Secrets of the shaking palsy

Parkinson's disease might have much in common with Alzheimer's disease, prion diseases and other protein-aggregation disorders. **Jim Schnabel** investigates.

The first thing you notice might be a slight twitching in your finger. You imagine that it will go away, but it worsens, turning into a tremor of your hand and eventually your entire arm. Your ever-clenching muscles begin to feel stiff, sore and weak. You find it harder to lift things. In time, you become reluctant even to get up from a chair and walk. Your posture begins to slump forward. Your gait shortens. Your speech slows. Your face, once expressive, becomes an immobile mask.

Over the years, in the absence of medication, these symptoms will inexorably spread and worsen. Eventually, as the British physician James Parkinson wrote in An Essay on the Shaking Palsy in 1817, "The submission of the limbs to the direction of the will can hardly ever be obtained in the most ordinary offices of life"— such as feeding oneself, or grasping and lifting a glass of water. Even muscles that normally work automatically are affected: swallowing becomes difficult, drooling becomes common and, at the other end of the line, moving the bowels requires, as Parkinson put it, "stimulating medicines of very considerable power."

To this list of common symptoms noted by Parkinson, modern neurologists have added a diminished sense of smell, sleep disorders, restless-leg syndrome, depression, anxiety, hallucinations, fatigue, impotence, visual problems and, in late stages, a form of dementia. The actor Michael J. Fox, now a 20-year veteran of Parkinson's disease, has called it "the gift that keeps on taking."

Proximate causes

To the eponymous Dr Parkinson, it seemed that the malady was caused by damage in a muscle-controlling region of the brain. Yet nothing in his patients' histories pointed to a clear cause of this damage.

Nearly 200 years later, this mystery remains largely intact. Parkinson's disease obviously is influenced by the ageing process; in most populations, relatively few diagnoses are made in people younger than 40. Gender matters too, possibly via a protective effect of oestrogen: women have only two-thirds the risk of men of getting Parkinson's disease. Yet modern neurologists still describe the vast majority of Parkinson's disease cases as idiopathic, which is to say, they do not know the ultimate cause.

The most obvious progress has been made in understanding the proximate causes, particularly the cell deaths that explain the classic motor symptoms of the disease. These deaths occur in an evolutionarily ancient clump of neurons near the brainstem known as the substantia nigra (black substance), which normally synthesizes the neurotransmitter dopamine and pumps it into movement-regulating brain regions. Until 50 to 70% of these dopaminergic neurons have been killed by the disease, downstream regions can compensate to some extent, and symptoms are seldom noticed. Eventually, however, neurons in the striatum, immediately downstream of the substantia nigra, start to die in large numbers too. A complex cascade of dysfunction ensues, and the signals that allow or restrain muscle movements become abnormal — generating an involuntary tremor and making voluntary movements harder to initiate and carry out.

A popular misconception is that Parkinson's disease results merely from the deaths of substantia nigra dopaminergic neurons. It is true that these neurons seem most vulnerable to the disease. But autopsy studies have found neuronal losses and pathological signs of Parkinson's disease — insoluble clumps of protein known as Lewy bodies — in several other regions of the brain and even in the peripheral nervous system.

Some of the affected neurons are dopamine producers as, for example, in the ventral tegmental area, close to the substantia nigra. Ventral tegmental area neurons supply dopamine to the limbic system, "which is concerned with mood and sleep among other things," says Ray Chaudhuri, a consultant neurologist at King's College Hospital, London. "So a drop in limbic dopamine affects these functions as well, right from the beginning of the disease."

Other affected regions of the nervous system have little or no connection to dopamine. These include noradrenaline-making neurons in the locus coeruleus, serotonin-making neurons in the raphe nuclei, acetylcholine-making neurons in the nucleus basalis of Meynert and neurons that produce a variety of neurotransmitters in the dorsal motor nucleus of the vagus. Also affected are non-dopaminergic neurons in the olfactory bulb and intestines. "It's clear now that the Parkinson's pathology is widespread," says Michel Goedert, a neurobiologist at the Medical Research Council Laboratory of Molecular Biology in Cambridge, United Kingdom.

Autopsy studies by neuroanatomist Heiko Braak and colleagues at Goethe University in Frankfurt have suggested that this pathology usually flows in a characteristic pattern, with the first affected brain regions being the olfactory system and the gut-connected dorsal motor nucleus of the vagus[1]. Researchers are looking at the loss of the sense of smell and constipation, together with other early symptoms such as depression and so-called rapid eye movement sleep-behaviour disorder, as warning signs of Parkinson's disease that might make possible diagnosis and treatment years before current methods allow (see page S11).

α-synuclein

What is the ultimate cause of all these symptoms? What is it that loads certain neurons with Lewy bodies and kills them over time? Unfortunately, just as James Parkinson found, the easier kinds of analysis have not been very fruitful. Epidemiologic studies have highlighted a number of possible factors, hinting for example that higher pesticide exposure increases Parkinson's disease risk, whereas cigarette smoking and coffee drinking lower it. Environmentally, however, there has been no 'smoking gun'. At the same time, studies of twins have suggested that Parkinson's disease usually does not run in families.

Yet it does run in some families in forms that closely resemble the more common sporadic form of Parkinson's disease, and, since 1997, genetic studies of these families have yielded some key clues. In that year, a team led by researchers at the US National Institutes of Health made headlines when they linked an early-onset form of Parkinson's disease in several Greek and Italian families to mutations in a gene on chromosome 4 (ref. 2). The gene normally codes for a short protein called α-synuclein.

α-synuclein was found to reside predominantly in the 'presynaptic' or output terminals of neurons. In substantia nigra dopaminergic

CHARLES KRUPA/AP PHOTO

Actor Michael J. Fox has suffered from Parkinson's disease for more than 20 years.

neurons, it seemed to play a role in the release of dopamine from these terminals. Yet its precise function was (and still is) unknown. Almost immediately after the gene findings were made, a group led by Goedert and Maria Grazia Spillantini at the University of Cambridge determined that the tiny fibrils making up Lewy bodies are, for the most part, constituted from stuck-together α-synuclein proteins[3].

Further research uncovered two other α-synuclein mutations in Parkinson's disease families, and it was eventually determined that two of the three mutations made α-synuclein proteins more likely to aggregate into insoluble fibrils. A further set of familial cases harboured α-synuclein genes that were not mutated but appeared in duplicate or triplicate, so that their cells simply overproduced the protein — increasing its concentration and, again, making it more likely to start sticking together.

"All this brought α-synuclein to centre-stage in the disease," Goedert recalls.

Clues to α-synuclein's role

One of the first steps in investigating α-synuclein's role was to produce knockout mice that lacked the gene. Surprisingly, such mice turned out to develop relatively normally. They seemed healthy, had a normal lifespan, did not develop any Parkinson's disease-like condition and did not overproduce other synuclein proteins to compensate for their lack of α-synuclein. In fact, they held up better than normal mice when challenged with a toxin that predominantly kills dopaminergic neurons in the substantia nigra. This implied that α-synuclein is not a protective factor the loss of which leads to Parkinson's disease, but instead somehow drives the disease. Its presence in a stuck-together form in disease-affected brain regions hinted, moreover, that its harmfulness arises when individual α-synuclein proteins begin to aggregate.

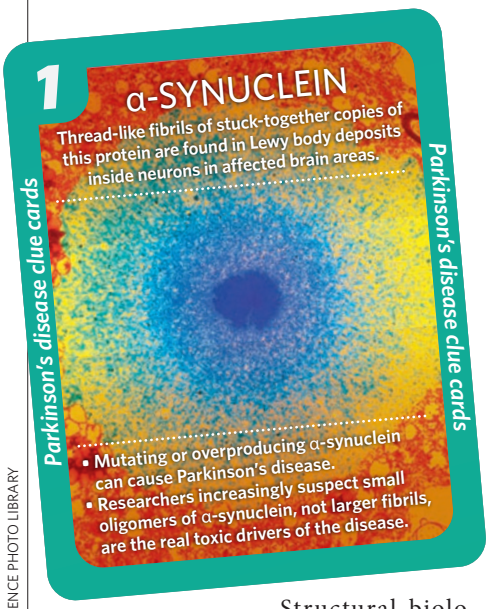
Further studies of families with Parkinson's disease added weight to this idea. Two other genes found to be mutated in such families turned out to code for proteins (parkin and UCH-L1) involved in the ubiquitin–proteasome system, which is a major waste-disposal system inside cells. A third such gene coded for a protein (LRRK2) that appears to be needed by the autophagy–lysosome pathway, which is a second major waste-disposal system. Both these disposal systems would be expected, when working normally, to prevent an excessive build up of α-synuclein aggregates.

Parkinson's as an amyloid disease

In 2008, two research teams, in Europe and America, separately reported unexpected findings in the autopsied brain tissue of several Parkinson's disease patients[4,5]. Years earlier, the patients had received experimental transplants of embryonic, dopamine-producing neurons. The hope had been that these cells would permanently boost dopamine levels in the motor-regulating region of the striatum, thus reversing some of the major symptoms of the disease.

The patients had seemed to benefit for a time. At autopsy, however, the researchers found that many of the transplanted neurons — which by then were chronologically no older than their teens — contained Lewy body-like inclusions made of α-synuclein fibrils, and otherwise showed signs of degeneration. The implication was that, over time, the Parkinson's disease process had spread from the hosts' brain cells to the young transplanted cells, in an infection-like manner.

For some researchers, this idea was not as far out as it might have seemed. In fact, a general theory concerning disease-associated protein aggregates had been developing since the late 1990s. These aggregates were formed of different proteins in different diseases: α-synuclein in Parkinson's disease, Lewy body dementia and multiple system atrophy; amyloid-β and tau protein in Alzheimer's disease; polyglutamine protein in Huntington's disease; and amylin protein in type 2 diabetes, to name just a few. Despite their different protein sequences, these aggregates — in their final, insoluble forms — exhibited the same 'β-pleated sheet' pattern in atomic-structure studies. As such, they were known as amyloids.



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Structural biologists had determined that amyloid formation can begin when a certain type of sticky segment within a protein becomes exposed enough to glue itself to identical segments on other copies of the same protein. Most proteins are large enough to tuck such segments safely away within their folds. Amyloid-prone proteins such as α-synuclein, however, tend to be small and unfolded[6].

It seems that when a few of these sticky segments come together to form a stable nucleus — perhaps a rare event — they begin to attract any accessible segment within range. Initially, these segments and their host proteins form small and still-soluble groupings known as oligomers. Some or all of these go on to form long, insoluble stacks or protofibrils, which might break in two, with each fragment forming a new nucleus for growth. In this way the amyloid-forming process can spread through tissues as far as the supply of vulnerable proteins and the weakness of cellular defences allow.

The thinking was that in some cases this process would simply overload tissues with insoluble amyloidal 'gunk', whereas in other cases it would generate aggregates that were actively toxic to cells. The clearest examples of the latter were seen in prion disorders such as Creutzfeldt–Jakob disease (CJD). CJD prion clusters can render the brain shrunken and sponge-like within months. They also are hardy enough to survive digestive enzymes and ordinary detergents — which is why CJD is so transmissible.

More recently, there has been direct evidence that aggregates associated with common neurodegenerative diseases can spread 'infectiously' from neuron to neuron, even if they are much less hardy and transmissible than prions. Two teams of researchers, one including Goedert, separately reported this in 2009 for aggregates of Alzheimer's disease-associated tau protein[7,8].

In the same year, a team of researchers from the University of California, San Diego and South Korea's Konkuk University reported similar findings for α-synuclein aggregates in mouse neuronal cell cultures. The cells that contained these α-synuclein aggregates soon died[9].

Like many other researchers, Goedert now suspects that α-synuclein aggregation is the primary event in Parkinson's disease — perhaps triggered by toxins or other environmental factors inside the intestines and sinuses. Most of the pathology in the brain appears typically to emanate from the dorsal motor nucleus, which is connected to intestinal neurons via the vagus nerve. In principle, α-synuclein aggregation could spread up this nerve and thereafter into most of the other affected regions. "The apparent selective killing of neurons in the disease," says Goedert, "could come from the fact that some neurons are better than others at defending themselves against these aggregates."

Precisely how do these aggregates harm neurons? That remains one of the big unanswered questions in Parkinson's disease research. Even if the α-synuclein fibrils found in affected neurons are not biochemically toxic, says Goedert, they might cause harm simply by taking up space and blocking normal transport pathways, especially within the narrow confines of axons and dendrites.

Some researchers suspect that a more direct toxicity is at work, not from the larger, insoluble fibrils but from the smaller, still-soluble oligomers. Cellular and animal-model studies suggest that α-synuclein oligomers can be toxic[10] — just as amyloid-β oligomers seem to be toxic in Alzheimer's disease and prion oligomers seem to be toxic in CJD.

Moreover, the three α-synuclein mutations so far seen in familial Parkinson's disease all appear to boost the protein's propensity to form oligomers, even if they do not always boost the formation of insoluble fibrils. Such findings hint that α-synuclein in its fibrillary, end-stage form might in fact be protective. "Lewy bodies might be marking the luckier cells that have avoided the problem by essentially sequestering these oligomers," says Peter Lansbury, a neurologist at Harvard Medical School, who co-founded a biotechnology company to develop oligomer-clearing therapies.

Mitochondrial dysfunction

A separate clue to the source of toxicity in Parkinson's disease appeared in Maryland in 1976, in the brain of Barry Kidston. A chemistry graduate student, Kidston had been synthesizing a heroin-like compound known as MPPP for his own use — but made one batch too many. It contained a contaminant, MPTP, which both in Kidston's case and in later outbreaks among drug users caused severe Parkinson's disease-like motor symptoms within a few days[11].

As researchers later discovered, one of MPTP's metabolites in the brain is preferentially taken up by substantia nigra dopaminergic neurons. It then enters and damages their mitochondria — bacteria-like factories that float within cells and use oxygen to synthesize ATP, an essential chemical-energy molecule. The damage caused by this toxin does not merely lower mitochondrial energy output, it also leads mitochondria to overproduce hydrogen peroxide and other reactive, oxygen-based chemicals. The resulting oxidative stress can harm cells or even trigger the cellular suicide process known as apoptosis.

Prompted by the MPTP discovery, researchers soon found signs of mitochondrial dysfunction in affected brain tissues from autopsied Parkinson's disease patients. They hypothesized that a chronic exposure to an environmental toxin with an MPTP-like mechanism might be the cause. They saw pesticides as likely suspects, because MPTP was chemically related to some, and epidemiological studies hinted that pesticide exposure could increase Parkinson's disease risk. In 2000, researchers in the laboratory of Tim Greenamyre at Emory University in Atlanta reported that one common pesticide, rotenone, did indeed have effects similar to those of MPTP, and could be used to produce a Parkinson's disease-like condition in rodents[12].

By this time, studies of families with genetic forms of Parkinson's disease had begun, and it was soon clear that three of the proteins mutated in these families — parkin, PINK1 and DJ-1 — were localized to

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mitochondria or protected cells from mitochondria-related oxidative damage.

Could this 'mitochondrial dysfunction hypothesis' stand on its own? A pesticide, such as rotenone, would find its way into a variety of cells, not just the neurons commonly affected in Parkinson's disease. Why would it cause a specifically parkinsonian pattern of damage?

"That's an intriguing question that nobody really has the answer for," says Greenamyre, whose laboratory is now at the University of Pittsburgh.

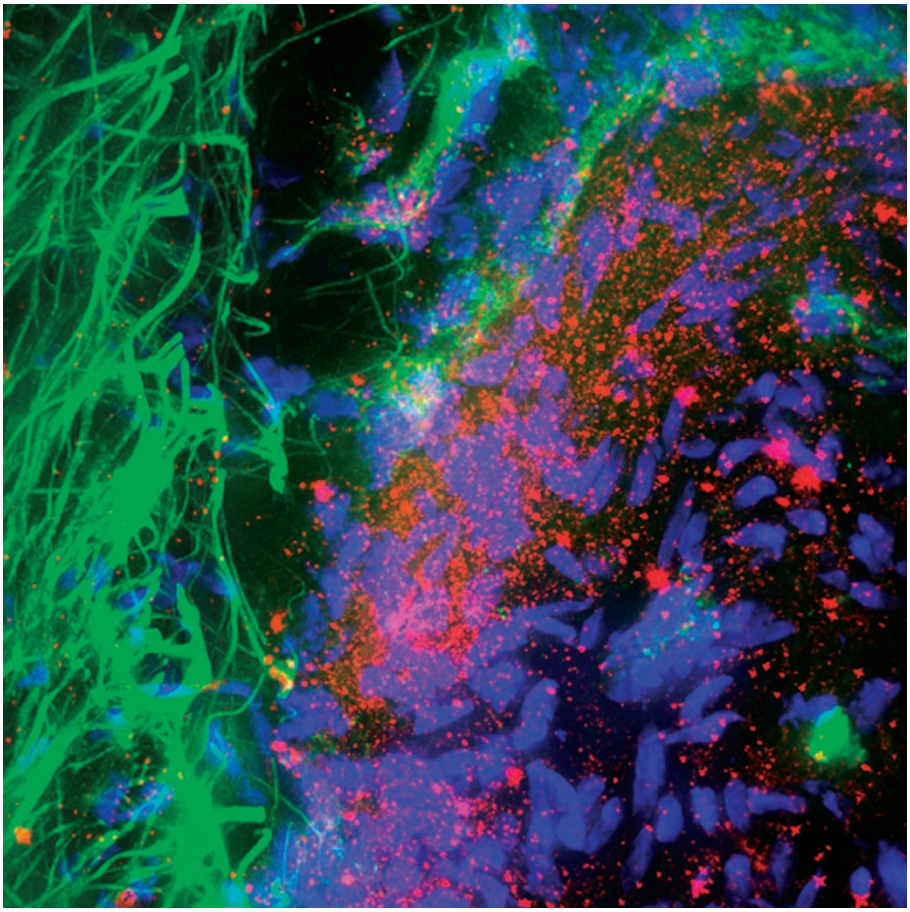
There are some clues, however. Neurons are more vulnerable than other cell types because, when fatally damaged, they are not usually replaced. Moreover, the neuronal types most affected in Parkinson's disease are projection neurons with long, thin, poorly-myelinated axons and many output synapses, implying a high vulnerability to energy-supply disruption.

Another possible clue is a cell-darkening compound known as neuromelanin. Formed from the oxidative-breakdown products of dopamine and other neurotransmitters, it accumulates during normal ageing in several of the brain regions hardest hit by Parkinson's disease, and thus might be a marker of existing oxidative damage — implying a high vulnerability to further damage.

Dopamine's oxidative by-products are viewed as a particularly likely source of such damage — and dopamine is found not only in dopamine-synthesizing neurons but also, as a precursor molecule, in noradrenaline-synthesizing neurons in the locus coeruleus and elsewhere. Oxidative by-products known as quinone compounds are thought to account for most of dopamine's natural toxicity. "We've shown that these dopamine quinones are able to get into mitochondria and disrupt function," says Greenamyre. 6-hydroxydopamine, another mitochondria-damaging dopamine derivative, is commonly used as a neurotoxin to produce a rodent model of parkinsonian motor symptoms.

Neurons normally prevent dopamine from oxidizing by packing it into vesicles. When mitochondria are damaged, this dopamine-packing process is impaired, perhaps by the fall in available ATP energy. This means that mitochondrial damage can lead to more unpackaged dopamine, the oxidized products of which then create more mitochondrial damage. "And you have a vicious cycle," Greenamyre says.

A protein known as VMAT2 normally handles this vesicle-packing process for dopamine and several other neurotransmitters. Last year, Gary Miller and colleagues at Emory University reported that transgenic mice expressing relatively low levels of VMAT2 develop a Parkinson's disease-like condition including movement abnormalities, loss of smell, slowed digestive functions, changed sleep patterns, and behaviour suggestive of anxiety and depression[13]. "They show symptoms and pathology that look much closer to the human disease than a lot of the existing models," says Miller.



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Section of a Parkinson's disease brain. Neuron nuclei (blue) and dendrites (green) are surrounded by α-synuclein (red).

A unified hypothesis?

So, does Parkinson's disease arise from mitochondrial dysfunction or from toxic clusters of α-synuclein? The answer might be a bit of both, because the two factors clearly can interact. When mitochondrial dysfunction is caused by rotenone or by familial Parkinson's disease genetic defects, it usually leads to the accumulation of α-synuclein fibrils in affected neurons. Recent studies have shown that α-synuclein can somehow get into mitochondria and impair their function, can bind to vesicles and, perhaps in aggregated form, can interfere with the safe packaging of neurotransmitters[14].

Thus, it is conceivable that Parkinson's disease begins with either mitochondrial dysfunction or α-synuclein aggregation, but can eventually be driven by both, in a self-reinforcing spiral that ends in extensive cell death.

The initial subtlety of these factors would explain why the pathogenesis of Parkinson's disease has been so difficult to pin down. The ageing process and its broad declines in function, combined with a mild but long-term exposure to a pesticide or other toxin, and perhaps a genetic predisposition towards moderate overproduction of α-synuclein, might be all that is needed to nudge a critical mass of nerve cells past their parkinsonian tipping points.

With this unified hypothesis in prospect, the research community might be at or near its own tipping point, where a consensus can finally form around animal models, disease biomarkers and therapeutic strategies. "The lack of consensus around the pathogenic mechanism has held back Parkinson's research," says Lansbury. "Yet these seemingly competing hypotheses may all along have been complementary."

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