1. Preamble

My father F. was diagnosed with Parkinson's disease (PD) about three years ago, in June 2009 . He was 74, in good general health conditions and without any further relevant pathology. In the preceding two years, he had been complaining of some muscular rigidity and indistinct uneasiness, so that he got deeply convinced that he was affected by some neurological disease. On the other hand, the symptoms he claimed were so generic that they were given no importance by both his family doctor and his relatives, which on the contrary blamed his anxious and hypochondriac mood.

Then, two events occurred almost simultaneously, that seem somehow correlated with the income of more evident Parkinsonian symptoms which in the end led to the actual diagnosis of PD.

The first event was the decision to quit smoking, in September 2008, some 6 months prior of early PD symptoms. The second event was a shock induced by a serious illness that affected his wife, i.e. a crisis of hemolytic anemia that required prolonged hospitalization. It was during this period (January 2009) that F. started exhibiting marked resting tremor in his right hand. It is remarkable that, as mentioned, even though he lamented several symptoms in advance, tremor was by no way present, and the onset of this symptom was sudden. The events are summarized in this table.

Event	**Date**
Generic uneasiness, anxiety, muscular rigidity	Since 2007
Quitted smoking	September 2008
Wife's illness	January 2009
Resting tremor onset	January 2009
PD diagnosed	June 2009

At present, F. is treated with levodopa and pramipexole (Mirapexim), and the disease is well controlled. However, since than I have been puzzled by the question whether there is an actual correlation of either event and the onset of tremor in PD. More in general, I was wondering if there is a correlation between smoking and PD risk. Whereas I found little evidence that an emotive shock may be correlated with the onset of PD symptoms, I was quite surprised to understand that there is a large amount of papers on the relationship between smoking and PD.

2. Correlation between smoking and PD

That some relationship exists between smoking habits and the risk of developing PD is quite recognized. For example, in a 1985 paper by G. Cazzato et al. *(Riv Neurol. 1985 Mar-Apr;55(2):79-87, "Smoking and Parkinson's disease," Cazzato G, Capus L, Monti F, Carraro N.)*, it is reported that "*tobacco smokers are at a lower risk of incurring Parkinson's disease than are non smokers and a high percentage of Parkinsonians stop smoking before the onset of the disease*." This claim was supported by a study performed on 110 hospital patients with PD and 110 other patients. *"In contrast to controls, fewer patients ever smoked cigarettes (39.1% versus 7.3%); moreover, many Parkinsonians (82%) stopped smoking before the onset of the disease."* In fact, we all were quite surprised by F.'s decision of quitting smoking, as it was only loosely motivated by health reasons (he was not experiencing significant breath problems). Without any scientific knowledge, but only based on direct observation, F.'s relatives were quite convinced of the role of his anxious mood induced by preexisting PD. I was surprised when I read, in the same cited paper, that *"the high percentage of patients with PD who stopped smoking might be explained by premorbid behavioral modifications related to the biochemical cerebral changes already present in the preclinical stage of the disease."*

Several epidemiological independent observations set the relative risk of a smoker having PD to approximately 0.5 that of a non-smoker. The effect of smoking on PD symptoms has also been investigated, showing that smoking reduced the tremor, rigidity, bradykinesia and gait disturbances observed in PD, although effects were transient and short lasting. In another report, improvement was noted in two elderly patients with PD treated with the nicotine gum and patch for several months. Variable results have been obtained in more recent small-scale clinical trials.

However, there is by no way accordance on the reasons underlying this fact. Some biochemical papers suggest a protective role for cigarette smoke against the development of PD. This claim is supported by animal studies, which have shown that the brain of rats exposed to cigarette smoke have reduced concentrations of pro-neurotoxins supposedly involved in the cause of PD, and that nicotine prevented parkinsonism in rodents. Moreover, other epidemiological studies (*Int J Neurosci. 1993 Jun;70(3-4):193-7. "Cigarette smoking: effects on cognitive functions and drug-induced parkinsonism in chronic schizophrenia.", Sandyk R.*) point out that patients with PD who smoke are less likely to develop dementia or dyskinesias. It is also well known that nicotine, besides having effects on the autonomic nervous systems, which are responsible for its negative effect on cardio circulatory system, has several effects on the central nervous systems (CNS). Many studies, mentioned by J. Le Houezec et al. (*Clin Chest Med. 1991 Dec;12(4):681-99. "Basic and clinical psychopharmacology of nicotine'"Le Houezec J, Benowitz NL*), point out effects of nicotine on human cognitive functions, with improvement in attention, learning, reaction time, and problem solving. Nevertheless, in this paper methodological problems are reported, which make it impossible to discriminate whether such improvements are due to a beneficial effect of nicotine itself, or simply to relief of symptoms of abstinence. However, this paper underlines a facilitatory action of nicotine on human performance. Hence, it may be possible that use of nicotine masks opposite effects due to dopamine lack.

On the other hand, other epidemiological hypotheses claim either that there is selective mortality of smokers with PD, i.e. death occurs at an accelerated rate, resulting in fewer smokers with PD - this idea is coherent with the fact that PD is most common in the older age (*it is worth noticing that this hypothesis has been ruled out by subsequent, more detailed epidemiological studies*), or that, again, the latent phase of PD, before symptoms become apparent, involves subtle personality changes producing the so-called *Parkinsonian personality* and an associated aversion to cigarette smoking. The changes may be a consequence of progressive nigrostriatal dopamine depletion resulting eventually in PD. Moreover, among the numerous non motor symptoms of PD, anxiety and depression are among the most recognized. In (*Eur J Neurol. 2008 Apr;15 Suppl 1:21-5. "Depressive symptoms in Parkinson's disease." Lemke MR*) it is stated that depression occurs in approximately 45% of all patients with PD, does not correlate with the stage of motor deficits (hence, may be present in a latent stage of the disease, even many years before the appearance of motor symptoms). Consequently, patients with subclinical PD are less prone to initiate smoking or more likely to quit.

In summary, cigarette smoking is for sure inversely associated with the risk of PD. These results are consistent across study designs and geographical settings and ***MAY reflect OR NOT*** a protective effect of smoking on PD risk. Whether these inverse associations correspond to protective effects of components of cigarette smoke, or whether they reflect changes in behavior that precede the motor manifestations of the disease, is not clear at present. The hypotheses to be investigated are:

a. Smoking is neuroprotective.
b. A genetic predisposition that increases the risk for PD simultaneously decreases the likelihood of smoking.
c. Inherently lower dopamine levels in predestined PD patients cause them to be less prone to addiction.
d. A combination of the above elements.

3. Is nicotine involved in PD prevention?

Even though cigarette smoking contains plenty of substances with a potential effect on the CNS, nicotine is the most natural candidate, due to its renown stimulatory effects on the CNS, mediated by the activation of neuronal acetylcholine-gated ion channel receptors (nAChRs, nicotinic acetylcholine receptors). Several studies using culture systems have revealed a protective effect of nicotine against neurotoxicity mediated by β-amyloid, glutamate, ethanol, trophic factor deprivation, etc.

Although PD is considered mostly a dopaminergic disorder, it is now clear that multiple CNS systems are involved in its pathogenesis. Lewy bodies[1] have been identified in numerous non-dopaminergic brain regions including the locus coeruleus, raphe nuclei, thalamus, amygdala, olfactory nuclei, pedunculopontine nucleus, and cerebral cortex. Significant declines have been observed in molecular markers of many neurotransmitters and neuromodulators, such as enkephalin, somatostatin, cholecystokinin, and substance P, as well as changes in the noradrenergic, glutamatergic, serotonergic, and cholinergic systems. Results from animal studies supports the assumption that nicotine or, in general, nAChR agonists may prove useful for treatment of PD-like symptoms, as NAChR stimulation can ameliorate motor behavior in animals with

nigrostriatal damage. Treatment with nicotine or a nicotinic agonist improves the action of levodopa in monkeys with nigrostriatal damage, and also ameliorates attention and executive cognitive functions. Most interestingly, studies using cultured ventral mesencephalic dopaminergic neurons showed that nicotine pre-treatment attenuates *in vitro* toxicity induced by MPP+, the metabolite of MPTP that selectively destroys dopaminergic terminals (*see Appendix 1*). Results have also been obtained *in vivo* using animal models. For example, nicotine pre-treatment protects rats against nigrostriatal degeneration induced by the dopaminergic neurotoxin 6-hydroxydopamine, by mechanical lesions, or induced by MPTP. Nicotine-induced protection against striatum damage is also observed in MPTP-treated nonhuman primates, a more significant model to the human disease. A chronic nicotine treatment improved a variety of neurochemical markers in MPTP-lesioned animals, including tyrosine hydroxylase, the dopamine and vesicular monoamine transporters, dopamine levels and nAChR expression. In addition, nicotine treatment normalized lesion-induced overactivity of the nigrostriatal pathway and preserved synaptic plasticity lost with nigrostriatal damage.

In (*J Neurochem. 2007 Jan;100(1):180-90, "Nicotine partially protects against paraquat-induced nigrostriatal damage in mice; link to alpha6beta2* nAChRs" Khwaja M, McCormack A, McIntosh JM, Di Monte DA, Quik M.*) they assessed the interplay between smoking and exposure to pesticides in a rodent model of nigrostriatal damage. Mice were administered nicotine as they were treated for 3 weeks with the pesticide paraquat (*see Appendix 1*). They showed that nicotine administration significantly protected against nigral cell damage, with only a 16% decline in nigrostriatal neurons against 25% in mice treated with paraquat alone. Moreover, paraquat treatment decreased of 14% the striatal dopamine transporter, and also this effect is partially prevented by nicotine. These changes in the striatal dopamine transporter were related to those in a select striatal α6β2* nicotinic receptor (nAChR) subtype. In contrast, striatal α4β2* nAChRs were not decreased with paraquat treatment, suggesting they are on a differential subset of dopaminergic terminals. The results show that nicotine treatment partially protects against paraquat-induced declines in nigrostriatal dopaminergic neurons to which a select population of α6β2* nAChRs are localized. This also points out the importance of characterizing the nAchR populations expressed in the striatum.

(Note[1]: a Lewy body is an *aggresome*, i.e. an abnormal protein aggregate found within nerve cells in PD. It is composed of several proteins such as alpha-synuclein, ubiquitin, neurofilament protein, alpha B crystalline, and it is likely to form when the cellular degradation machinery (proteasome) is impaired or overwhelmed. An aggresome forms in response to a cellular stress which generates a large amount of misfolded or partially denatured protein: hyperthermia, overexpression of an insoluble or mutant protein, etc.).

Addendum: Types of nAchR involved and their possible impairment in PD. NAChRs are pentameric ligand gated ion channels made of different combinations of α and β subunits to form heteromeric receptors, and select α subunits to form homomeric receptors. There is a requirement for α subunits in the receptor complex, as these possess the recognition site for acetylcholine. The β subunit does not bind acetylcholine, however it modulates the interaction of the ligand with the α subunit, thereby affecting the physiological and pharmacological properties of the receptor.

In order to understand how nAChR activation is involved in neuroprotection and/or symptomatic improvements in PD, knowledge of the anatomical relationship between the striatal cholinergic and dopaminergic systems is critical. Two major neuronal cell types are present in the striatum. These include the GABAergic medium spiny projection neurons that comprise the greater majority (95%) of neurons, as well as a much smaller population of interneurons (5%) of which the majority are GABAergic and about a third cholinergic. These larger cholinergic interneurons form a dense network in striatum that closely overlaps with dopaminergic terminals. Under physiological conditions, the cholinergic interneurons are tonically active. They release acetylcholine, which subsequently interacts with nAChRs on striatal cell bodies and nerve terminals, including dopaminergic projections from the substantia nigra and glutamatergic afferents from the cortex. *Stimulation of nAChRs at these sites results in dopamine release mediated through various nAChR subtypes.*

In the rodent nigrostriatal system, several nAChR subtypes have been identified, among which the major subtypes are α6α4β2β3, α6β2β3 and α4β2* (*indicates the possible presence of other subunits in the receptor complex). In contrast, α7 nAChRs are present at a much lower density. Multiple nAChRs have also been identified in striatum of nonhuman primates, with predominant presence of α6β2* and α4β2* subtypes. The major nAChR subtypes appear similar to those in the rodent

and include α6α4β2β3, α6β2β3 and α4β2, with only very low α7 nAChR expression. Although studies in human striatum are more limited, the nAChR subtypes appear to be similar to those in the rodent and monkey nigrostriatal systems. Combined data suggest the presence of α4β2*, α6β2* and α7 nAChRs in human striatum.

A key question is which kind of alteration is induced on nAChRs by nigrostriatal damage in PD. Investigations carried out using nonhuman primates with MPTP-induced nigrostriatal damage, revealed that a moderate lesion led to declines predominately in α6β2* nAChRs that closely paralleled reductions in the dopamine transporter. With severe nigrostriatal damage (≥90%), decreases were also evident in α4β2* nAChR although they were of smaller magnitude compared to those in α6β2* nAChRs. There was no change in α7 nAChRs, while the α5 and β4 subunit proteins are not expressed in monkey striatum. In summary, α6β2* nAChRs appear particularly vulnerable to nigrostriatal damage as they are selectively decreased with a moderate lesion. In contrast, α4β2* subtypes are affected only with severe degeneration, while α7 nAChRs are unchanged. Human results in brains of PD cases were similar.

The α6β2* nAChR population may be of particular relevance to nigrostriatal function and to PD. In fact, its localization is fairly restricted to the dopaminergic nigrostriatal and a few other catecholaminergic systems. In addition, declines in α6β2* nAChRs closely parallel the loss of the striatal dopamine transporter. Recent findings suggest that the α6α4β2β3 nAChR subtype represents a marker for neurons particularly vulnerable to nigrostriatal damage. Nicotine stimulation of the remaining α6α4β2β3 nAChRs may enhance function lost with nigrostriatal damage. On the other hand, nicotine is well known to desensitize or block nAChRs raising the question whether nicotine may induce its beneficial effects by blocking aberrant nAChR-mediated activity. This latter interpretation would suggest that an α6α4β2β3 nAChR antagonist (and not an agonist) may be the drug of choice to counteract the effects of nigrostriatal injury and/or provide symptomatic improvements.

4. Possible biochemical action mechanisms of nicotine

Neurotrophic factors. In (J. *Neurochem. 1998 Dec;71(6):2439-46 "Nicotine prevents experimental parkinsonism in rodents and induces striatal increase of neurotrophic factors." Maggio R, Riva M, Vaglini F, Fornai F, Molteni R, Armogida M, Racagni G, Corsini GU.*), it is shown that nicotine induces the basic fibroblast growth factor-2 (FGF-2) and the brain-derived neurotrophic factor in rat striatum. As trophic factors have been reported to be neuroprotective for dopaminergic cells, such data suggest that the increase in neurotrophic factors is a possible mechanism by which nicotine protects from experimental parkinsonisms.

Modifications in dopamine release/utilization (indirect antioxidant effect). Cigarette smoke may stimulate dopamine release and upregulate nicotinic receptors through nicotine. A study has correlated smoking with low prolactin levels in post menopausal women, possibly suggesting a role of nicotine as a dopamine stimulating factor. In (*N Y Acad Sci. 2004 Dec;1035:316-34. "Nicotinic receptor modulation for neuroprotection and enhancement of functional recovery)* it is stated that the action of nicotine on dopamine release may explain the variable effects of nicotine in animal models of PD, pointing out neuroprotective properties of both nicotinic agonists and antagonists. This may be explained by recalling the multiplicity of nAChR types present in the nigrostriatal system. Janson and Moller found that chronic nicotine treatment in rats at concentrations similar to those found in smokers result in a functional desensitization of nicotinic receptors located on dopaminergic neurons. This could lead to reduced burst firing of nigral dopaminergic neurons as well as reduced dopamine utilization. In *(Cell Mol Neurobiol. 1988 Sep;8(3):285-91, "Hypothesis: a nicotine-dopamine interaction linking smoking with Parkinson's disease and tardive dyskinesia' "Kirch DG, Alho AM, Wyath*) it is reported that, although acute doses of nicotine have been shown to facilitate dopamine release, recent chronic nicotine treatment may actually decrease CNS dopamine turnover in the striatum. This may protect against free radical formation from catecholamine oxidation (less free radical formation and thus less oxidative stress) that in turn damages striatal neurons – see next paragraph.

Direct antioxidant effect. A large body of literature supports a role for ROS and oxidative stress and neuronal loss in the substantia nigra. Much of this data is linked to the mitochondrial dysfunction of complex I associated with this disease. There have been numerous reports of increases in lipid, DNA and protein oxidative changes in PD and animal

models of PD. In addition, there are reports of decreases in antioxidants such as GSH, increased iron content and activation of NFkB. Toxins that increase ROS in the substantia nigra such as MPTP also produce PD-like symptoms and pathology in man and animals. Its metabolite MPP+, is actively transported into DAergic neurons by the dopamine transporter. Within these neurons MPP+ enters mitochondria, and selectively inhibits mitochondrial respiration at complex I of the electron transport chain. Chronic infusion of rotenone, a selective complex I inhibitor, also reproduced behavioral (e.g. hypokinesia and rigidity) and neuropathological features of PD in rats.

Nicotine reduces ROS generation from mitochondria, and prevents neurotoxin induced mitochondrial swelling and cytochrome c release in vitro through inhibition of the mitochondrial PT pore. The antioxidant properties of nicotine may be involved in neuroprotection; e.g. nicotine may be protective against PD through complex formation with Fe^{2+}, thus yielding Fenton inactive Fe^{2+} and less oxidative stress. In (*Biochim Biophys Acta. 1999 Jul 7;1454(2):143-52 "In vitro and in vivo studies investigating possible antioxidant actions of nicotine: relevance to Parkinson's and Alzheimer's diseases," Linert W, Bridge MH, Huber M, Bjugstad KB, Grossman S, Arendash GW*) it is postulated that nicotine can affect the formation of the neurotoxin 6-hydroxydopamine resulting from the addition of dopamine to Fenton's reagent (i.e., Fe^{2+} and H_2O_2). Thus, under certain circumstances, nicotine can strongly affect the course of the Fenton reaction. The results of these studies suggest that the beneficial/protective effects of nicotine in PD may be, at least partly, due to antioxidant properties.

Smoking also induces cytochrome P450 enzymes (responsible for biomodification and detoxification of foreign substances and increased metabolism of xenobiotics) that may be involved in the metabolism and detoxification of neurotoxins such as MPTP. Shahi et al. observed that cigarette smoke exposure, besides partially protecting against MPTP-induced striatal dopamine depletion in mice, at the same time causes cytochrome P450 induction. P450 induction alone also had a protective effect. However, it is not agreed that nicotine is the only substance in cigarette smoke that is able to induce cytochrome P450 enzymes. In (*Neuroepidemiology. 1993;12(2):114-6." Induction of cytochrome P-450 enzymes via tobacco smoke: a potential mechanism for developing resistance to environmental toxins as related to parkinsonism and other neurologic diseases.", Gresham LS, Molgaard CA, Smith RA*) the induction of cytochrome P450 enzymes is demostrated by polyaromatic hydrocarbons in tobacco smoke.

Many authors have observed that cigarette smoke exposure inhibits monoamine oxidase-B (MAO-B) in several animal tissues and human platelets. Although post-mortem studies have not revealed differences in MAO-B activity between PD patients and controls, Fowler et al. showed that smoking reduced levels of MAO-B *in vivo* in various brain regions (including the basal ganglia). As oxidation of dopamine by MAO-B leads to the formation of hydrogen peroxide, its inhibition might lead to less oxidative stress. MAO-B is also required for the conversion of MPTP to the active neurotoxin MPP+. The presence of the MAO-B allele I has been associated with PD, thus also suggesting that a particular variant of this enzyme may be important in a predisposition to the disease.

Other substances in cigarette smoke may also exert a neuroprotective effect: 4-phenyl-pyridineand hydrazine have been shown to protect from MPTP-induced neurotoxicity, possibly through a competitive mechanism. In (*Chem Res Toxicol. 2001 May;14(5):523-7. "Neuroprotection in the MPTP Parkinsonian C57BL/6 mouse model by a compound isolated from tobacco.", Castagnoli KP, Steyn SJ, Petzer JP, Van der Schyf CJ, Castagnoli N Jr.),* 2,3,6-trimethyl-1,4-naphthoquinone (TMN) has been identified as a MAO-A and MAO-B inhibitor present in tobacco smoke. Results of experiments provide evidence that this compound protects against the MPTP-mediated depletion of neostriatal dopamine levels in mouse. These results support the hypothesis that the inhibition of MAO by constituents of tobacco smoke may be related to the decreased incidence of PD in smokers. Other quaternary N-methylated nicotine derivatives may have a similar effect. Calne and Langston proposed that carbon monoxide in cigarette smoke could act as a radical scavenger and thereby reduce PD risk. Kessler and Diamond hypothesized that nicotine could be converted to nicotinic acid by an as yet unidentified pathway.

Genetic lack in detox enzymes reduce smoking attitude. Another possible explanation for less smoking among later PD patients would be an inherent inability to metabolize certain toxins, which could influence the inclination to smoke. Both enzymatic and molecular genetic studies have found a higher prevalence of poor homozygous and heterozygote

debrisoquine metabolizers among PD patients than controls. Debrisoquine hydroxylase is a P450 enzyme involved in the detoxification of numerous xenobiotics, among which MPTP. Cholerton et al. found that healthy homozygous poor metabolizers of debrisoquine (but not heterozygotes) were less able to convert nicotine to cotinine. Barbeau et al. reported that PD patients in their study who were heterozygotes or homozygous poor metabolizers of debrisoquine had either never smoked or had smoked for a shorter time period than normal metabolizers. Sulphur oxidation and conjugation is defective more frequently among PD patients as compared to controls. In addition, there is evidence that N-methylated pyridines tend to accumulate in PD patients as compared to controls, possibly due to a block in further metabolism by P450 oxidation reactions or oxidation through monoamine oxidase B. Nicotine is also metabolized to an N-methylated pyridine derivative, which might theoretically be perceived as unpleasant and lead to less smoking in predestined PD patients. Alternatively, accumulation of this metabolite could saturate the system completely and thereby reduce the formation of other more toxic N-methylated pyridines, thus having a true protective effect.

5. Can this be exploited for therapy?

Pure nicotine has no known carcinogenic properties and can be administered in numerous ways including transdermal patches and tablets. As discussed, nicotine acts on cholinergic receptors which are depleted in PD, and that interact closely with several neurotransmitters including dopamine. Hence, whereas there is no doubt that tobacco smoking can be harmful and no-one should be encouraged to smoke, and that nicotine has many harmful side-effects, it may have therapeutic value or at least be a useful tool for future drug development.

In *(Expert Rev Clin Pharmacol. 2011 Jul;4(4):429-36.Can nicotine be used medicinally in Parkinson's disease? Thiriez C, Villafane G, Grapin F, Fenelon G, Remy P, Cesaro P.)* it is observed that nicotine has clear motor effects when associated with L-DOPA, reducing L-DOPA-induced dyskinesias. However, clinical trials have yielded inconclusive results so far, and are hampered by different designs and small cohorts. There is still no agreement on the daily dosage of nicotine or the method of administration. Together, these data suggest that nicotine or nicotinic receptor drugs have therapeutic potential for PD, although the specific treatment regimens remain to be determined.

We have seen that striatal α6β2containing nAChRs are particularly susceptible to nigrostriatal damage, with a decline in receptor levels that closely parallels losses in striatal dopamine. In contrast, α4β2-containing nAChRs are decreased to a much smaller extent under the same conditions. These observations suggest that development of nAChR agonists or antagonists targeted to α6β2-containing nAChRs may represent a particularly relevant target for PD therapeutics.

6. Conclusions

From this brief resume of the state of the art, I have understood that there may be a relationship between nicotine and protection against PD. The possible role of nicotine in dopamine release, nAchR modulation, prevention of oxidative stress may explain the reasons why, after quitting smoking, the symptoms of a latent disease may become apparent. However, I have still doubts related to the involved time constant (i.e., whether the suppression of a neuroprotective substance can exert its effects in a time as short as six months). The theory that an underlying Parkinsonian personality trait underlies the behavior of still asymptomatic patients is fascinating, and it is indeed possible that the real situation implies a mixture of both effects.

APPENDIX 1: MPTP

(from www.wikipedia.org)

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin precursor to MPP+, which causes permanent symptoms of PD by destroying dopaminergic neurons in the substantia nigra of the brain. It has been used in study disease models in various animal studies. While MPTP itself does not have opioid effects, it is related to MPPP, a synthetic opioid drug with effects similar to those of morphine and meperidine (Demerol). MPTP can be accidentally produced during the illicit manufacture of MPPP, which is how its Parkinson-inducing effects were first discovered. Injection of MPTP causes rapid onset of Parkinsonism, hence users of MPPP contaminated with MPTP will develop these symptoms.

MPTP itself is not toxic, and as a lipophilic compound can cross the blood-brain barrier. Once inside the brain, MPTP is metabolized into the toxic **MPP** (+1-methyl-4-phenylpyridinium) by the enzyme MAO-B of glial cells. MPP+ kills primarily dopamine-producing neurons in the pars compacta of the substantia nigra. MPP+ interferes with the oxidative phosphorylation in mitochondria (complex I of the electron transport chain), causing depletion of ATP and cell death, and the buildup of free radicals, toxic molecules that contribute further to cell destruction. It also inhibits the synthesis of catecholamine, reduces levels of dopamine and cardiac norepinephrine, and inactivates tyrosine hydroxylase. The chloride of MPP+ has been used as a herbicide under the trade name **cyperquat** and is structurally similar to the herbicide paraquat.

MPP+ quite selective ability to cause neuronal death in dopaminergic cells can be explained through a high-affinity uptake process in nerve terminals normally used to reuptake dopamine after it has been released into the synaptic cleft. The dopamine transporter moves MPP+ inside the cell. The resulting gross depletion of dopaminergic neurons has severe implications on cortical control of complex movements. MPTP causes parkinsonism in primates including humans, while rodents are much less susceptible and rats are almost immune. It is believed that the lower levels of MAO-B in the rodent brain's capillaries may be responsible for this.

The neurotoxicity of MPTP was hinted at in 1976 after Barry Kidston, a 23-year-old chemistry graduate student in Maryland, synthesized MPPP with MPTP as a major impurity, and self-injected the result. Within three days he began exhibiting symptoms of PD. The National Institute of Mental Health found traces of MPTP in his lab. They tested the substances on rats, but due to rodent tolerance for this type of neurotoxin nothing was observed. Kidston's parkinsonism was successfully treated with levodopa but he died 18 months later from a cocaine overdose. Upon autopsy, destruction of dopaminergic neurons in the substantia nigra was discovered.

In 1982, seven people in Santa Clara County, California were diagnosed with Parkinsonism after having used MPPP contaminated with MPTP. The neurologist J. William Langston in collaboration with NIH tracked down MPTP as the cause, and its effects on primates were researched. Langston documented the case in his 1995 book *The Case of the Frozen Addicts*. Langston *et al.*(1984) found that injections of MPTP in squirrel monkeys resulted in parkinsonism, symptoms of which were subsequently reduced by levodopa. The symptoms and brain structures of MPTP-induced Parkinson's are fairly indistinguishable to the point that MPTP may be used to simulate the disease in order to study PD and possible treatments within the laboratory. Mouse studies have shown that susceptibility to MPTP increases with age. Knowledge of MPTP and its use in reliably recreating PD in experimental models has inspired scientists to investigate the possibilities of surgically replacing neuron loss through fetal tissue implants, sub thalamic electrical stimulation and stem cell research, all of which have demonstrated initial, provisional successes. It has been postulated that PD may be caused by minute amounts of MPP+-like compounds from ingestion or exogenously through repeated exposure and that these substances are too minute to be detected significantly by epidemiological studies. In 2000, another animal model for PD was found. It was shown that the pesticide and insecticide rotenone causes parkinsonism in rats by killing dopaminergic neurons in the substantia nigra. Like MPP+, rotenone also interferes with complex I of the electron transport chain.

MPTP was first synthesized as an analgesic in 1947 by Ziering *et al.* by reaction of phenylmagnesium bromide with 1-methyl-4-piperidinone. It was tested as a treatment for various conditions, but the tests were halted when Parkinson-like symptoms were noticed in monkeys. In one test of the substance, two of six human subjects died. MPTP is used in industry

as a chemical intermediate; the chloride of the toxic metabolite MPP+ was turned into the herbicide cyperquat, that is structurally similar to Paraquat.

Paraquat is the trade name for ***N*,*N*′-dimethyl-4,4′-bipyridinium dichloride**, one of the most widely used herbicides in the world. Paraquat is toxic to human beings and animals. Research has shown that it is linked to development of PD. Although first synthesized in 1882, paraquat's herbicidal properties were not recognized until 1955. Paraquat was first manufactured and sold in early 1962, and is today among the most commonly used herbicides. It is quick-acting, non-selective, and kills green plant tissue on contact. It is redistributed within the plant, but does not harm mature bark. Herbicides are used to protect crops by controlling a wide range of annual and certain perennial weeds that would otherwise compete with the crop for water, nutrients, and light.

In the United States, paraquat is available primarily as a liquid in various strengths. It is classified as "restricted use," which means that it can be used only by licensed applicators. The European Union approved the use of paraquat in 2004. Subsequently Sweden, supported by Denmark, Austria, and Finland, brought the European Union commission to court. In 2007, the court annulled the directive authorizing paraquat as an active plant protection substance. As a consequence, paraquat has been forbidden since 2007.

Paraquat is often used in science to catalyze the formation of ROS, more specifically, the superoxide free radical. Paraquat will undergo redox cycling in vivo, being reduced by an electron donor such as NADPH, before being oxidized by an electron receptor such as dioxygen to produce superoxide. A large majority (93%) of fatalities from paraquat poisoning are cases of intentional self-administration, i.e., suicides. In third world countries, paraquat is a major suicide agent. The reason is due to its widespread availability, low toxic dose (10 ml or 2 teaspoons is enough to kill) and relative low cost. There are campaigns to control or even ban paraquat outright, and there are moves to restrict its availability by requiring user education and the locking up of paraquat stores. Pure paraquat, when ingested, is highly toxic to mammals, including humans; potentially leading to acute respiratory distress syndrome (ARDS), and there are no specific antidotes. However, fuller's earth or activated charcoal is an effective treatment, if taken in time. Death may occur up to 30 days after ingestion. Diluted paraquat used for spraying is less toxic; thus, the greatest risk of accidental poisoning is during mixing and loading paraquat for use. In acute toxicity studies using laboratory animals, paraquat has been shown to be highly toxic by the inhalation route and has been placed in Toxicity Category I (the highest of four levels) for acute inhalation effects. However, the EPA has determined that particles used in agricultural practices (400 to 800 μm) are well beyond the respirable range and therefore inhalation toxicity is not a toxicological endpoint of concern. Paraquat is toxic (Category II) by the oral route and moderately toxic (Category III) by the dermal route. Paraquat will cause moderate to severe eye irritation and minimal dermal irritation, and has been placed in Toxicity Categories II and IV (slightly toxic) respectively for these effects. Even a single swig, immediately spat out, can cause death from fibrous tissue developing in the lungs, leading to asphyxiation. According to the Center for Disease Control, ingesting paraquat causes symptoms such as liver, lung, heart, and kidney failure within several days to several weeks that can lead to death up to 30 days after ingestion. Those who suffer large exposures are unlikely to survive. Chronic exposure can lead to lung damage, kidney failure, heart failure, and esophageal strictures. Long term exposures to paraquat would most likely cause lung and eye damage, but reproductive/fertility damage was not found by the United States Environmental Protection Agency (EPA) in their review. In 2011, a US National Institutes of Health study showed a link between paraquat use and Parkinson's disease in farm workers. A co-author of the paper said that paraquat increases production of certain oxygen derivatives that may harm cellular structures, and that people who used paraquat, or other pesticides with a similar mechanism of action, were more likely to develop PD. Paraquat-induced toxicity in rats has also been linked to Parkinson's-like neurological degenerative mechanisms. A study by the Buck Institute showed a connection between exposure to paraquat and iron in infancy and mid-life Parkinson's in laboratory mice. Paraquat also induces oxidative stress in invertebrates such as Drosophila melanogaster. Paraquat-fed flies suffer early-onset mortality, and significant increases in superoxide dismutase activity.

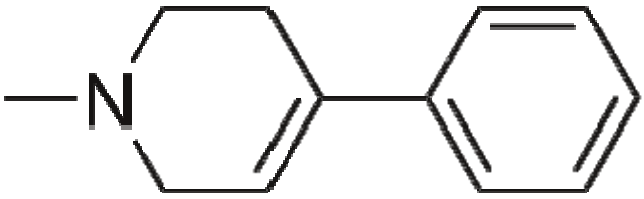

Figure 1: MPTP

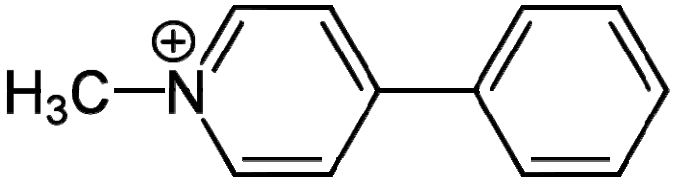

Figure 2: MPP+

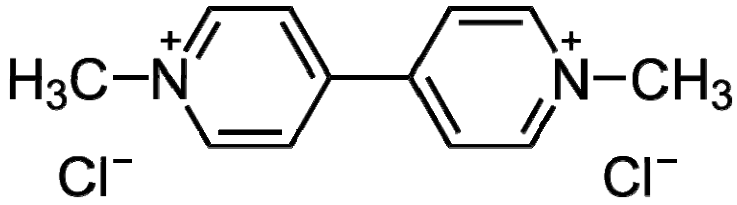

Figure 3: paraquat