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Parkinson's Disease: A Review

By

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Abbreviations:
AOE, antioxidant enzymes
ARJP, autosomal recessive juvenile parkinsonism
BBB, blood brain barrier
BG, basal ganglia
DA, dopaminergic
L-DOPA, L-3, 4-dihydroxyphenyalinine
LB, Lewy bodies
MAO-B, monoamine oxidase-B
MLT, melatonin
MPP^+, 1-methyl-4-phenylpyridinium
MPTP, methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PD, Parkinson's Disease
RIA, radioimmunological assay
ROS, reactive oxygen species
SN, substantia nigra

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Abstract:

Parkinson's disease (PD) is a neurodegenerative disorder that is becoming one of the most debilitating diseases in the country. The major endpoint associated with the neurodegeneration is the depletion of dopaminergic neurons in nigrostriatal system. The exact mechanism of neuronal depletion is not entirely clear, but several explanations including the genetic, toxin and free radical theories have been proposed to explain the abnormality, each with convincing evidence to support its claim. The Free Radical Theory stands apart because it has also been proposed as a mechanism in aging. Current treatment for PD is designed to increase dopamine levels in the affected brain regions and relieve symptoms associated with the disease. Extensive research into the etiology of PD is continuously providing new ideas for improving or expanding therapeutic applications. I have included some of my own ideas for experiments that may prove beneficial in PD treatments and could be used to improve the quality of life of patients.

Introduction:

Parkinson's disease (PD), or paralysis agitans, was first described by the English physician James Parkinson in 1817. It is a progressive neurodegenerative disorder of the basil ganglia (BG) that is characterized by resting tremor, muscular rigidity, bradykinesia, and loss of postural reflexes [1-2]. Early indications suggest that PD began after age 40, with a peak age of onset around 60 years of age. Parkinson's disease affects over 1 million people in the US alone and it is estimated that 1 in 200 persons will get PD during their lifetime with the risk increasing with age making it the second most common neurological disorder observed in the elderly population [3].

Two major categories of PD are the late onset form (idiopathic) usually occurring over age 55 and the early onset form (familial) seen in some cases as early as 40 years of age [4]. The pathological hallmark feature seen in most cases of PD is the presence of intracellular inclusions commonly referred to as *Lewy bodies* (LB), and the major biochemical alteration observed is the depletion of dopaminergic (DA) neurons in the nigrostriatal system accounting for the motor movement related symptoms [1].

The symptoms associated with PD are not manifested until more than 70% of the DA neurons have been depleted lending to a long preclinical stage. There is no cure for PD, but current treatment focus on alleviating the symptoms by either restoration of depleted dopamine and stimulation of DA pathways. There are three major theories to explain the neurodegeneration process seen in DA neurons and they include The Genetic Theory [4], The Toxin Theory [5-6] and The Free Radical Theory [5-6].

This review will focus on the disease biology, theories of causation, treatment modalities and new experiments in PD research.

Biology of Disease:

a. Pathology:

Parkinson's disease is characterized pathologically by a loss of pigmented cells in the substantia nigra (SN) and the formation of eosinophilic intracellular inclusions termed LB [1]. The SN is a heavily pigmented with neuromelanin that is produced from a slow non-enzymatic process based on the autoxidation of and spontaneous polymerization of dopamine. When dopamine is lost, the neuromelanin is phagocytized and disappears. Human midbrain scans clearly show the differences in the appearance of a normal brain that is highly pigmented in the DA regions and a Parkinsonian brain suffering a loss of pigmentation in those same regions (Figure 1A). Figure 1B is a depiction of a typical LB.

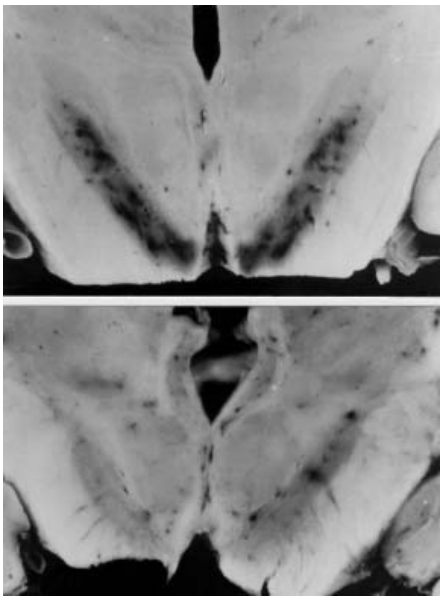


Figure 1A. Appearance of normal (upper) and Parkinsonian (lower) midbrain. Adapted from [7].

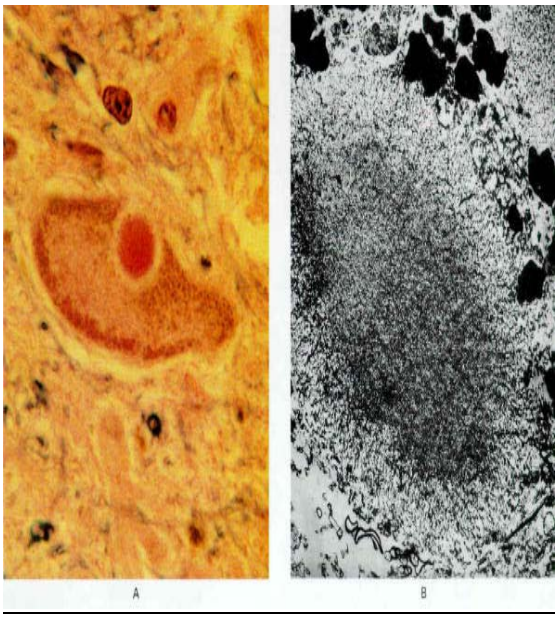


Figure 1B. Typical Lewy Body (A), Ultrastructural examination (B). Adapted from [8].

b. Biochemical Alterations:

The SN, a midbrain structure, is considered part of the BG complex based on its close proximity to the striatum (putamen and caudate nuclei). The BG nuclei are portions of the

midbrain that are involved in motor movement and their degeneration are the basis for the symptoms of PD. BG depletion of dopamine, an inhibitory neurotransmitter, is the principal biochemical alteration in PD.

Symptoms in BG disorders result from an imbalance of dopaminergic (inhibitory) and cholinergic (excitatory) activity of the caudete putamen. PD, a degeneration of DA_nigrostriatal pathway causes dopamine depletion in the BG and relative excess cholinergic activity in the feed back circuit (Figure 2).

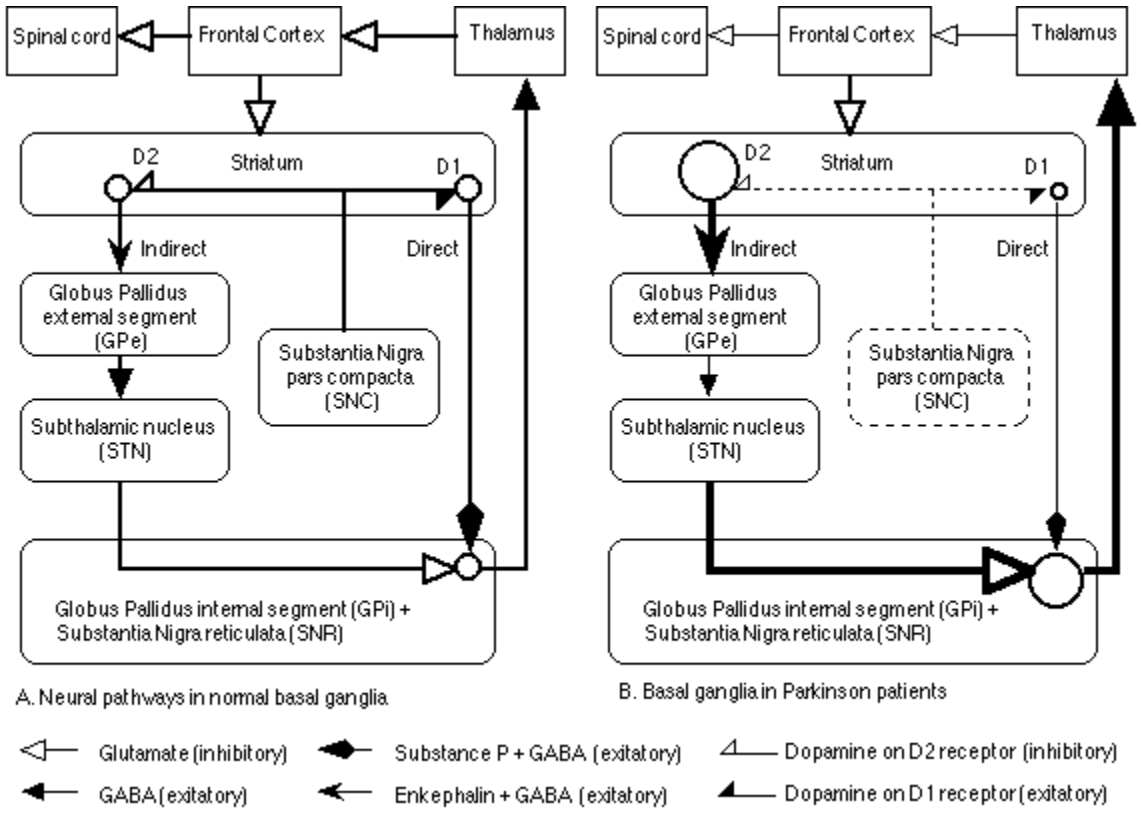


Figure 2. Model for the basal ganglia in persons with normal motor control (A) and Parkinson's disease (B), Adapted from [8].

Dopamine is a hormone-like substance that is an important neurotransmitter. When present in small quantities, it facilitates critical brain functions. It affects brain processes that control movement, emotional response, and the ability to experience pleasure or pain. The

degeneration of nigrostriatal DA neurons and the depletion of dopamine are the major pathological features of PD. Neurons containing DA are clustered in the mid brain area with major concentration in the SN. Figure 3 is a depiction of how DA neuron depletion leads to motor dysfunction.

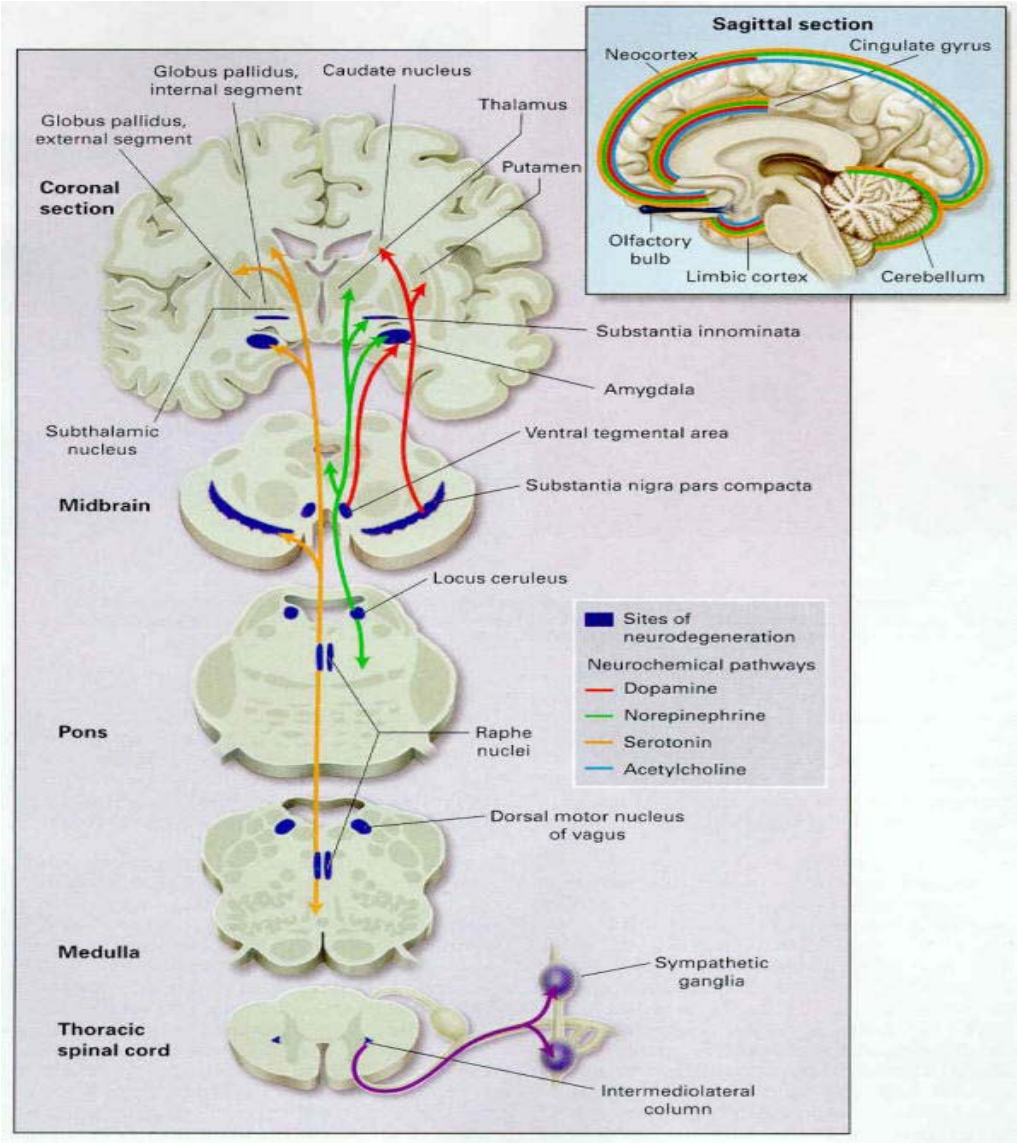


Figure 3. The sites of neurodegeneration and neurochemical pathways involved in PD. Adapted from [9].

Treatment:

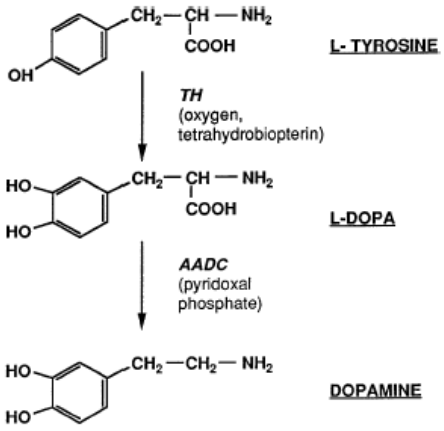
The management of PD can be divided into three categories: (1) protective treatment, (2) symptomatic treatment and (3) restorative or regenerative treatment [8].

a. Protective Therapy:

Several approaches have been used to slow the progression of PD, they have not been successful. Selegiline a selective MAO-B inhibitor has been shown to delay the onset of disability in some cases and its effects were later attributed to relieving symptoms rather slowing disease progression. High doses of the antioxidant vitamin E were also ineffective in slowing the disease progression. The pathogenesis of PD is not entirely understood, therefore, the approaches used may not be adequate.

b. Symptomatic Therapy:

A variety of drugs provide dramatic relief from the symptoms. These drugs work by stimulating the remaining cells in the SN to produce more DA or by inhibiting some of the acetycholine that is produced. The gold standard in drug therapy is L-DOPA (L-3, 4-dihydroxyphenyalinine) which is the precursor for dopamine. L-DOPA is synthesized from tyrosine via tyrosine hydroxylase (TH) (Scheme 1).



Scheme 1. Dopamine Synthesis. Adapted from [10]

L-DOPA is a simple chemical found naturally in plants and animals. Nerve cells use L-DOPA to replenish dopamine which itself cannot be given since it does not cross the blood-brain-barrier (BBB).

Although L-DOPA is efficacious in treating PD, there are several side effects. The most common are nausea, vomiting, hypotension, involuntary movements and restlessness. One approach to alleviating the side effects is to administer the drug more often and in smaller dosages. Long term L-DOPA therapy is usually associated with the "wearing-off" fluctuation and or the more malignant type swing, the "on-off effect" which is unrelated to dosage schedules. Some aspects of PD research involve the determination of tolerance of patients to L-DOPA therapy. Since this is the mainstay treatment for the disease, it is imperative that we understand the mechanisms of actions for L-DOPA tolerance so that patients can be treated more effectively.

c. Surgical and Restorative Therapy:

The knowledge gained in the understanding of the pathophysiological aspects of PD is responsible for the surgical procedure development. The objective of treatment is to disrupt the activities of the motor related regions of the brain. It is usually reserved for advanced stages of the disease.

Theories of DA Neuronal Degeneration:

a. The Genetic Theory:

Parkinson's disease was thought to have a hereditary link since its initial discovery in the 1800's [4]. The importance of the hereditary link to the pathogenesis of PD was further emphasized with the discovery of the candidate gene on chromosome 4. The gene product of the identified candidate gene is Alpha-Synuclein (α-synuclein). The genetic linkage to chromosome

4q was found in a large Italian family (Contursi kindred) with apparent autosomal dominant LB disease. Later it was discovered that a missense mutation in the α-*synuclein* gene at the 53rd codon where alanine is being substituted with theronine (A53T). This mutation in affected individuals resulted in early onset PD at age 45. A second mutation in the α-*synuclein* gene, A30P, where alanine is replaced by proline at the 34th codon caused PD in a family of German descent [4].

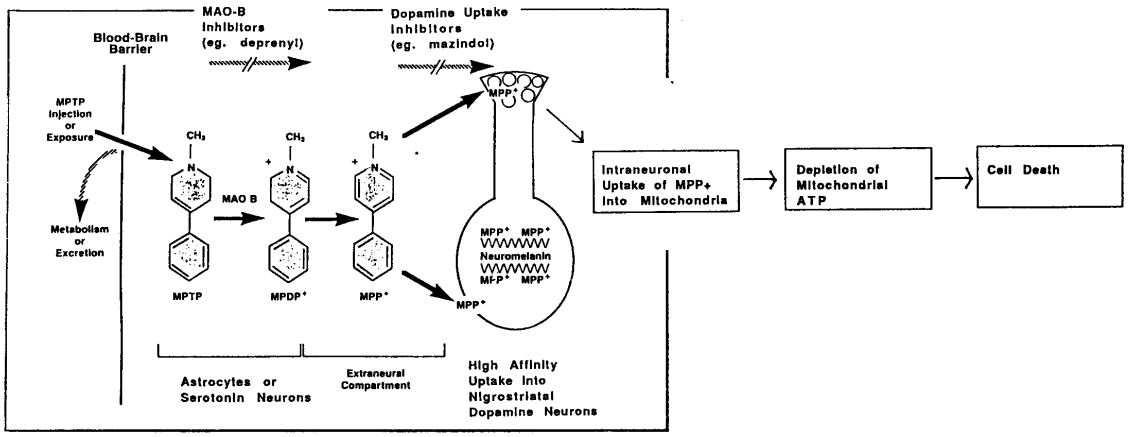
Transgenic α-*synuclein* models have been created. Mice lacking the gene have functional defects in the nigrostriatal system [11]. Transgenic *Drosophilia* with either human wild type or mutant α-*synuclein* cannot climb properly and have intracellular inclusions resembling LB in some DA neurons [12].

At least two other mutated genes associated with the PD syndrome have been identified. A *parkin* gene mutation linked to chromosome 6q25-27 results in autosomal recessive juvenile parkinsonism (ARJP) seen in 50% of familial cases with a typical onset at 40 years of age 13]. Mutations in the *DJ-1* gene mapped to chromosome 1p36 are also related to ARJP [14]. Even thought the evidence presented supports the theory, only a small number of cases of PD can be attributed to genetic factors.

b. The Toxin Theory:

Many toxins that produce damage to the BG and SN result in neurological disorders that include PD. One toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) selectively target DA neurons and has been used as a primate model for investigating PD. MPTP is lipid soluble and readily crosses the BBB and enter brain cells. Due to its amphiphilic nature, it is captured in acidic organelles such as the lysosomes of astrocytes. MPTP is not toxic itself, but is oxidized to a toxic product. The proposed mechanism of the neurotoxic effects of MPTP is illustrated in

Scheme 2. After exposure or injection of MPTP, it is oxidized to 1-methyl-4-phenylpyridinium (MPP^+) by the monoamine oxidase B (MAO-B) enzyme outside the dopaminergic neurons. MPP^+ is then transported and concentrated by the DA uptake system. Once inside the DA neuron, MPP^+ exerts its toxic affect by acting as a mitochondrial poison by inhibiting complex 1 of the electron transport chain. This provides a possible mechanism for degeneration of DA neurons in PD.



Scheme 2. Hypothesized mechanism of neurotoxicity of MPTP. Adapted from [2].

c. <u>The Free Radical Theory:</u>

Free radicals are atoms or molecules with unpaired electrons. They are relatively unstable under physiological conditions and can be highly reactive. The oxygen related species commonly referred to as reactive oxygen species (ROS) consist of superoxide ($O_2^{\bullet-}$) and the hydroxyl radical ($HO^{\bullet}$). Hydrogen peroxide (H_2O_2) is also considered as a ROS, but it is not a free radical.

Generally, production of ROS either occurs accidentally or by deliberate synthesis although the precise amounts generated are still uncertain [6]. Accidental generation includes

mechanisms such as leakage of electrons from mitochondrial electron transport chain, microsomal cytochromes P450 and their electron donating enzymes, and autoxidation of catecholamines, ascorbic acid, and reduced flavins. Usually mitochondria reduce 95 % of the O_2 consumed by cells to H_2O by the sequential transport of 4 electrons. Under normoxic conditions an estimated 1-2% of the mitochondrial electron flow leaks off to form $O_2^{\bullet-}$ which dismutates to H_2O_2. Deliberate synthesis of ROS mainly due to their production by activated phagocytes.

Under normal conditions the antioxidant enzymes consisting of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are capable of maintaining a balance between the ROS generated during normal processes that is usually not present under pathological conditions. When the ROS produced exceeds the capacity of AOEs to remove them, oxidative stress occurs. It is at this instance that free radicals or ROS are potentially damaging to biomolecules such as DNA, lipids and proteins. In most cases the damaging effects of $O_2^{\bullet-}$ depend on the simultaneous presence of H_2O_2 [17]. H_2O_2 is a powerful oxidizing agent and is potentially damaging to cells. It is capable of transversing plasma and nuclear membranes thereby contributing to DNA adduct formation. The $HO^{\bullet}$ radical is the most reactive radical known to biology [6]. It can attack and damage almost every molecule found in living cells.

The Free Radical Theory of PD suggests that DA neuron cell death is attributed to an imbalance in ROS and AOE. A long list of markers exist and are currently being used for assessing oxidative stress: (1) lipid peroxidation, (2) formation of protein carbonyls, (3) loss of reducing substrates such as glutathione, (4) changes in AOE, and (5) oxidative damage to DNA [11]. Several studies used to provide evidence to support oxidative stress in the pathogenesis [6, 17] of PD take advantage of these markers and a discussion of a few investigations will be presented.

Increased levels of cholesterol lipid hydroperoxides were detected in brain tissues extracts from SN of PD subjects compared to control subjects detected by high performance liquid chromatography. There was as much as a 10-fold increase in tissue extracts in PD versus controls. This study suggests that an early component of lipid peroxidation could be associated with oxidative stress in PD [15].

An investigation into the extent and distribution of nucleic acid oxidative damage in the SN neurons of PD patients compared to aged matched controls using 8-hydroxyguanosine (8OHG) as a histochemical marker demonstrated that the proportion of 8OHG immunoreactive SN neurons in PD patients was significantly greater than the controls [16].

The evidence supporting this theory is enormous and provide convincing proof that it is involved in the pathogenesis of PD.

Author's Experimental Ideas:

In the genetic models for PD causation, I think it would be prudent to investigate whether the missense mutation A30P, where alanine is replaced by proline at the 34th codon in the α-synuclein gene was some how related to the transcription factor hypoxia inducible factor 1 (HIF-1) since it is activated by hydroxylation of proline residues in response to oxygen to determine whether some other mechanism was involved in neuronal degeneration. The experimental design could be set up as follows:

- Transgenic mice with wild type or mutated α-*synuclein.*
- Expose to normoxic and hypoxic conditions.
- Measure HIF-1 expression by measuring protein levels using western blot with anti-HIF-1 antibody.

- Measure the expression of the Oxy R and Sox proteins using methods as in previous step.
- Results generated maybe confer something about the redox state in the genetic model.

Parkinson's disease treatment is mostly symptomatic due to the long preclinical stage of approximately 20 years and the false negatives in diagnosis. It would be advantageous to develop a biomarker that could be used to identify PD before substantial neuronal depletion has occurred. I think that protective treatment could be more beneficial if we found something that works. For example melatonin (MLT) shows great promise in scavenging free radicals and may be useful in PD treatment. An experimental design would be as follows:

- Treat PC-12 undifferentiated and differentiated cells with MLT by adding to culture media. Use untreated cells as a control.
- Collect cells after some time frequency.
- Measure AOE via enzymatic assay and MLT levels via radioimmunological assay (RIA)
- Determine if there is a difference in MLT
- Since MLT is lipophilic and endogenous in mammals, it maybe useful as a biomarker in PD.
- Repeat the experiment. This time induce oxidative stress before MLT application.
- Measure AOE and MLT as before.
- Determine if this approach is beneficial to research based on results generated.

Conclusion:

Parkinson's disease is a debilitating neurological disease for which there is no cure. It is increasingly becoming the second most common neurological disorder observed in the elderly population with Alzheimer's disease being number one [3]. PD affects over 1 million people in the US alone and it is estimated that 1 in 200 persons will get PD during their lifetime. Age is the greatest risk factor for developing PD and with the increased longevity in the population; PD incidences are destined to increase.

The Free Radical Theory is convincing as an explanation of neurodegeneration in PD and should be explored more extensively in the future..

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