discharge of striatal neurons (<1 spikes/s by the projection neurons and 4–10 spikes/s by the tonically active neurons (TANs), the cholinergic interneurons of the striatum resembles cortical discharge rates. However, this slow discharge contrasts strikingly with the high frequency (50–80 spikes/s) discharge of GPe, GPi and SNr neurons. In all these structures the firing rate is irregular (Poisson-like). Furthermore, neuronal oscillations are seldom observed in normal awake subjects (DeLong, 1971; Elias *et al.*, 2007).

Studies exploring the relationship between the spiking activity of basal ganglia neurons and movements have found even more unexpected results. The akinesia associated with PD suggests that the basal ganglia may play a critical role in movement initiation. Nevertheless, most basal ganglia neurons change their firing rate after initiation of stereotyped, over-learned, stimulus-triggered movements (Putamen: (Crutcher and DeLong, 1984; Alexander and Crutcher, 1990); GP: (DeLong, 1971; Anderson and Horak, 1985; Mink and Thach, 1991); SNr: (Schultz, 1986)), and do not have any exclusive or consistent relationships to movement parameters such as start/end, velocity or amplitude (Mink and Thach, 1991). The data regarding basal ganglia discharge related to voluntary, self-initiated movements (Romo, Scarnati and Schultz, 1992; Schultz and Romo, 1992) are still scarce and under debate. Together with the inconclusive findings of lesion studies of the output structures of the primate basal ganglia (Horak and Anderson, 1984; Mink and Thach, 1991; Kato and Kimura, 1992) the physiological results revealing late timing of basal ganglia discharge has led to the surprising conclusion that the basal ganglia do not initiate movements (Mink, 1996). Rather, basal ganglia inhibitory control of the thalamic-cortical network probably enables and facilitates the execution of learned and semi-automatic movements. Recent advances of our understanding of the role of striatal dopamine (Schultz, 2007; Calabresi *et al.*, 2007; Arbuthnott and Wickens, 2007) and acetylcholine (Morris *et al.*, 2004; Cragg, 2006; Wang *et al.*, 2006) indicate that these movements are those acquired and shaped by implicit learning (Bar-Gad, Morris and Bergman, 2003; Daw and Doya, 2006).

PHYSIOLOGICAL STUDIES IN THE BASAL GANGLIA NETWORKS OF MPTP-TREATED PRIMATES AND HUMAN PATIENTS WITH PARKINSON'S DISEASE

Early physiological studies of Parkinsonian MPTP-treated monkeys reported increases in the discharge rate within the GPi (Miller and DeLong, 1987; Filion and Tremblay, 1991) and the STN (Bergman *et al.*, 1994) as opposed to a decrease in discharge rate in the GPe (Miller and DeLong, 1987; Filion and Tremblay, 1991). Reversed trends of pallidal discharge rates in response to dopamine replacement therapy have been reported in both human patients (Hutchinson *et al.*, 1997; Merello *et al.*, 1999) and primates (Heimer *et al.*, 2006; Filion, Tremblay and Bedard, 1991; Papa *et al.*, 1999). The possible role of these rate changes in the pathophysiology of PD has been verified by the subsequent findings showing that inactivation of STN and GPi could improve the motor symptoms in Parkinsonian animals (Bergman, Wichmann and DeLong, 1990; Aziz *et al.*, 1991) and human patients (Machado *et al.*, 2006).

These findings contributed to the formulation and the popularity of the direct/indirect model of the basal ganglia (see above). Nevertheless, several studies have failed to find the expected significant changes of firing rates in the pallidum (Boraud *et al.*, 2002), thalamus (Pessiglione *et al.*, 2005) or motor cortical areas (Goldberg *et al.*, 2002) of MPTP monkeys. This and other inconsistencies with the assumptions and the predictions of the direct/indirect rate model have attracted more attention to the potential role of other aspects of neuronal activity, such as firing patterns and neuronal synchronization in the pathophysiology of PD. MPTP monkeys show an increase in the fraction of basal ganglia neurons that discharge in bursts. These bursts are either irregular or oscillatory and have been found in the STN, GPe, GPi and also in the primary motor cortex (Bergman *et al.*, 1994; Raz, Vaadia and Bergman, 2000; Miller and DeLong, 1987; Filion and Tremblay, 1991; Goldberg *et al.*, 2002; Boraud *et al.*, 2001; Wichmann and Soares, 2006). In most cases the cells tend to oscillate at the tremor frequency as well as at double or even triple the tremor frequency (Bergman *et al.*, 1994; Raz, Vaadia and Bergman, 2000; Heimer *et al.*, 2006). Nevertheless, these studies have failed to reveal a significant fraction of neurons whose oscillations are consistently coherent with the simultaneous recorded tremor (Raz, Vaadia and Bergman, 2000; Heimer *et al.*, 2006). Both STN inactivation (Wichmann, Bergman and DeLong, 1994) and dopamine replacement therapy (Heimer *et al.*, 2006) significantly ameliorate the 4–7 Hz tremor and reduce the GPi 8–20 Hz oscillations, indicating the critical role of beta range, higher frequency oscillations, rather than the tremor frequency oscillations, in tremor generation.

Physiological studies of simultaneously recorded neurons in the pallidum (Raz, Vaadia and Bergman, 2000; Heimer *et al.*, 2006; Nini *et al.*, 1995; Heimer *et al.*, 2002), as well as in the primary motor cortex (Goldberg *et al.*, 2002), among striatal TANs and between TANs and pallidal neurons (Raz *et al.*, 1996; Raz *et al.*, 2001) in MPTP-treated monkeys demonstrate that their pair-wise cross-correlograms become peaked and oscillatory. The abnormal pallidal synchronization decreases in response to dopamine replacement therapy (Heimer *et al.*, 2006). In

most cases, the maximal power of the synchronous oscillations was found to be at double the tremor frequency (Raz, Vaadia and Bergman, 2000; Heimer *et al.*, 2006; Raz *et al.*, 1996; Raz *et al.*, 2001). Similar, double tremor frequencies were observed also in the STN of both akinetic-rigid- and tremor-dominant human PD patients undergoing DBS procedures (Moran, Bergman, Israel, Bar-Gad, unpublished results). These correlation studies therefore suggest that striatal dopamine depletion induces abnormal coupling of basal ganglia loops, but mainly in a higher, beta frequency range.

As in the MPTP primate, single unit studies of the basal ganglia of human PD patients (performed during electrophysiological mapping of the target area for therapeutic implantation of stimulating electrodes) have reported a high fraction of GPi and STN cells oscillating at the tremor frequency (Hutchison *et al.*, 1997; Weinberger *et al.*, 2006; Levy *et al.*, 2002). However as in the primate, the human studies (Lemstra *et al.*, 1999; Hurtado *et al.*, 1999; Hurtado *et al.*, 2005) show that these oscillations are not fully coherent with the simultaneously recorded tremor. Advanced time-dependent phase correlation techniques have been applied to pairs of tremor-related GPi single units and EMG of PD patients undergoing stereotactic neurosurgery. Analysis using short sliding windows shows that oscillatory activity in both GPi oscillatory units and muscles occurs intermittently over time. There is partial overlap in the times of oscillatory activity but, in most cases, no correlation has been found between the times of oscillatory episodes in the two signals. Phase-locking analysis reveals that pallidal oscillations and tremor are punctuated by phase slips, which have been classified as synchronizing or desynchronizing. The results of this high-level quantitative characterization of PD tremor and pallidal oscillations can be explained by either a very dynamic connectivity from the basal ganglia to the periphery, or by tremor generators downstream of the basal ganglia. The sharp contrast between this transient and inconsistent pallidal-tremor synchronization and the high synchronicity found between thalamic Vim neurons and the tremor (Lenz *et al.*, 1988) suggest that pallidal neurons cannot be viewed as the tremor generators, or as simple encoders of the proprioceptive feedback of the tremor.

PHYSIOLOGICAL STUDIES OF POPULATION ACTIVITY IN THE BASAL GANGLIA NETWORKS OF DOPAMINE-DEPLETED ANIMAL MODELS AND HUMAN PATIENTS WITH PARKINSON'S DISEASE

Synchronization of basal ganglia neuronal activity is also evident in the local field potentials (LFP) recorded in the basal ganglia of PD patients through macro-electrodes used for high-frequency stimulation of these structures. These oscillations occur mainly in the high beta range (15–30 Hz) and following treatment with levodopa shift to higher frequencies (40–70 Hz) in the gamma range (Kuhn *et al.*, 2006; Hammond, Bergman and Brown, 2007). In line with both the single unit and the LFP studies, magnetoencephalographic (MEG) studies (Timmermann *et al.*, 2003) of tremor-type PD patients have revealed a strong coherence between the tremor and activity in the motor and sensory cortices and the cerebellum at tremor frequency, and an even stronger coherency at double tremor frequency. Spectra of coherence between thalamic activity and cerebellum as well as several other brain areas have revealed additional broad peaks at around 20 Hz.

Studies of LFPs recorded from the frontal cortex and STN of rats following 6-OHDA lesions of midbrain dopamine neurons (Sharott *et al.*, 2005) have revealed significant increases in the power and coherence of beta-frequency oscillatory activity. Administration of the dopamine receptor agonist apomorphine to these dopamine-depleted animals suppressed the beta-frequency oscillations, and increased coherent activity at gamma frequencies in the cortex and STN. Thus, the pattern of synchronization between population activity in the STN and cortex in the 6-OHDA-lesioned rodent model of PD is closely paralleled to that seen in PD human patients and the primate MPTP model.

Recordings of both LFPs and multi-neuronal activity from microelectrodes inserted into the STN in PD patients during functional neurosurgery suggests that the discharges of some of the neurons in the STN are locked to beta oscillations in the LFP (Kuhn *et al.*, 2005). LFPs probably represent the synaptic input to a neural structure and its subthreshold slow activity. The discrepancies between LFP oscillatory activity as compared to neuronal activity (both in their frequency domains, prevalence and power) may be due to the fact that even quite strong synchronized inputs (as reflected in the LFP) may lead to weak correlations in the neuronal discharge. On the other hand, correlations can be very low at the neuronal pair-wise level, but still summate and become substantial at the population (LFP) level (Goldberg *et al.*, 2004; Schneidman *et al.*, 2006). We therefore conclude that the hallmark of the dopamine-depleted basal ganglia network is its abnormally strong synchronized state.

ADVANCED NEUROSURGICAL TREATMENTS OF PARKINSON'S DISEASE: WHAT THEY TELL US ABOUT PD PHYSIOLOGY

The most important prediction of the dual pathway model of the basal ganglia (see above) and the finding of

abnormal tonic firing rate in the basal ganglia of MPTP monkeys was that ablation or inactivation of the GPi or STN should lead to the alleviation of parkinsonian symptoms. Inactivation of the GPi would remove the excessive inhibitory drive to the frontal cortex (via the thalamus) and the resulting suppression of voluntary movements. Similarly, destroying the STN would remove the excessive excitatory drive to the GPi. This would normalize GPi output, thereby reducing the inhibition of the frontal cortex.

These predictions were in fact supported by early pallidal ablation therapy of PD. Pallidotomy, which had been largely abandoned since the advent of levodopa therapy, was shown to be very successful at alleviating akinesia and rigidity of human patients (Laitinen, Bergenheim and Hariz, 1992; Lozano *et al.*, 1995; Vitek and Bakay, 1997). Injection of excitatory amino acid antagonists into the GPi of MPTP-treated monkeys was shown to reverse the motor symptoms of parkinsonism (Graham *et al.*, 1990). STN permanent lesions and temporal inactivation (by injection of the GABA agonist, muscimol) were shown by several groups to reverse parkinsonian symptoms in MPTP primates (Bergman, Wichmann and DeLong, 1990; Wichmann, Bergman and DeLong, 1994; Aziz *et al.*, 1991; Guridi *et al.*, 1993; Guridi *et al.*, 1996). The primate results set the stage for the introduction of subthalamotomy as a treatment for PD patients (Obeso *et al.*, 1997; Gill and Heywood, 1997) and for the development of deep brain stimulation (DBS) of the STN as an alternative treatment for PD. DBS of the STN and the GPi have been successful at alleviating parkinsonism both in PD patients (Limousin *et al.*, 1995; Pollak *et al.*, 1996; Kumar *et al.*, 1998) and in MPTP primates (Benazzouz *et al.*, 1996). Today DBS is preferred by neurosurgeons over ablative surgery due to its reversibility and parameter tuning capabilities (Lang and Lozano, 1998; Gross *et al.*, 1999). There are indications that GPi stimulation is best suited to treat levodopa-induced dyskinesia (LID) and rigidity, whereas STN stimulation is best fit for treatment of akinesia, rigidity and tremor (Limousin *et al.*, 1995; Pollak *et al.*, 1996; Kumar *et al.*, 1998; Benabid *et al.*, 1994). However, significant reduction of the dopamine replacement therapy is better achieved with STN DBS, and therefore this is the procedure of choice in most centers. An updated description of surgical techniques and patient management during DBS surgery for advanced PD has been published as Supplement 14 to Volume 21 of *Movement Disorders*, 2006 (see also Chapter 7: Surgery for Parkinson's Disease, this volume).

The mechanism of DBS on neuronal discharge is still debated. Early studies (Ranck, 1975; Stoney, Thompson and Asanuma, 1968) showed that when metal microelectrodes are used, the susceptibility of nerve fibers is much higher than that of the cell bodies, suggesting that microstimulation activates bypassing fibers. In any case, the classical interpretation of the effect of micro-stimulation on the motor cortices has been that the stimulation induces excitation of the cortex or cortico-spinal axonal pathways and thus evokes movement of different body parts. Since the effect of DBS in PD is paradoxically similar to the effect of lesions (e.g., neuronal inactivation) the question of the actual effect of DBS has been recently addressed both in the MPTP primate model and in human PD patients. In the MPTP primate, DBS in the GPi has been shown to reduce the discharge rates to within the normal range (Boraud *et al.*, 1996). Similar results have been described in rodent studies (Benazzouz *et al.*, 2000) and in human PD patients (Dostrovsky *et al.*, 2000; Maltete *et al.*, 2007). There are several possible mechanisms to explain these results: depolarization block of the stimulated neurons, stimulation of bypassing inhibitory pathways, and/or induction of GABA release from the terminals of the GPe projection neurons, thereby inhibiting the target GPi neurons. A major difference between human DBS and primate or human micro-stimulation is that the former uses macro-electrodes (with impedances in the ranges of a few kΩ). The current densities around the stimulating macro-electrodes are much smaller than with micro-stimulation, and therefore the mechanisms of clinical macro deep brain high-frequency stimulation are still debated.

Application of DBS to the STN of MPTP primates has also generated conflicting results. In one early study, DBS was found to differentially affect the mean discharge rates in the GPe and GPi for several hours after the DBS: it caused an increase in the former and a decrease in the latter (Hayase *et al.*, 1996). In a more recent study the mean firing rates increased in both pallidal segments during STN DBS (Hashimoto *et al.*, 2003) and GPi DBS lead to suppression of thalamic discharge (Anderson, Postupna and Ruffo, 2003). DBS with macro-electrodes has been recently shown in rats *in vivo* to directly depolarize the membrane potential of the STN neurons (Garcia *et al.*, 2003). Thus, DBS may enforce high-frequency homogenous (in time and space) discharge in the basal ganglia and "jamming" of their abnormal output rather than inhibition (Benabid, 2003; Bar-Gad *et al.*, 2004; Miocinovic *et al.*, 2006).

In summary, the precise neuronal mechanism of DBS (e.g., effects on neurons or fibers in the area of electrode, effect of the stimulated area or remote structures, etc.) is still an open issue. In any case, the similar effects of thalamic and STN/GPi DBS, despite the GABAergic connectivity between these two structures, supports the hypothesis that the main effect of DBS is mediated by enforcing a constant spatio-temporal firing pattern, rather than modulation of discharge rate, on pallidal or thalamic neurons. This enables the cortical network to ignore the pathological synchronized oscillatory "noisy" drive of the basal ganglia, and to provide a compensation for their missing gating signal.

SUMMARY AND CONCLUSIONS

In this review we have explored the possible relationships between basal ganglia neuronal activity and PD clinical symptoms. PD is the result of dopamine depletion in the striatum—the input stage of the basal ganglia. Akinesia, rigidity and rest tremor are the major motor symptoms of PD. Nevertheless, cumulative clinical and experimental evidence support the view that they are not generated by identical neuronal mechanisms. Following striatal dopamine depletion, many basal ganglia neurons develop synchronous oscillations at the tremor frequency and at their higher harmonics, as well as in the beta range. However, the PD tremor does not strictly follow the basal ganglia oscillatory activity. The recent demonstration of anatomical connections between the cerebellum and the basal ganglia, both at the cortical, striatal and brain stem level may suggest that the cerebellum is associated with the movement disorders classically described as pure basal ganglia disorders. The critical role of the cerebellar output in the generation of PD tremor has been demonstrated by primate lesion studies and the efficacy of Vim intervention in treatment of PD tremor. These findings, along with the physiological studies of the normal basal ganglia indicating that the basal ganglia do not initiate movements, strengthen the supposition that the abnormal synchronous beta-range (higher than tremor frequency) oscillations in the basal ganglia provide noisy input to the frontal cortex. The abnormal driven activity of the frontal cortex hinders the normal functioning of the motor cortices and hence leads to PD akinesia. We further suggest that rigidity and tremor are generated by basal ganglia downstream mechanisms struggling to compensate for PD akinesia. The stronger association between akinesia and rigidity as opposed to the more independent nature of PD tremor may indicate that rigidity and tremor are generated by different mechanisms.

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