LECTURE 04: Response to injury

Cells in the CNS vary in their response to injury. Neurons are the most sensitive. They have a very high metabolic rate and need a constant supply of oxygen or they will die. Depriving a neuron of blood flow for even as short a period of time as 6-8 minutes will cause the neuron to die. Neurons do not regenerate. Healing in the brain is different from the rest of the body. There are no fibroblasts in the brain and so when there is necrosis, there is no scarring, no fibroplasia as we see in the other body systems. Instead, the part of the brain that has undergone necrosis just gets soft and is eventually consumed and cleaned up by the microglia, leaving a hole in the brain.

There is a special term for necrosis in the brain: malacia.

It appears as a big soft spot within the brain. There is very little area for the brain to expand. Consequently if there is inflammation, or edema, or hemorrhage, or a tumor, the brain becomes slightly bigger than normal, and it presses against the dura mater and the cranium. This pressure compromises the blood flow and neurons may die because they do not get the oxygen they need because the blood vessels are compressed.

Edema and Vascular Disturbances

The brain is a dense structure closely bounded by bone. There is NO room for the brain to expand. As a result when there is swelling of the brain, and edema is the most common reason for swelling, there is no place for the brain to go. It enlarges, and presses against the cranium. The pressure causes a decrease in blood flow, and hypoxia of the neurons then occurs. As neurons become hypoxic and die, the functions they are responsible for also stop. So, when the center of the brain responsible for heartbeat or respiration gets compromised, life gets compromised also.

What are the reasons for edema in the brain?

- Inflammation can always generate edema.

- A space-occupying mass such as a hematoma or a tumor will cause edema to form.

- Hypoxia will cause the cells to function poorly, they develop cell swelling, and there is resulting edema.

- Hydrocephalus, which happens when the drainage of CSF is blocked, will lead to more fluid and pressure As the brain swells, the cerebellum gets pushed backwards and can protrude out through the foramen magnum. Below are sagittal sections of two brains. The one on the left is normal. The brain on the left has had some edema, there was so much pressure that the cerebellum got pushed out through the foramen magnum and it has a kind of “tail” now. We call this “coning” of the cerebellum, it is a very dangerous sequela to

pulmonary edema. Because when the cerebellum pushes through and compresses the brain stem, it can compromise the cardiovascular centers there and, uh oh, the heart stops.

Some of the most sensitive areas to hypoxia are the neurons in the cerebral cortex. As these become compromised, animals will often exhibit seizures. Any kind of trauma to the brain can cause hemorrhage. Because the brain is soft, the blood tends to continue flowing from a damaged vessel , and can create a space-occupying problem in the brain. Because this blood build-up occurs quickly, it can cause acute brain swelling and death. A common place to bleed is the leptomeninges, a hematoma forms within the leptomeninges (below the dura). The dura is tough collagen, it can't stretch, and so the brain gets compressed. These lesions are called subdural hematomas, and they are a common cause of death in any kind of head trauma.

In humans, a common cause of brain problems is STROKE. This happens when an embolus (usually from atherosclerosis) breaks off of the aorta and travels up the carotid artery. It gets stopped in the smaller vessels of the brain, and creates and infarct. The endothelium breaks down in this zone and then there is hemorrhage. We don't see stroke in domestic animals because they do not get atherosclerosis.

Infectious Diseases

BACTERIA

Bacteria tend to reach the brain primarily by traveling there through the bloodstream. They often do not penetrate into the brain, but they will cause a purulent inflammation in the meninges, which affects function. So most bacterial infections of the central nervous system are MENINGITIS. Bacterial meningitis is most common in young farm animals, especially calves and lambs, and can include a variety of organisms. Most common route of infection into the animal is through the open umbilicus. There is a septicemia, and organisms settle in the meninges.

There are some other ways that bacteria can reach the brain:

o Extension from otitis (inflammation of the ear)

o Traveling up the cranial nerves from the oral cavity, this is how Listeria gets to the brain

Most bacteria that cause problems in the brain are not specific brain infections, that is, the bacteria are not specifically "looking" for brain. A notable exception is LISTERIOSIS. When there is Listeria in the feed, and any oral erosion, the Listeria bacteria will get into the submucosal tissue of the oral cavity, find the cranial nerves, and move up the nerves to infect the brainstem. Once in the brainstem, they grow as microabscesses.

VIRUSES

There are several viruses that have neurons or other nervous system cells as their specific target.

NEUROSCIENCE

Rabies

The disease is spread to humans from another animal, commonly by a bite or scratch. Infected saliva that comes into contact with any mucous membrane is also a risk. Globally almost all cases are the result of a dog bite. The rabies virus grows in the brain and the salivary gland of infected animals. So, when an infected animal bites another animal (including a human), the rabies virus is inoculated. The virus moves into the nerves, and then moves UP the nerves, toward the spinal cord, at a very slow rate, only about 3mm per day.

Once in the brain, it will infect various neurons, causing disease that differs according to the species. In cattle, sheep, goats, and horses, it is usually "dumb" rabies, with animals being very slow and almost stuporous. In dogs and wildlife, the virus is more likely to cause the "furious" rabies, with animals becoming very aggressive, and more likely to spread the virus through biting. It moves to the salivary glands about the same time as it is in the brain.

The diagnosis of rabies requires fluorescent antibody testing or histopathology. Using an antibody specific for rabies, and impression smear of brain from an animal infected by rabies will have fluorescence. By histopathology, Negri bodies may be visible in brain sections:

There are several other viruses that will cause encephalitis in animals. Many are hard to distinguish from one another. Most will create lymphocytic inflammation in the brain, leading you to think that it is probably a viral infection (by contrast, bacterial infections of brain and meninges will have mostly neutrophils in the inflammation). Some examples of viral diseases that might cause an animal to display neurologic signs because brain tissue is infected:

Bovine herpes encephalitis, cattle West Nile encephalitis, horses Louping ill, sheep Tick-borne encephalitis, dogs and horses Caprine arthritis-encephalitis, goats

FUNGUS

There are several fungi from the environment that, once they gain access to the body, may settle out in brain. There are almost always lesions in other organs as well, there are no fungi that are specific for brain.

Aspergillus might be the most common fungal infection among domestic animals.

PARASITES

Toxoplasma is a common infection among all mammalian species. The cat is the definitive host and it is shed in cat feces. The organism will encyst in tissues of other mammals. The organism usually lies quietly in tissue, causing no problems. However, sometimes the cysts in the brain will rupture and create necrosis and inflammation, especially in young animals. The dog tapeworm, Echinococcusgranulosus, can cause serious problems in sheep (and also humans). The intermediate form is cystic and can occur in many body tissues, including brain, although liver and lung are affected far more frequent

NEUROSCIENCE

Nutritional, Metabolic and Toxic Disorders

Thiamin deficiency Thiamin is also known as B1. It is essential for metabolism in many organs, including brain. Ruminants are especially sensitive to a deficiency of thiamin. If there is not sufficient thiamin in the diet, the neurons in the cerebral cortex will die. The lesion is often called polioencephalomalacia.

Lead poisoning Animals that have access to old batteries or fuel can develop lead poisoning. This is seen most commonly in cattle. It is directly toxic to neurons and astrocytes, so the damage is primarily in the cerebral cortex.

Copper deficiency

- Pregnant sheep that do not have enough copper in their diet (or, too much molybdenum, which keeps copper from being absorbed), may give birth to lambs that have severe incoordination. Lack of sufficient copper during gestation will cause inadequate development of the white matter (axons and myelin) throughout the brain. Animals cannot walk, have very poor balance, and eventually die.

- If a growing animal does not receive enough copper in the diet, there will also be damage to the white matter, and this shows up in goat kids and lambs as ataxia. There is severe axonal degeneration and lack of re-myelination.

Hepatic encephalopathy

When the liver fails to function adequately, there is excess ammonia in the circulation, and this is toxic to astrocytes. The astrocytes degenerate, and brain edema develops.

Botulism Clostridium

Botulinum produces one of the most powerful toxins known. Even a very small amount of ingested toxin will cause paralysis. This is considered intoxication, not an infection. The toxin acts by preventing the release of acetylcholine from the end of the axon onto the motor endplate.

Neurodegeneration

Neurodegenerationis the umbrella term for the progressive loss of structure or function of neurons, including death of neurons. Many neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's, Alzheimer's, and Huntington's occur as a result of neurodegenerative processes. Such diseases are incurable, resulting in progressive degeneration and/or death of neuron cells. As research progresses, many similarities appear that relate these diseases to one another on asub-cellular level. Discovering these similarities offers hope for therapeutic advances that could ameliorate many diseases simultaneously. There are many parallels between different neurodegenerative disorders including atypical

protein assemblies as well as induced cell death. Neurodegeneration can be found in many different levels of neuronal circuitry ranging from molecular to systemic.

PARKINSON'S DISEASE

Introduction

In his classic 1817 monograph "Essay on the Shaking Palsy," James Parkinson described the core clinical features of the second most common age-related neurodegenerative disease after Alzheimer's disease (AD).

Clinical Characteristics of PD

PD is a progressive disease with a mean age at onset of 55, and the incidence increases markedly with age, from 20/100,000 overall to 120/100,000 at age 70. In about 95% of PD cases, there is no apparent genetic linkage (referred to as "sporadic" PD), but in the remaining cases, the disease is inherited. Over time, symptoms worsen, and prior to the introduction of levodopa, the mortality rate among PD patients was three times that of the normal age-matched subjects. Furthermore, most PD patients suffer considerable motor disability after 5–10 years of disease, even when expertly treated with available symptomatic medications.

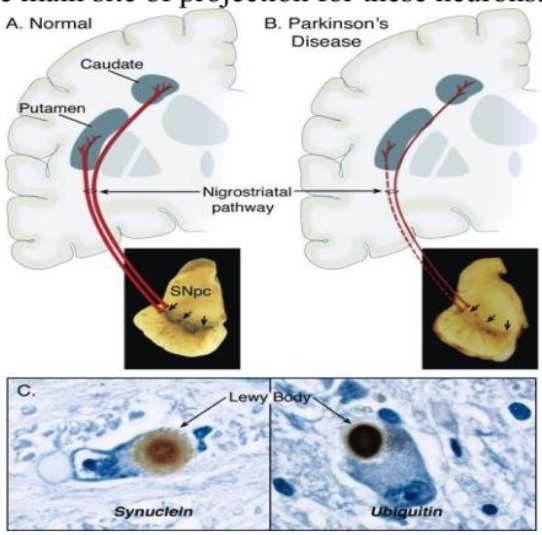
Clinically, any disease that includes striatal DA deficiency or direct striatal damage may lead to "parkinsonism," a syndrome characterized by tremor at rest, rigidity, slowness or absence of voluntary movement, postural instability, and freezing . PD is the most common cause of parkinsonism, accounting for ~80% of cases.

PD tremor occurs at rest but decreases with voluntary movement, so typically does not impair activities of daily living. Rigidity refers to the increased resistance (stiffness) to passive movement of a patient's limbs. Bradykinesia (slowness of movement),hypokinesia (reduction in movement amplitude), and akinesia (absence of normal unconscious movements, such as arm swing in walking) manifest as a variety of symptoms, including paucity of normal facial expression (hypomimia), decreased voice volume (hypophonia), drooling (failure to swallow without thinking about it), decreased size (micrographia) and speed of handwriting, and decreased stride length during walking. Bradykinesia may significantly impair the quality of life because it takes much longer to perform everyday tasks such as dressing or eating. PD patients also typically develop a stooped posture and may lose normal postural reflexes, leading to falls and, sometimes, confinement to a wheelchair. Freezing, the inability to begin a voluntary movement such as walking (i.e., patients remain "stuck" to the ground as they attempt to begin moving), is a common symptom of parkinsonism. Abnormalities of affect and cognition also occur frequently; patients may become passive or withdrawn, with lack of

initiative; they may sit quietly unless encouraged to participate in activities. Responses to questions are delayed, and cognitive processes are slowed ("bradyphrenia"). Depression is common, and dementia is significantly more frequent in PD, especially in older patients.

Neurochemical and Neuropathological Features of PD

The pathological hallmarks of PD are the loss of the nigrostriatal dopaminergic neuronsand the presence of intraneuronalproteinacious cytoplasmic inclusions, termed "Lewy Bodies" (LBs) . The cell bodies of nigrostriatal neurons are in the SNpc, and they project primarily to the putamen. The loss of these neurons, which normally contain conspicuous amounts of neuromelanin , produces the classic gross neuropathological finding of SNpcdepigmentation . At the onset of symptoms, putamenal DA is depleted ~80%, and ~60% of SNpc dopaminergic neurons have already been lost. The mesolimbic dopaminergic neurons, the cell bodies of which reside adjacent to the SNpc in the ventral tegmental area (VTA), are much less affected in PD . Consequently, there is significantly less depletion of DA in the caudate (, the main site of projection for these neurons.



Neuropathology of Parkinson's Disease

(A) Schematic representation of the normal nigrostriatal pathway (in red). It is composed of dopaminergic neurons whose cell bodies are located in the substantianigra pars compacta (SNpc; see arrows). These neurons project (thick solid red lines) to the basal ganglia and synapse in the striatum (i.e., putamen andcaudate nucleus). The photograph demonstrates the normal pigmentation of the SNpc, produced byneuromelanin within the dopaminergic neurons.(B) Schematic representation of the diseased nigrostriatal pathway (in red). In Parkinson's disease, the nigrostriatal pathway degenerates. There is a marked loss of dopaminergic neurons that project to the putamen (dashed line) and a much more modest loss of those that project to the caudate (thin red solid line). The photograph demonstrates depigmentation (i.e., loss of dark-brown pigment neuromelanin; arrows) of the SNpc due to

the marked loss of dopaminergic neurons.(C) Immunohistochemical labeling of intraneuronal inclusions, termed Lewy bodies, in a SNpcdopaminergic neuron. Immunostaining with an antibody against α-synuclein reveals a Lewy body (black arrow) with an intensely immunoreactive central zone surrounded by a faintly immunoreactive peripheral zone (left photograph). Conversely, immunostaining with an antibody against ubiquitin yeilds more diffuseimmunoreactivity within the Lewy body (right photograph).

Experimental support for the concept of dying back includes the observations that in MPTP-treated monkeys the destruction of striatal terminals precedes that of SNpc cell bodies, and in MPTP-treated mice, protection of striatal terminals prevents the loss of SNpc dopaminergic neurons

Lewy Bodies

Another pathologic hallmark of PD is the Lewy body,

aneosinophilic inclusion identified within neurons.

On histologic stains, Lewy bodies have an eosinophilic core, and a surrounding pale halo.

They are usually rounded, although their shape can be pleiomorphic, and they are generally 5to 25 m in diameter.

They usually are observed within the cell soma, but also can be seen in neurites or free in the extracellular space.

Lewy bodies are commonly observed in the brain regions showing the most neuron loss in PD, including SN, locus coeruleus, the dorsal motor nucleus of the vagus, and the nucleus basalis of Meynert, but they are also observed in neocortex, diencephalon, spinal cord, and even peripheral autonomic ganglia.

Another major antigenic feature of Lewy bodies is the expression of cellular proteins involved in protein degradation, including ubiquitin , and the proteasome . Presence of these antigens has been hypothesized to represent efforts on the part of the cell to degrade the abnormal protein aggregate.

-synuclein gene

Following the identification of mutations in the -synuclein gene in a few cases of familial PD, it was discovered that -synuclein is a component of Lewy bodies.

Etiology of PD

Aging

The possible role of aging in the pathogenesis of PD is suggested by its usual occurrence in late middle age, and by marked increases in its prevalence at older ages.

Environmental Factors

Consideration of a role for environmental factors in the cause of PD was given major impetus with the discovery in 1983 that exposure to MPTP is capable of inducing parkinsonism in humans. The possible role of environmental factors has been addressed by a number of epidemiologic studies. Many of these studies have shown associations between rural residence, well-water drinking, or herbicide/pesticide exposure and the risk of developing PD However, the precise role played by any specific compounds has remained elusive.

Genetic Factors

For many years, genetic factors were considered unlikely to play an important role in the pathogenesis of PD. This concept was based largely on twin studies conducted in the early 1980s that demonstrated a very low rate of concordance for the disease among identical twins. Nevertheless, many investigators recognized that PD could occasionally be identified in families. The most important advances in PD research in recent years have been the identification of specific disease-causing mutations, making it possible for the first time to begin to explore pathogenesis at the molecular level.

Synuclein

Two missense mutations $Ala^{53} \rightarrow Thr$ (A53T) and $Ala^{30} \rightarrow Pro$ (A30P) in α-synuclein cause dominantly inherited PD.

Mutations in α-synuclein have not been found in sporadic PD. So the concept that α-synuclein-mutant and sporadic PD share common pathogenic mechanisms rests predominantly on the observation that α-synuclein is a major component of LBs in sporadic PD.

The normal physiological role of α-synuclein

prevalent presynaptic protein may modulate synaptic vesicle function.

In striatal dopaminergic terminals, α-synuclein participates in the modulation of synaptic function.

Biochemical and biophysical evidence is also consistent with a role for α-synuclein in cellular membrane dynamics. The fact that α-synuclein is abundant in LBs suggests that its propensity to misfold and form amyloid fibrils may be responsible for its neurotoxicity in pathological situation such as PD and that pathogenic mutations endow it with a toxic gain of function.

Parkin

Loss-of-function mutations in the gene encoding parkin cause recessively inherited parkinsonism .

Heterozygote mutations in parkin may also lead to dopaminergic dysfunction and later onset of PD .

Pathologically, parkin-related PD is characterized by loss of SNpc dopaminergic neurons, but it is not typically associated with LBs.

It is uncertain how loss of parkin function leads to dopaminergic neuron degeneration, but clues are emerging from the identification of its normal function.

Parkin,

a 465 amino acid protein, contains two RING finger domains separated by an in-between RING (IBR) finger domain at the C terminus and an ubiquitin-like homology domain at the N terminus. The presence of an IBR led to the finding that parkin is an E3 ubiquitin ligase , a component of the ubiquitin-proteasome system that identifies and targets misfolded proteins to the proteasome for degradation. The upstream ubiquitin ligases (E1 and E2) cooperate nonspecifically to tag misfolded proteins with a single ubiquitin, while E3 ligases confer target specificity by binding to specific molecules or classes of molecules facilitating the polyubiquitination necessary for targeting to the proteasome. Many parkin mutations abolish this E3 ligase activity, suggesting that the accumulation of misfolded parkin substrates could be responsible for the demise of SNpc dopaminergic neurons in PD.

Ubiquitin C-Terminal Hydrolase-L1

This enzyme catalyzes the hydrolysis of C-terminal ubiquityl esters and is thought to play a role in recycling ubiquitin ligated to misfolded proteins after their degradation by the proteasome.

A dominant mutation (I93M) in UCH-L1 was identified in one family with inherited PD.

Additionally, a polymorphism (S18Y) of UCH-L1 appears to be protective for the development of PD. Both the I93M mutation and the S18Y polymorphism alter UCH-

L1 ligase activity in a manner consistent with the hypothesis that impaired activity of the ubiquitin proteasome system is critical in PD pathogenesis:

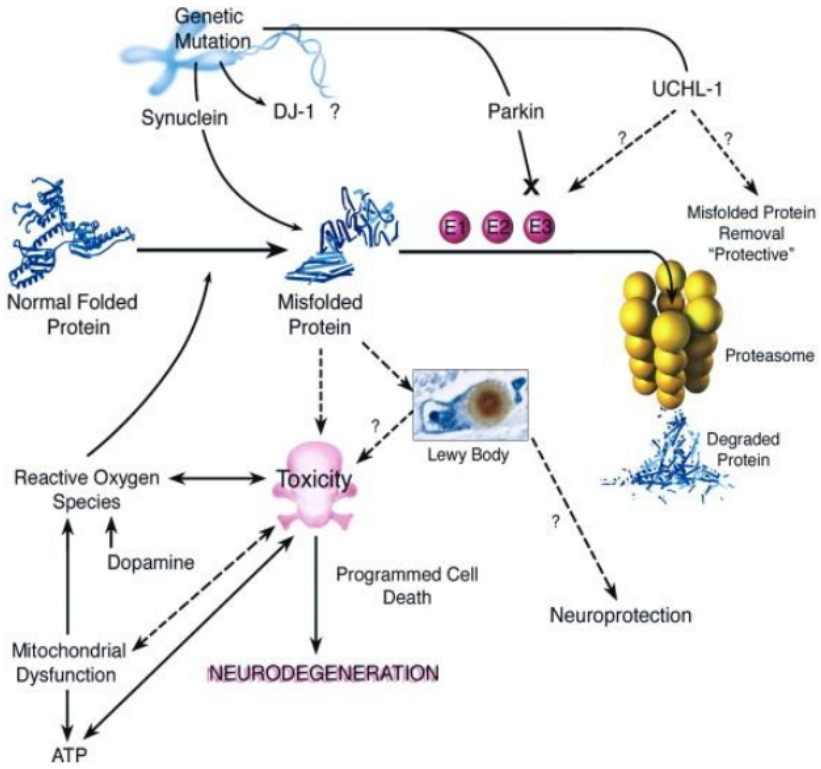
DJ-1

DJ-1 mutations were identified in two consanguineous pedigrees with autosomal recessive PD . One family carried a deletion predicted to abolish protein function, while the other harbored a missense mutation that results in the insertion of a proline into an α-helical region. Expression of this proline mutant form of DJ-1 appears to lead to its accumulation in mitochondria , and DJ-1 has been implicated as a cellular monitor of oxidative stress .

Pathogenesis of PD

Whatever insult initially provokes neurodegeneration, studies of toxic PD models and the functions of genes implicated in inherited forms of PD suggest two major hypotheses regarding the pathogenesis of the disease. One hypothesis posits that misfolding and aggregation of proteins are instrumental in the death of SNpc dopaminergic neurons, while the other proposes that the culprit is mitochondrial dysfunction and the consequent oxidative stress, including toxic oxidized DA species.

Potential points of interaction are diagrammed in Figure 2. The finding that oxidative damage to α-synuclein can enhance its ability to misfold and aggregate is one example of such an interaction. Another uncertain issue is whether the multiple cell death-related molecular pathways activated during PD neurodegeneration ultimately engage common downstream machinery, such as apoptosis, or remain highly divergent. Clearly, this issue is of great consequence in deciding about possible therapeutic strategies for PD.



Mechanisms of Neurodegeneration

A growing body of evidence, suggests that the accumulation of misfolded proteins is likely to be a key event in PD neurodegeneration. Pathogenic mutations may directly induce abnormal protein conformations (as believed to be the case with α-synuclein) or damage the ability of the cellular machinery to detect and degrade misfolded proteins (Parkin, UCH-L1); the role of DJ-1 remains to be identified. Oxidative damage, linked to mitochondrial dysfunction and abnormal dopamine metabolism, may also promote misfolded protein conformations. It remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Lewy body). Controversy exists regarding whether Lewy bodies promote toxicity or protect a cell from harmful effects of misfolded proteins by sequestering them in an insoluble compartment away from cellular elements. Oxidative stress, energy crisis (i.e., ATP depletion) and the activation of the programmed cell death machinery are also believed to be factors that trigger the death of dopaminergic neurons in Parkinson's disease**.**

Misfolding and Aggregation of Proteins

The abnormal deposition of protein in brain tissue is a feature of several age-related neurodegenerative diseases, including PD. Protein aggregates could directly cause damage, perhaps by deforming the cell or interfering with intracellular trafficking in neurons. Protein inclusions might also sequester proteins that are important for cell survival. If so, there should be a direct correlation between inclusion formation and neurodegeneration.

Mitochondrial Dysfunction and Oxidative Stress

The possibility that an oxidative phosphorylation defect plays a role in the pathogenesis of PD was fueled by the discovery that MPTP blocks the mitochondrial electron transport chain by inhibiting complex I. Subsequent studies identified abnormalities in complex I activity in PD.

In vitro studies indicate that such a complex I defect may subject cells to oxidative stress and energy failure.

Nearly 100% of molecular oxygen is consumed by the mitochondrial respiration, and powerful oxidants are normally produced as byproducts, including hydrogen peroxide and superoxide radicals.

Inhibition of complex I increases the production of the ROS superoxide, which may form toxic hydroxyl radicals or react with nitric oxide to form peroxynitrite. These molecules may cause cellular damage by reacting with nucleic acids, proteins, and lipids. One target of these reactive species may be the electron transport chain itself, leading to mitochondrial damage and further production of ROS.

Several biological markers of oxidative damage are elevated in the SNpc of PD brains . Also, the content of the antioxidant glutathione is reduced in the SNpc of PD brains , consistent with increased ROS, although this could also indicate a primary reduction of protective mechanisms against ROS.

The presence of ROS would increase the amount of misfolded proteins, increasing the demand on the ubiquitin-proteasome system to remove them. Dopaminergic neurons may be a particularly fertile environment for the generation of ROS, as the metabolism of DA produces hydrogen peroxide and superoxide radicals, and autooxidation of DA produces DA-quinone, a molecule that damages proteins by reacting with cysteine residues. Mitochondria-related energy failure may disrupt vesicular storage of DA, causing the free cytosolic concentration of DA to rise and allowing harmful DA-mediated reactions to damage cellular macromolecules. Thus, DA may be pivotal in rendering SNpc dopaminergic neurons particularly susceptible to oxidative attack. Nevertheless, despite the literature documenting mitochondrial dysfunction and indices of oxidative damage in tissue from PD patients, all of these observations are correlative in nature, and the supportive data from postmortem studies of PD patients suffers from the fact that such specimens primarily consist of glial cells and nondopaminergic neurons, as most dopaminergic neurons die long before these specimens become available. There are no data that convincingly link a *primary* abnormality of

oxidative phosphorylation or ROS generation with PD. Furthermore, parkinsonism is rare in many diseases known to result from mutations directly affecting oxidative phosphorylation ("mitochondrial cytopathies"). When parkinsonism is encountered in these diseases, it is generally accompanied by other symptoms not typical of PD. Therefore, many of the oxidative phosphorylation and ROS abnormalities documented in PD tissues could be nonspecific features of dying cells.

Mode of Cell Death

How do cells ultimately die in PD? Does a common downstream pathway mediate all PD-related cell loss, or is there significant heterogeneity in the pathways activated in different sick neurons in a single patient, or among different patients with PD? The answers to these questions are important for the rational development of therapeutic strategies for PD.

Programmed cell death (PCD):

- In PCD intracellular signaling pathways are activated to cause cell demise.
- Although physiological PCD is crucial during normal development and as a homeostatic mechanism in some systems (e.g., immune system), dysregulation of this pathway in the brain may contribute to neurodegeneration.
- Investigators have explored the possibility that PCD occurs in PD autopsy specimens by searching for neurons that display features of apoptosis, a morphological correlate of PCD.
- Some studies of PCD in PD have measured molecular components of PCD instead of relying on morphological criteria. The PCD molecule Bax demonstrate an increased number of Bax-positive SNpc dopaminergic neurons in PD, and compared to controls, there is increased neuronal expression of Bax in PD, suggesting that these cells are undergoing PCD.

- Other molecular markers of PCD are altered in PD, including the activation of caspase-8 and caspase-9. Taken together, these studies suggest that the PCD machinery is activated in postmortem PD tissue.

Modeling PD in Animals

While recent genetic discoveries have led to significant insight into molecular pathways of likely importance in PD pathogenesis, these discoveries have not contributed to an understanding of other important aspects of the disease. Why is there a relatively selective loss of dopaminergic neurons in PD? Is the toxicity provoked by these disease alleles a cell-autonomous effect in dopaminergic neurons? What is the role of aging in both sporadic and inherited PD, or posed differently, why does it take many decades even for inherited PD to

develop? Does pharmacological or genetic manipulation of the ubiquitin-proteasome pathway prevent (or provoke) dopaminergic neurodegeneration? Do the different genetic forms of PD display unique responses to cell-based (e.g., stem cell) or pharmacological therapies? What is the relationship between the neurodegeneration provoked by disease allele-related pathways and that occurring in sporadic PD? Although aspects of these questions can be assessed in PD patients, postmortem tissue, and in vitro systems, it is clear that these and related questions will be addressed most powerfully in animal models.

The crucial requirement for a disease gene-based model of PD (also referred to as an "etiologic model") is the adult onset of relatively specific and progressive dopaminergic neuron degeneration. A behavioral correlate of the nigrostriatal dopaminergic pathway degeneration is also desirable but, in rodents, will not likely parallel the motor deficits of PD because rodents do not develop typical parkinsonism. Alternatively, behaviors that involve striatal function, such as habituation to a novel environment or the ability to learn a stimulus-response paradigm, may be useful in assessing the striatal dopaminergic function. Because motor system organization differs in rodents and humans, the value of a particular behavioral phenotype depends upon its relationship to striatal dopaminergic function rather than apparent similarity to a symptom of PD. Specifically, behaviors claimed to result from striatal DA deficiency should improve with DA replacement. The formation of LBs is also a desirable but not essential feature. While LBs are characteristic of PD, they are not specific, are not found in a minority of clinically defined PD cases, and are not seen in parkin-related PD.

Other valuable approaches to modeling PD in animals do not depend on disease-related genes. These "pathologic models" use toxins or non-PD-related genetic mutationsto mimic the selective degeneration of dopaminergic neurons or exploit the loss of dopaminergic neurons that normally occurs in rodents during early postnatal development. These strategies are based on the premise that dopaminergic neurons have a stereotyped death cascade that can be activated by a range of insults or developmental signals. Clearly defining this cascade of events may lead to the identification of new molecules of potential relevance to PD pathogenesis or treatment. Most notable is the MPTP model, partially because of the striking similarity between PD and individuals intoxicated with MPTP. Finally, "symptomatic" or "pathophysiologic" models recapitulate the motor symptoms of PD and are used to develop symptomatic therapies or to study circuit-related questions. Only nonhuman primates accurately mimic the motor symptoms of PD and are therefore the only suitable animal for such studies.

Toxin-Based Models

Among the neurotoxins used to induce dopaminergic neurodegeneration, 6-hydroxydopamine (6-OHDA), MPTP, and more recently paraquate and rotenone have received the most attention. Presumably, all of these toxins provoke the formation of ROS. Rotenone and MPTP are similar in their ability to potently inhibit complex I, though they display significant differences, including, importantly, their ease of use in animals. Only MPTP is clearly linked to a form of human parkinsonism, and it is thus the most widely studied model.

MPTP: False Narcotic, Real Parkinsonian Toxin

- In 1982, young drug users developed a rapidly progressive parkinsonian syndrome traced to intravenous use of a street preparation of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), an analog of the narcotic meperidine (Demerol).
- MPTP was the responsible neurotoxic contaminant, inadvertently produced during the illicit synthesis of MPPP in a basement laboratory.
- In humans and monkeys, MPTP produces an irreversible and severe parkinsonian syndrome characterized by all of the features of PD, including tremor, rigidity, slowness of movement, postural instability, and freezing.
- In MPTP-intoxicated humans and nonhuman primates, the beneficial response to levodopa and development of long-term motor complications to medical therapy are virtually identical to that seen in PD patients. Also similar to PD, the susceptibility to MPTP increases with age in both monkeys and mice.
- Studies show that, as in PD, monkeys treated with low-dose MPTP exhibit preferential degeneration of putamenal versus caudate dopaminergic nerve terminals. Similarly, MPTP damages the dopaminergic pathways in a pattern similar to that seen in PD, including relatively greater cell loss in the SNpc than the VTA and a preferential loss of neurons in the ventral and lateral segments of the SNpc ; this regional pattern is also found in MPTP-treated mice.
- Also reminiscent of PD , dopaminergic neurons that contain neuromelanin are more susceptible to MPTP-induced degeneration . Neuromelanin may contribute neurodegeneration in PD and MPTP-treated monkeys by catalyzing ROS formation through an interaction with iron selectively in pigmented neurons . A variety of organic molecules interact with neuromelanin, including pesticides, MPTP, and MPP+ , so it may contribute to toxicity of pigmented neurons by acting as a depot for toxic compounds.
- The monkey MPTP model does not include two characteristic features of PD. First, neurons are not consistently lost from other monaminergic nuclei, such as the locus coeruleus, a typical feature of PD . Second, although intraneuronal inclusions resembling LBs have been described , classical LBs have not been demonstrated

convincingly in the brains of MPTP-intoxicated patients or monkeys . These cases were exposed to acute regimens of MPTP, so the lack of LB-like formation in MPTP-intoxicated humans and monkeys may reflect the fact that in these cases dopaminergic neurons were rapidly injured. Despite these neuropathologic shortcomings, the monkey MPTP model is the gold standard for the assessment of novel strategies and agents for the treatment of PD symptoms. For example, electrophysiologic studies of MPTP monkeys revealed that hyperactivity of the subthalamic nucleus is a key factor in the genesis of PD motor dysfunction

- This seminal discovery led to the targeting of this structure using chronic high-frequency stimulation procedures (also called deep brain stimulation) to effectively ameliorate the motor function of PD patients whose symptoms cannot be further improved with medical therapy.
- In addition, MPTP-treated monkeys Gash et al. 1996 and Kordower et al. 2000 were used to demonstrate that the delivery of glial-derived neurotrophic factor (GDNF) both significantly limits MPTP-induced nigrostriatal dopaminergic neurodegeneration and can lead to behavioral recovery when given to previously lesioned animals. These studies form the basis for current attempts to use GDNF in PD patients.
- Because of practical considerations, MPTP monkeys have not generally been used to explore the molecular mechanisms of dopaminergic neurodegeneration; the MPTP mouse model is typically used for such studies.