Chapter 2

Fetal and Environmental Basis for the Cause of Parkinson's Disease

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1. Introduction

In Parkinson's disease (PD) dopamine producing neurons in the substantia nigra, pars compacta of the midbrain and with their axons projecting to the neostriatum degenerate. PD is classified as being familiar when it is known to be the result of genetic abnormalities, and this represents about 5 to 10 percent of all cases. The other cases are idiopathic, represent 90 – 95 percent of all cases of PD and the causes are unknown. The expression of the specific symptoms of idiopathic PD vary among individuals, and may be accompanied with other brain disorders, including Alzheimer's type dementia, depression and amyotrophic lateral sclerosis (ALS). The common relationship among all of the degenerative disorders is that all are caused by failure of specific functions that are under the control of identifiable neuronal sets, with relatively low population number of larger neurons that usually occur in clusters and with far reaching axons. These neurons are well represented by the nigrostriatal dopamine neurons, and the degeneration of the neuronal set represents the major pathology of PD. They are also represented by the basal nucleus of Meynert acetylcholine neurons with major projections to the cerebral cortex that degenerate in Alzheimer's disease (AD), and by the upper and lower motor neurons with projections to the brainstem, spinal cord or motor-end plate, that degenerate in ALS. These neuronal sets have specific prenatal and fetal periods for their neurogenesis, migration and axonal extension during which they acquire their specific phenotype that can be influenced by internally and externally derived biochemical forces, including toxins and excesses and deficiency of regulatory factors that will shape the physiological and functional destiny of these neuronal sets. If the influence is of a positive or enhancing nature, the neuronal set will turn out to be functionally superior or with exceptional resilience and longevity and will impart an enhanced character to the individual. However, if the influence is deleterious it will cause harm to the neuronal set and likewise will influence the character of the individual. For the latter, deficiencies may occur at sub-threshold level, may continue in a subliminal and a graded way and may

compromise resilience and functional longevity, finally serving as the 'weak link' and pairing with deteriorating changes that occur during aging to cause diseases, such as Parkinson's disease. Whereas the gene has inherent command over the variation of biological forms and some biological outcomes, it is the interacting entities derived from the environment that really sway functional outcomes. Toxins, that may be endogenous or exogenous, represent a set of these environmental factors and quite likely are responsible for the cause of idiopathic PD and other degenerative disorders. So, this chapter will discuss the idea, supported by experimental findings, that the substantia nigra dopamine neurons that deteriorate to the point of causing idiopathic PD were impaired early in life at a sub-threshold level. This occurs during the vulnerable stage of neurogenesis, neuronal development and neuronal migration. The exposures of the substantia nigra dopamine neurons to toxic or harmful influences early in life cause sub-threshold harm, and further exposures to stress during aging cause additive insults that precipitate the symptoms of PD. The early insults, the naturally low population of nigrostriatal neurons, the continuous functional demands placed on the few nigrostriatal DA neurons and the far-reaching nature of the axonal projections render the nigrostriatal DA neurons vulnerable. The high content of cytoskeleton and their kinases seen as pathological markers for various degenerative disorders (McGee and Steele, 2011) indicate that axonal damage to far-reaching neurons is a preeminent occurrence in PD.

2. Major symptoms and the proposed causes for Parkinson's disease

The major clinical symptoms of Parkinson's disease (PD), an age-related disorder, are resting tremors, hypokinesia, rigidity and postural instability (Tetriakoff, 1919: Foix and Nicolesco, 1925) caused by the degeneration of the nigrostriatal (NS) dopaminergic pathway and the depletion of dopamine (DA) (Greenfield and Bosanquet, 1953; Hornykiewicz, 1966). The pathological features include extensive (about 70% or more) loss of dopaminergic neurons in the pars compacta of the substantia nigra, the presence of inter-cytoplasmic inclusions known as Lewy's bodies and gliosis. It was reported also that norepinephrine (NE) (Erhinger and Hornykiewicz, 1960) and serotonin (5-HT) Bernheimer et al., 1961) levels are decreased and that acetylcholine neurotransmission (Yahr, 1968) is increased. A small population of PD cases is caused by genetic abnormalities, involving alpha–synuclein (Polymeropoulos et al, 1997; Papadimitrior et al, 1999 and Kruger et al,1998, Dauer et al, 2002), ubiquitin (Leroy et al, 1998) and apolipoprotein E (APOE), (Kruger et al, 1999). Changes in chromosome 2p13 (Gasser et al, 1998), cyp2D6 (Kruger et al, 1999; Christensen et al, 1998; Kosel et al, 1996; Bon et al, 1999, Sabbagh et al, 1999) as well as mitochondria tRNA (A4336G) (Epensperger et al, 1997) have also been reported. The mutation of the parkin gene is closely associated with juvenile PD (Kitada et al, 1998), which has about eight variants (Lansbury and Brice, 2002). It should be noted however, that multiple other PD cases have been screened and they did not harbor mutations (Giasson et al, 2000), but gene mutations may serve as vulnerable markers, superimposed by environmental factors and age-related wear-and–tear. The root-cause of idiopathic PD is unknown, but various factors are implicated, including the oxidation of dopamine, free radical-mediated oxidative injury,

mitochondrial abnormalities, excitotoxins, over exposure to manganese (Chu et al, 1995; Hochberg et al, 1996) and carbon monoxide, the intake of beta-methylaminoalanine (Spencer, 1987), benzyl-tetra-hydroisoquinolines and tetra-hydroprotoberines (Caparros-Lefebvre and Steele, 2005), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Davis et al, 1979), methanol (Guggenheim et al, 1971). As well as the potent methylating agent, methylazoxymethanol (Ince and Codd, 2005) and excess methylation via high utilization of the endogenous S-adenosyl-L- methionine in the brain (Charlton and Way, 1978; Charlton et al, 1992; Charlton and Mack, 1994).

2.1. Aberrations in non-basal ganglia systems

In PD the basal ganglia is the primary affected structure, but lesions have been identified in the locus ceruleus (Selby, 1968; Alvord et al, 1974), the hypothalamus (Jagar and Bethlem, 1969; Ohama and Ikuta, 1976; Langston and Forno, 1978), the dorsal motor nucleus of vagus (Eadie, 1963; Vanderhaegen et al 1970), the sympathetic ganglia (Jagar and Bethlem, 1960; Vanderhaeghen et al., 1970; Rajput and Rozdilsky, 1970 and Forno and Norvill, 1976) and in the adrenal medulla (Jager, 1969) as well. Furthermore, Lewy's bodies, the standard marker for PD, have been seen in the cerebral cortex, anterior thalamus, hypothalamus, amygdala, basal forebrain, dorsal motor nucleus of vagus, adrenal medulla and locus ceruleus. The clearly un-circumscribed localization of lesions in the patients or victims of PD means that the changes or the incidents that cause the dopaminergic cell loss in the nigrostriatal system may not specifically target the basal ganglia, but instead the nigrostriatal dopaminergic neurons may be more vulnerable or sensitive. In other words, the factors that are involved in the cause of, at least, some cases of PD may also cause harm to other cell populations, but the basal ganglia neurons are more vulnerable and will die when other neuronal sets remain alive and function normally. This means that a state of vulnerability or sensitization may exists for PD and that the occurrence of damage to other neuronal pool may help to explain the variation in the expression of the PD syndrome.

3. The fetal basis hypothesis for Parkinson's disease

PD is age-related but a large percentage of the older population does not suffer from the disorder, although aging is accompanied with pronounced and progressing reduction in motor and other functions. The age-dependent increase in the frequency of essential tremor (Elble 1995; Koller and Huber, 1989), the occurrence of kyphotic posture, diminished arm swing, shorter strides (Murray et al, 1969; Elble et al, 1992; Elble et al, 1991, bradykinesia (Waite et al, 1996) and slowed reaction time (Weiss 1965; Welford, 1977) are signs found to be associated with aging, but the abnormalities are distinguishable from the changes that occur in PD. This suggests that, during normal aging and as a rule, the nigrostriatal DA neurons do not deteriorate to the point of causing PD. Therefore, it is very possible that for PD symptoms to be expressed in the aged, some primary changes that render the nigrostriatal DA neurons vulnerable occur during the earlier life of the PD patients and serve as the underpinning for the deleterious age-related changes that normally occur. So, the functional age-related changes pair with the early predispositions to precipitate the

symptoms of PD. Furthermore, there is the high probability that the causes of the vulnerability that occur early in life are based on chance and occur during a critical period when nigrostriatal dopamine neurons are structurally responsive to endogenous and exogenous toxic type of interventions.

3.1. Chance encounter of the nigrostriatal neurons with harmful factors

It is proposed that chance encounter of factors with the NS DA neurons at critical times during their development eventually shape the long-term outcome of the neuronal pool. If the encounter decreases the longevity of the neurons idiopathic PD will occur. This will underlie the sporadic feature of idiopathic PD, and the nature of the early encounter will determine the pathological characteristics. So, the cluster of PD cases caused by the outbreak of the epidemic encephalitis lethargic in 1919 that killed about one million people worldwide and left millions more 'frozen' with the symptoms of PD and which decline rapidly after 1925 (Ravenholt et al, 1982) represent a special but a typical set of parkinsonism. The Guam Parkinson's dementia complex (PDC)-amyotrophic lateral sclerosis (ALS) syndrome proposed to be caused by the toxins contained in flour prepared from the cycad plant (Spencer et al, 1987) suggests a syndrome that is caused by long–term exposures that target the nigrostriatal neurons, motor neurons and basal nucleus of Meynert acetlycholinergic neurons. In these cases the diversity in the character of the syndrome is a reflection of the neuronal sets that were harmed. So, the individuals that develop idiopathic Parkinson's disease, and likely other neurodegenerative disorders, were marked early in life for the disorder. The early process may be synonymous to natural selection that occurs by chance, and helps to define the variation of phenotypes among a population. In the case of PD, the variation may be defined by the magnitude of the reduction in the number of nigrostriatal dopaminergic neurons, and/or deficiencies in the metabolic capability or resilience of the neurons. Therefore, the nigrostriatal DA neurons of the PD patients may have experienced early exposure to environmental, nutritional and/or metabolic toxic interventions. This early exposures may result in DA neurons that lack the reserve capacity to survive during the natural life of the individual, but they function at a level of output that is above the threshold at which the symptoms of PD occur (pre-threshold). During the progression of time or during aging, however, subtle but accumulative changes occur that further damage the nigrostriatal DA neurons and the additive effects precipitate PD-like symptoms. **Thus, the fetal basis hypothesis proposes that by chance early interventions render the nigrostriatal neurons sensitive, susceptible or vulnerable, characteristics that enable changes involving the wear-and-tear of living or the exposure to toxins or traumatic events later in life to take a toll on the vulnerable NS neurons and cause PD.**

3.2. High workload may explain the vulnerability of the nigrostriatal neurons

The normal population of nigrostriatal pigmented neurons is relatively low, showing a mean value of 163,238 ± 42,372 in normal human (Ma et al, 1997). The relatively low population number of the nigrostriatal neurons and the high workload placed on these specialized cells play a role in their metabolic durability. This relationship may help to

explain the rapid decline in the ability to effectively execute rapid and skillful movement-related skills as a function of aging. This is evident in the short time that a competitive athlete can maintain his or her exceptional ability. A 100-meter runner, for example, is normally competitive for only one or two olympic game and skillful ballet dancers are young people. Even the ability to play the game of golf requires skills that deteriorate to non-competitiveness by the time the athlete reaches early middle age. So, even under normal living condition the nigrostriatal neurons are under moment-by-moment demands by the motor and other functions that they control, and their capability naturally deteriorates in time. The demands placed on these neurons by muscles, for example, are continuously occurring, even during sleep, since skeletal muscle activities are maintained for limb and eye movements. Demands on the nigrostriatal neurons are continuous during regular activities and increased during stress-related physical activities, so, these neurons never rest, unlike neurons that control functions such as hearing, vision and cognition that are at rest at least during sleep. Therefore, while other neuronal sets with less stressful functions and without experiencing an early assault will age at a regular rate, the functional stress imposed on already susceptible dopamine neurons, during the process of living, will cause them to deteriorate at a fast rate to below the threshold that maintains normal functions. This means, therefore, that the prenatal exposure hypothesis will explain cases of juvenile PD that occur at about the age of forty years, in patients that are functionally normal high into the thirties. So, early markers for juvenile PD that are known to be caused by genetic abnormalities, likely exist long before the occurrence of the PD symptoms. The early markers may exist as subtle but serious sub-threshold genetic nigrostriatal abnormality that is below the threshold at which PD symptoms are expressed. So, as compared to idiopathic PD, that has its onset about in the sixth decade, juvenile PD, because of it more serious early impairments, requires a shorter duration of time before the added stress induces threshold level nigrostriatal damage. The overall analogy, therefore, means that at least two stages or two sets of factors or groups of factors are involved in PD:

1. The first stage: the predisposing/sensitization/susceptible/vulnerable stage.
2. The second stage: the inducing/precipitating/superimposing stage.

Again, the first stage is defined by subtle or sub-threshold level of adverse changes that start early in life and form the weak link for the second stage, defined by stressful events occurring later in life and coupled with the first stage to cause the expression of the disease symptoms. It should be noted that normal functional and age-related existence may cause enough stress to produce the 'added-on' second stage damage to the nigrostriatal neurons in individuals with early stage predisposition.

4. The predisposing, sensitization, susceptible or vulnerable stage of the hypothesis

Normally, immature neurons or neuroblast are subject to chemical and mechanical influences that cause them to migrate to various locations in the nervous system, to extend axonal and dendritic processes toward other cells and then to make and break synaptic

connections with these cells before a final pattern of branching and connections are established (Levitan and Kaczmarek, 2002). Moreover, factors released by other cells influence the type of neurotransmitter the neuron will synthesize and the specific type and mixture of receptor, ion channels and other proteins that determine the characteristics of the fully differentiated neurons (Levitan and Kaczmarek, 2002). Along with or besides the normal pattern of development that occur, the differentiating and young neurons may be subjected to toxic and interfering influences that shape them for life. There could be failure in the normal process of apoptosis, that acts via cytochrome c, caspase 9, caspase 3 and other cellular constituents, to cause cellular pruning and to allow the remaining neurons to survive and to be properly organized.

In general, brain neurons are known to be susceptible or vulnerable to insults during prenatal and the early postnatal stage of the life of the individual. This is the basic reasons for the practice of protecting the pregnant mother, new born and young children from chemical and other potentially harmful exposures. For the midbrain dopaminergic system, the most susceptible time is likely to be the period of neurogenesis, proliferation and migration of the cells to produce the nigrostriatal dopaminergic phenotype. These midbrain dopamine neurons are generated early during development, first in the midbrain-hindbrain junction (Voorn et al, 1988), and they migrated radially to their final position in the ventral midbrain to form the substantia nigra, the ventral tegmental area and the retrorubal nuclei (Perrone-Capano and di Porzio 1996). Tyrosine hydroxylase (TH) immunoreactivity is used to identify those dopamine tegmental neurons, and the first appearance of the TH marker is regarded as the birth of the tegmental cells, which occurs on embryonic day 9 for the mouse. The periods close to the birth of these neurons are likely to be a very critical window through which the environment causes long-term changes to the cells and to the motor performance of the organism. In fact, it is these types of manipulations that may be relevant in causing diseases and in enhancing special features related to the functions of the basal ganglia, and they will have effects similar to natural selection and imprinting.

The signal for the differentiation of the NS DA neurons is through a protein called the sonic hedgehog (SHH). The amino-terminal product is the inductive moiety. SHH is produced by the floor plate cells and induces the dopaminergic phenotype (Hayes, et al., 1995). The signal for the SHH protein can be antagonized by increasing the activity of cyclic AMP-dependent protein kinase A. High activity of cAMP blocked the induction of dopamine neurons (Hayes et al, 1995), therefore it could be reasoned that other molecules, e.g. environmental toxins, that modulate cyclic AMP-dependent protein kinase A will interfere with cellular differentiation and migration of these emerging DA neurons. Biomolecules may also affect the metabolic and structural components of the emerging DA neurons, resulting in different degrees of effects that may be enhancing or detrimental to the functions and longevity of the new born DA neurons. If the modulation enhances the metabolism and functions of the nigrostriatal neurons it is expected that the adult may possess motor features that are superior in functions, and will endure to advance ages. On the other hand if the modulations impair metabolism and functions of the nigrostriatal neurons, it is expected that the adult will possess motor features that fail early in life to produce PD symptoms. So,

the severity of the prenatal impairment will dictate the age of onset of PD symptoms. Susceptible type of impairments that are most severe, and do not result in death of the fetus, will be closest to the threshold at which PD symptoms are seen, so patients with early onset or juvenile PD may be endowed with sub-threshold but severely impaired NS system that developed early in life.

In summary, the period for the reorganization of the cellular membranes, organization of the chromatid for cell division, the synthesis of structural proteins, production of sub-systems for neurotransmitter synthesis and storage and the synthesis of molecules for intracellular transport and cell movement make the emerging dopaminergic cells well exposed to interfering factors and incidents. During this transforming cellular period the lack of essential metabolites, exposure to inappropriate metabolites and to exogenous and/or endogenous toxins can interfere with the molecular processes to cause permanent changes to the differentiating and migrating cells, that will reduce the resilience of the cell population. The affected neuronal set will become sensitive, susceptible, predisposed or vulnerable to the "wear-and-tear" of living or to toxic type of interventions that are encountered later in life. So, harmful basal ganglia neuronal changes that occur early in life could set the stage and shape the destiny of the individuals to the development of PD.

The dopamine neurons that are degenerated in PD have as their distinguishing feature long axons that project from the substantia nigra in the midbrain to the neostriatum in the forebrain region. One of the key sub-structures of the axon is cytoskeleton. Since they are involved in major cytoarchitectural changes during the development of the nigrostriatal dopamine neurons, the cytoskeleton and other associated molecules, including the kinases, are prime targets for modifications that will determine the outcome of the nigrostriatal dopaminergic neurons.

4.1. The involvement of cytoskeleton and alpha-synuclein as axonal constituents

The cytoskeleton proteins are important structures in the developmental and maintenance of the basal ganglia dopaminergic neurons. They support cellular shape, axonal and dendritic extensions, trafficking and transportation of macromolecules. More importantly, they allow the neurons to extend their reaches and influences far distances from the soma in the midbrain to the striatum in the forebrain region. So, the cytoskeleton serves to distinguish the new nigrostriatal dopaminergic neurons from the parent parochial cells and is the key components that enable the neurons to be functional; noting that the cell bodies may be correctly in place in the substantia nigra, but they will be non-functional without their far-reaching axons. So, by virtue of their relative cyto-architectural and functional significance, cytoskeleton synthesis and assembling ought to be one of the most vulnerable features affected by agents that interfere with the differentiation and proliferation of the far-reaching nigrostriatal dopaminergic neurons. Accordingly the molecules of the cytoskeleton protein classes, (i) microtubules, (ii) neurofilaments and (iii) microfilaments are seen as prime targets. Their vulnerability may help to explain why key markers of neurodegenerative disorders are mostly insoluble remnants of cytoskeleton protein. Lewy bodies, the major pathological marker for PD are composed principally of neurofilament

proteins, alpha synuclein, actin-like protein, microtubules associated protein 2 (MAT 2), microtubules associated protein 5 (MAT 5), syaptophysin, tubulin (Giasson et al, 2000). Lewy bodies are also reactive for cytoskeletal protein kinases, calcium/calmodulin-dependent protein kinase (Iwatsubo et al, 1991), cyclin-dependent kinase 5 (Nakamura et al, 1997) and stress activated protein kinases (Giasson et al, 2000).

The microtubules include the subunits, (i) alpha-tubulin and beta-tubulin and (ii) polymerization regulator proteins that include microtubule associated protein 2 and 5 (MAP2 and MAP5). Microtubules span the length of axon and dendrites, serving as the track for macromolecular transport. They are the major component of mitotic spindle, an organelle that participates in cell division and are of importance in the differentiation of cells to form the nigrostriatal dopaminergic neuronal phenotype. Microtubules also play an important role in cell movement. The subunit, tubulin, synthesized in the cell body is actively transported down the axon, so they are relatively easy target for interfering molecules, such as colchicines. Moreover, the turnover of microtubules requires the polymerization and depolymerization of the molecule. This is a cyclic process that is more stable in mature dendrites and axons but is active in dividing cells, which again is a potential target for molecules, such as colchicines and vinblastine. So, the process that involves polymerization and depolymerization of microtubules is a weak link in the life of a far-reaching neuron during which modifications of a permanent nature can be made.

The neurofilaments are the most abundant fibrillar components of axon (Schwartz, 1991). They include the light (L), medium (M) and heavy (H) molecular weight neurofilament subunit proteins. Neurofilaments are oriented along the length of the axons, are most abundant in axons and are critical for axonal extension, a feature that enables the DA cell bodies in the substantia nigra to extend their axons to the striatum. So, neurofilament proteins form the 'backbones of the nigrostriatal DA neurons and interference with the protein will likely cause significant and permanent change.

Microfilaments are made up of globular subunits of (i) beta-actin and (ii) gamma-actin. Actin plays a major role in the function of growth cones and in dendritic spines. High concentrations occur in dendritic spines and they are located just underneath the plasmalemma, together with a large number of actin binding proteins, including spectrin-fodrin, ankyrin, talin and actinin. They play key role in motility of growth cone during development, the generation of specialized micro domains on the cell surface and in the formation of presynaptic and postsynaptic morphological specializations. They undergo cycles of polymerization and depolymerization (Kandel, Schwartz and Jessel, 2000).

Alpha-synuclein is also a likely prime target for prenatal toxins. It is a heat stable protein associated with synaptic vesicles and axonal terminals (Withers et al, 1997). It plays important roles in neurotransmission, synaptic organization and neuronal plasticity (George et al, 1995). Alpha-Synuclein is the major building block for the fibrillary component of Lewy's bodies (Pollannen et al, 1993), the major antigenic component of Lewy's bodies (Baba et al. 1997; Spillantini et al, 1997) and may be critical for the expression of PD symptoms (van Duinen et al, 1999). It is also a component of the thread-like structures seen

in the perikarya of some neurons in the brainstem nuclei of the PD victims (Arima at al, 1998). It has been shown also that the association of alpha-synuclein with membrane promotes alpha synuclein aggregation (Lee et al. 2002) and that alpha-synuclein binds with dopamine transporters (Lee et al. 2001).

The interaction of the cytoskeleton proteins and other proteins of interest has been observed. For example, tubulin seeds the fibrillar form of alpha synuclein (Alim et al, 2002) and parkin has been shown to be a novel tubulin binding protein (Ren et al, 2003). It was also observed that 1-methyl-4-phenylpyridinium (MPP^+), the toxic metabolite of MPTP, reduced the synthesis of tubulin in PC12 cell model (Capelletti et al, 1999, Capelletti et al, 2000) and that MPP^+ inhibited tubulin polymerization (Capelletti et al, 2001), by specifically binding to tubulin in the microtubule lattice (Capelletti et al, 2005). Antibodies that recognize phosphorylated neurofilamant-M and neurofilaments-H also label Lewy's bodies, therefore the phosphorylation state of neurofilaments may be important in the formation of Lewy's bodies (Julien and Mushynski, 1998; Sternberger et al. 1983; Lee et al. 1987).

4.2. There may be a window of vulnerability for nigrostriatal dopamine neuronal sensitization

PD occurs in a relatively small number of the population, which may be so because a relatively short window of time exists during which the nigrostriatal DA neurons of the individual can be easily harmed. Such a window of vulnerability, we believe, is the period of differentiation, neurogenesis and migration of cells to form the nigrostriatal DA neurons, and this period occurs during gestational day 9-11 in mice. As mentioned above, the synthesis and laying down of cytoskeleton and neurotransmitter synthesis, storage, uptake and release capacities are likely the prime time during which the transforming cells are most vulnerable to toxic type of interference and inappropriate levels of metabolites and factors. So, idiopathic PD and some other degenerative disorders may have their origin in the fetus and the vulnerability may occur during pregnancy. This should not be seen as shifting the blame of having PD on pregnancy, but the fact is, pregnancy also produces the life and existence of the individual in the first place. So, the probability of having PD would be proportionate to the duration of the neurogenesis/neuronal development time, the number of pregnancy, the frequency by the individual encounter the toxic factor and the potency of the toxic encounter.

4.3. The susceptible stage may set the age of onset of PD and the severity of PD symptoms

If the rate of change is constant during the precipitating stage, it means that the more severe the sensitization, susceptible or vulnerable stage of affliction is, the earlier will the threshold reached for expressing the symptoms of PD. Thus, the age at which PD occurs may be directly related to the severity of the impairments that occur during the sensitization or the first stage affliction. So, juvenile PD may be marked by basal ganglia that were severely affected or were made less resilience by the changes that occur during the sensitization,

susceptible or vulnerable stage of affliction. The individuas whose basal ganglia are less severely affected during the sensitization, susceptible or vulnerable stage may experience a delay in the expression of PD symptoms, since more harm will need to be made during the precipitating stage to reach the threshold at which PD symptoms will be seen. So individuals with the least affected nigrostriatal system during the susceptible stage are those that may live without the experiencing the symptoms of PD. In other words, the severity of the changes that occur during the sensitization, susceptible or vulnerable stage may very well predetermine the age at which PD symptoms will occur and the severity of the symptoms.

4.4. The number of NS DA neurons may also determine the susceptibility to PD

The proposed early exposures of the basal ganglia may reduce the number of NS neurons in a random pattern, among the population, so that the average individual possesses a normal population of, say 120,000 (120K) NS DA neurons and with various fractions of the population having values above and below the 120K. Thus, a bell-shaped frequency distribution pattern will exist, with some individuals represented at the far left of the curve, say with 30K or 25%. The individuals among the population who will most likely develop PD would be those endowed with a low (pre-threshold) population of 30K NS DA neuronal subset and PD will occur following a reduction of merely 6K neurons, to 20% of the mean. This low population number of neurons, similar to the marginally resilience neurons mentioned above, would constitute the 1st stage or the sensitization, susceptible or vulnerable stage, and contributes to the cause of PD. During the wear-and-tear of aging, that involves the reduction of NS DA neurons, individuals with the 30K number of NS DA neurons will be those most likely to develop PD symptoms and also at an early age (juvenile). This analogy could form the basis for the early-onset to late-onset PD cases. It may also explain the PD-like dispositions that are exhibited by the very old, due to the chronic reduction of NS DA neurons. The population at the right of the bell shape curve may be those that live to old ages without basal ganglia impairments.

4.5. The coincidental involvement of other neuronal sets with the NS neuronal changes

When the NS DA neurons are made susceptible during the early stage of life other neuronal groups may also be harmed by the modifying factor(s) and the coincidence will determine the occurrence of other symptoms with the symptoms of PD. The coincidental involvement may occur if the window of exposure or neurogenesis for the basal ganglia DA neurons overlap the period of neurogenesis for other neuronal sets, or the period of exposure to the interfering factor/factors is long enough to overlap the period of neurogenesis of all neuronal sets. If that is the case all the neuronal sets will be harmed by the interfering factor/factors. For example, if the nucleus basilis of Meynert acetylcholinergic neurons and the mesolimbic or mesocortical catecholaminergic neurons are affected, as proposed for the NS DA neurons, these other neuronal sets will be scared early in life and succumb to the wear-and-tear of aging later in life. Such co-incident may explain the comorbidity of

Alzheimer-like dementia as well as depression with the occurrence of PD. It is of interest, therefore, that the Guam amyotrophic lateral sclerosis-parkinsonism-dementia that may be caused by toxins from the cycad plant (Spencer, 1987), may involve the early damage to upper motor and lower neurons, NS DA neurons and nucleus basalis of Meynert neurons and that the failure of the neuronal sets later in life precipitates the triage of symptoms. This may involve a longer time for the early exposure, which is reasonable because the toxin in cycad was taken in as food. So, the impairments of various neuronal sets during the stage of neurogenesis and neuronal development may help to explain the variations and complexity of the PD related syndrome.

4.6. Agents that may cause neuronal susceptibility

Parkinson's disease was described by James Parkinson in 1817, almost two centuries ago. So, if external factors are involved in the cause of PD they were in the environment during those early times and the factors would be widely distributed since the occurrence of idiopathic PD is universal. Moreover, since aging is the key risk factor for having PD, PD can be seen as the outcome of the changes that occur during the wear-and-tear of aging. As mentioned above, the best scenario is that the changes in aging coupled with early events that render the nigrostriatal neurons susceptible. Several agents or conditions may be involved in causing the NS DA neurons to be susceptible because all that is required is for the factor to cause damage to dividing and developing neurons, and for the factors to be available during the critical stage of the birth of the NS DA neuronal phenotype. The deficiency and excesses of otherwise normal metabolites, such as momentary fetal hypoxia during the development of the NS DA neurons may be all that is required to trigger the sensitization, susceptible or vulnerable stage. There may also be excesses of normal metabolites, since high activity of cyclic AMP can block the induction of dopamine neurons (Hayes et al, 1995).

It is highly likely that the susceptible phase occurs over a short period, which may help to explain the relatively low incidence of PD. We have used the toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), to model the sensitization stage in the mice (Muthian et al, 2010), so structurally similar agents to MPTP that occur in nature could affect the basal ganglia long before the synthetic MPTP became available as a toxicant. It is proposed, also, that agents such as colchicine and vincristine that have been in use as medicine for over 2000 years could have played a role as a sensitization factor for PD. Colchicine is an alkaloid from the Lily family, including Autumn lily or Colchicum autumnale and of the saffron family, that is still used today, as food coloring and cosmetics. Vincristine is an alkaloid obtained from the periwinkle plant. These two compounds are not known to target the nigrostriatal dopamine neurons, however, they bind to tubulin and prevent the polymerization of tubulin to form microtubules. By doing so, they interfere with cell division and are known to arrest cell division in the metaphase stage. It means that these agents will interfere with the division of the newly proliferating nigrostriatal dopamine neurons if they are administered during the period of neurogenesis. They will also interfere with cellular transport, cell polarization, cell growth and axonal extension that depend on the integrity of cytoskeleton proteins. These features are especially important for a group of cells, such as

the basal ganglia DA neurons that require their long axonal reaches to the striatum for their actions and effectiveness. By interfering with the assembling of the microtubules of the cells, colchicines and vincristine and now MPTP, via MPP^+, (Capelletti et al, 2005), will also impede and/or retard the new neurons from migrating to their place of destination in the substantia nigra, pars compacta. The phenomenon will also prevent the cells from extending their axons to their targets in the striatum. Since colchicines have been found to abolish retrograde transport in neurons resulting in the withdrawal of presynaptic terminals (Schwartz, 1991), these alkaloids will eventually result in cell death due to the lack of contact or contact inhibition. Today colchicines are used as a research tool and as a drug and the range of their toxicity is well known. Toxins, such as colchicines and vincristine are not disease specific, but they can cause a specific disease outcome based on the timing of their toxic effects to coincide with the vulnerable stage of a cellular substrate that underlie a specific disorder. For example, if a fetus is exposed to colchicines or vinblastine during the period of the neurogenesis and development of cells to produce the nigrostriatal dopaminergic phenotype, these neurons will be selectively harmed, and likely will result in PD later in life. If the effect of the toxin coincides with the birth of the nucleus basalis of Meynert neurons, Alzheimer's type dementia will occur. However, if the exposure time is extended to overlap both the birth of the nigrostriatal and acetylcholine neuronal sets the final symptoms will show parkinsonism and Alzheimer's like dementia.

4.7. Testing the prenatal sensitization, susceptibility or vulnerable concept

In studies designed to test the effects of toxin on the development of the midbrain neurons that are destined to become the nigrostriatal phenotype, we administered MPTP during the stage of neurogenesis, proliferation, migration and development of these DA cells. In the mouse, this period occurs during gestation day 9 - 11 and is marked by the appearance and maturation of TH-containing immunoreactive nigrostriatal neurons. The pregnant dams were treated with various dosages of MPTP or with phosphate buffered saline (PBS), as the control. We found that the dams treated with the 20 mg/kg and 30 mg/kg levels of MPTP, amounts that did not caused marked acute toxicity in the dams, caused very low to no full term pregnancy, suggesting that the higher dosage of MPTP may cause the pups to be aborted. For the 10 mg/kg of MPTP, however, the dams delivered normal looking pups, and this dosage was used to test the prenatal effects of MPTP.

4.7.1. Prenatal effects of MPTP on body weight, motor activity, TH and DA.

The outcome showed that the birth weights of pups born to dam that were exposed to prenatal 10 mg/kg of MPTP lagged behind the PBS control, but caught up within 4 weeks (Muthian et al, 2010). This recovery in birth weight and the appearance of the offspring indicated that they were in good physical health. The prenatal exposure to MPTP also reduced motor activity, measured as the total distance travelled, the movement time and the number of movements (Muthian et al, 2010) and Western blot detection showed that the exposure of the pregnant dams to MPTP at G9-11, that targeted the developing nigrostriatal dopamine neurons, reduced striatal tyrosine hydroxylase (TH) protein by 38%. DA and the

metabolites of DA were also studied in the brain of the 12 week old C57BL/CJ mouse offspring following the prenatal exposure to10 mg/kg of MPTP or to PBS (Muthian et al, 2010). As shown in table 1, the prenatal exposure to MPTP reduced the concentrations of striatal dopamine (DA), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) by 13.80%, 16.48% and 66.25%, respectively (Muthian et al, 2010). The level of dihydroxyphenylacetic acid (DOPAC) showed a slight increase (table 1).

	Dopamine and metabolites (ng/mg protein)			
Prenatal	DA	DOPAC	HVA	3-MT
Treatments	[%]	[%]	[%]	[%]
PBS	157.3 ± 17.30	5.2 ± 0.76	18.2 ± 0.80	1.60 ± 0.20
	[0.0]	[0.0]	[0.0]	[0.0]
MPTP	135.6 ± 4.80	5.9 ± .88	15.2 ± 0.80	0.54 ± 0.12
	[13.8]	[+13.46]	[16.48]	[66.25]

Table 1. Effects of prenatal MPTP on striatial DA, DOPAC, HVA and 3-MT. C57BL/6J dams were treated with 10 mg/kg MPTP or with PBS during G8-G12 to target the developing nigrostriatal dopamine neurons in the fetus. The table shows the levels of DA, DOPAC, HVA and 3-MT in the striatum of the 12 weeks old offspring. MPTP reduced DA, HVA and 3-MT, as compared to the values for the PBS group.

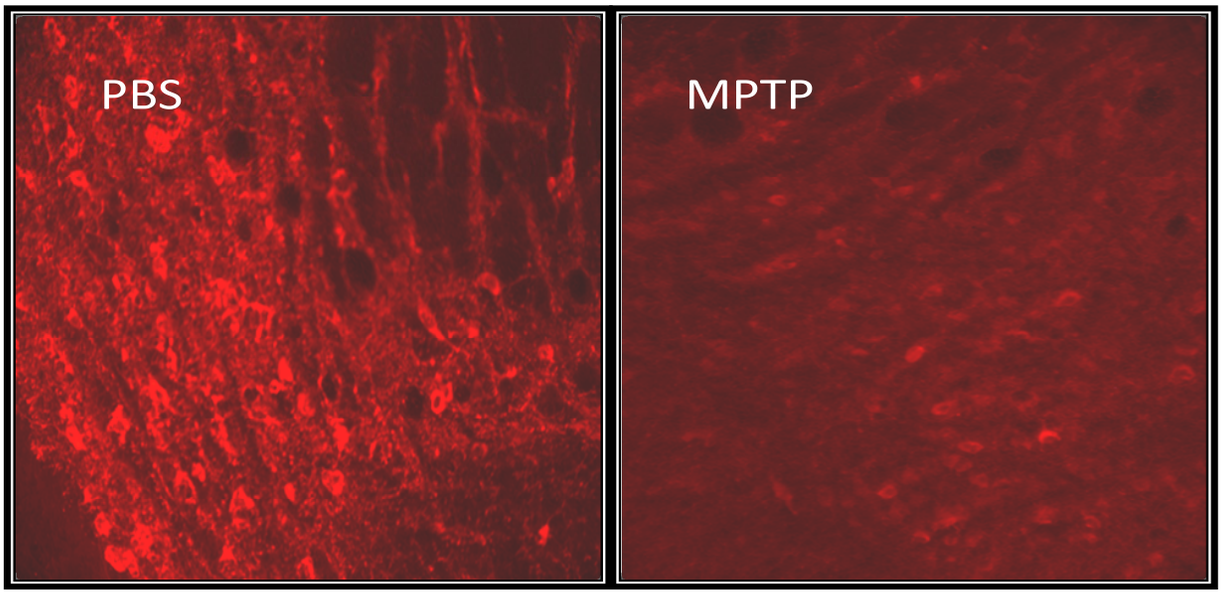

Figure 1. Substantia nigra, compacta of mice showing tyrosine hydroxylase immunoreactivity. The figure shows tyrosine hydroxylase (TH) immunoreactivity (I) in the substantia nigra compacta of a 12 weeks old mouse that was exposed to PBS (left) and one that was exposed to MPTP (right) in utero. The pregnant dam was treated during gestation days 8-12 and TH-I was determined in the 12 weeks old offspring.

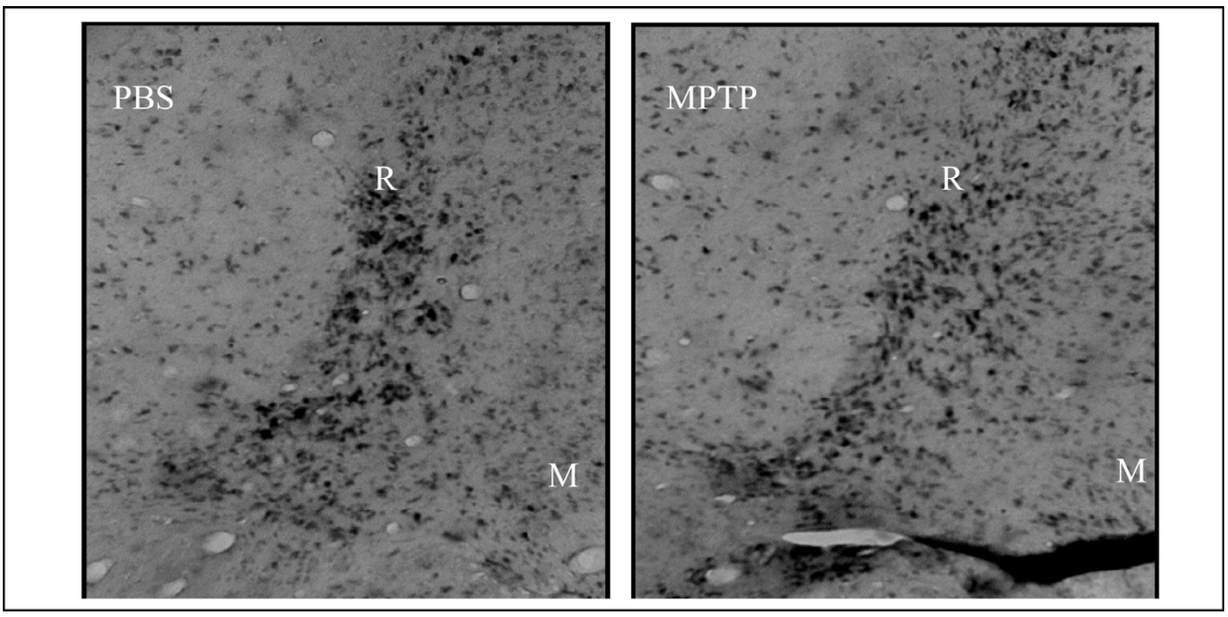

Figure 2. Nissl staining of the substantia nigra of mice exposed to prenatal PBS or MPTP. The Nissl staining highlights the cells (dots) of the substantia nigra, pars compacta. The overall morphology is closely similar, but the cellular composition of the PBS exposed mice are more concentrated within a defined zone in the compacta and with larger cells, as compared to the mice exposed to MPTP in which the smaller cells, especially within the rostro-medial (R-M) zone, are more abundant.

4.7.2. Prenatal MPTP on the in situ TH immunoreactivity in the substantia nigra

Figure 1 shows the effects of the prenatal exposure to MPTP on midbrain TH immunohistochemistry. Polyclonal antibodies against tyrosine hydroxylase (TH) were used to detect the changes that occurred in 12 weeks old mice offspring that were exposed to 10 mg/kg of MPTP, in utero, during G8-12 of the dam's pregnancy, when the midbrain neurons are developing the tyrosine hydroxylase phenotype. The results show that TH-like immunoreactivity was reduced in the midbrain substantia nigra of a mouse exposed to MPTP. The rostroventral section of the substantia nigra compacta was taken from horizontal slice of the mouse brain. The left section shows the TH immunoreactivity from a mouse offspring that was preexposed to PBS during G8-12 of the pregnant dam. The right section shows the TH inmmunoreactivity of a mouse offspring that was exposed to 10 mg/kg of MPTP during G8-12. The study shows that marked reduction of TH-I occurred in the mouse that was exposed in utero to MPTP (right).

4.7.3. Prenatal effect of MPTP on the Nissl Stained substantia nigra

The effect of prenatal exposure to MPTP on cellular distribution pattern in the substantia nigra, compacta of C57BL/CJ mice is shown in figure 2 as low magnification Nissl stained section of the 12 weeks old mice offspring. The differences in the cellular patterns for the PBS and the MPTP exposed animals were not marked, but cellular pattern seems to occur in

the compacta zone for the PBS control as compared to the mouse that was exposed to MPTP, in which more scattered smaller cells can be seen in the medial (M) to rostral (R) zone of the substantia nigra (figure 1). The proportion of neurons to glia cells are unknown and are yet to be determined.

5. The inducing, precipitating or superimposing stage of the hypothesis

PD shares some characteristics with aging and the incidence of PD is higher in the aged individuals, but only a relatively small number of elders (about 0.3%) developed full-blown PD, therefore, since PD is sporadic it would appear that a predisposition exists for the disorder. The individuals that developed PD may have been predisposed or susceptible throughout their lives, and they develop PD symptoms when metabolic changes associated with getting older caused further harms to the nigrostriatal DA neurons and reduced the number of neurons. The precipitating effects may be due to various factors, such as changes that allow molecules that serve normal functions early in life to become toxic via direct or indirect ways, such as the production of toxic byproducts, for example. The exposure to

DA and Metabolites (ng/mg protein)	Prenatal Exposure.	Postnatal MPTP Challenges (mg/kg)			
		0 (PBS)	10	20	30 mg/kg
DA	PBS	157.3 ± 17.3 [0.0]	141.0 ± 5.50 [10.35]	34.5 ± 1.7 [78.06]	16.40 ± 2.0 [89.57]
	MPTP 10mg/kg	135.6 ± 4.80 [13.80]	48.0 ± 7.10 [69.96]	28.0 ± 2.0 [82.20]	3.95 ± 1.0 [97.49]
DOPAC	PBS	5.2 ± 0.76 [0.0]	6.00 ± 1.00 [15.38]	3.3 ± 0.4 [36.53]	1.95 ± 0.41 [62.5]
	MPTP 10mg/kg	5.9 ± 0.88 [+13.46]	1.04 ± 0.96 [80.0]	0.46 ± 0.58 [91.15]	0.41 ± 0.33 [92.11]
HVA	PBS	18.2 ± 0.80 [0.0]	17.5 ± 1.00 [3.85]	9.84 ± 0.6 [45.93]	6.0 ± 0.47 [67.03]
	MPTP 10mg/kg	15.2 ± 0.80 [16.48]	9.4 ± 0.66 [48.35]	8.3 ± 2.1 [54.39]	4.7 ± 0.70 [74.17]
3-MT	PBS	1.6 ± 0.20 [0.0]	1.2 ± 0.15 [25.0]	0.75 ± 12 [53.22]	0.54 ± 0.11 [66.25]
	MPTP 10mg/kg	0.54 ± 0.12 [66.25]	0.45 ± 0.11 [65.38)	0.32 ± 0.05 [80.0]	0.32 ± 0.06 [80.0]

Table 2. Postnatal effects of MPTP in mice offspring exposed to in utero MPTP or PBS. Effects of postnatal MPTP (10, 20, 30 mg/kg) on striatal DA, DOPAC, HVA and 3-MT in 12 weeks old mice offspring exposed to prenatal MPTP or PBS. The percent changes based on the normal PBS population levels are enclosed by brackets below the respective concentrations. The results show that postnatal MPTP was more effective in reducing DA and its metabolites in the offspring that were exposed to prenatal MPTP. However, for the 20 and 30 mg/kg doses of MPTP the significance of the postnatal, precipitating concept was masked because those doses of MPTP also markedly reduced DA and its metabolites in the prenatal PBS offspring.

exogenous toxic insults may also occur. This is represented by the outbreak of the 1919 encephalitis lethargic epidemic (Ravenholt et al, 1992) that precipitated PD symptoms among some of those that were affected by the encephalitis virus. Whether the inducing, precipitating or superimposing stage is due to metabolic changes or exposure to toxins, it should be noted that the effects do not have to be specific to cause the expression of the specific symptoms of PD, since the incidence during the first stage marks or sensitizes the nigrostriatal system, accordingly, any toxin or any change that can cause further harm to neurons, even in a general way, will affects those neurons that were made fragile.

5.1. Testing the inducing, precipitating or superimposing stage

We have shown that MPTP can be used to model the inducing, precipitating or superimposing stage. This was demonstrated in our studies in which we found that the postnatal administration of MPTP to 12 weeks old offspring, that were exposed *in utero* to MPTP earlier, during the developmental stage of the NS DA neurons, showed dramatically reduced levels of DA and its metabolites, as compared to similar mice that were exposed to the PBS treatment. The magnitude of the changes matches the level seen in PD, when compared with the normal population, or the PBS controls (table 2). The 10 mg/kg dosage of MPTP given to the mice that were exposed to prenatal MPTP caused the most dramatic reduction of DA and its metabolites, as compared to the PBS control (Table 2, column 3 vs. 4 showing values for prenatal PBS vs. prenatal MPTP). The 20 and 30 mg/kg of postnatal MPTP markedly reduced DA in the prenatal exposed MPTP mice, but these dose levels of MPTP also caused dramatic reductions of DA and its metabolites in the prenatal PBS mice, as well, so the differences between the prenatal MPTP and the prenatal PBS were not as dramatic (Fig 2, column 3 vs. 5 and 6 showing values for prenatal PBS vs. pre natal MPTP).

6. Analogy that depicts the two stages of affliction hypothesis

The two stages of affliction hypothesis for PD may be best illustrated by an analogy of a motor vehicle tire that was manufactured with a specific defect due to poor quality steel cords imbedded in the carcass or the body of the tire, during a critical period in the manufacture of the tire. The tire shows all of the characteristics of normal tires, but on exposure to the roadway the frictions that cause normal wear in tires turn out to cause serious failure in the defective tire. An inspection of the failed tire will show specific failure of the steel cords. The subtle imperfection that occurs during the manufacture of the tire may be seen as the sensitization factor that tags the tire for the specific type of failure that occurs under normal usage. In this scenario, such a normal tire usage may constitute the period for the precipitating stage, the tire serves to depict the human brain, the cords depict the nigrostriatal dopamine neurons with their far-reaching axonal projections, and the roadway-frictions represent the wear-and-tear of living that increases as a function of age. The two stages of afflictions or the sensitization-precipitating hypothesis for PD may also explain the discordance for PD in monozygotic twins. The life-long personality difference between monozygotic twins discordant for Parkinson's disease suggests that the process

responsible for the disorders of PD has its inception early in life (Ward et al, 1983). The developmental personality of the member of the monozygotic twins who developed PD was found to be more introvert but since being an introvert is not usually abnormal within the population, it may be deduced that at least a second factor should be involved in causing the PD in the affected twin. The primary factor could be the early changes that render the nigrostrital DA neurons susceptible and also reflected or coincide with personality difference. The second factor for the disorder expression may be related to the regression in dopamine cells that occurs during aging (see McGree et al 1977).

7. Special cases of PD may involve early-life and multiple neuronal groups

The Guam amyotrophic lateral sclerosis-parkinsonism-dementia complex (ALS-PDC) may represent an incident of PD in which wide-scale neuronal damage occurred during the sensitization stage, and the wear-and-tear of living or the aberrations associated with aging take their toll later in life. In other words, the nigrostriatal dopaminergic neurons that were impaired during the fetal development degenerate to the threshold level that causes PD symptoms. Above threshold neuronal death also occurred for the nucleus basalis of Meynert acetylcholinergic neurons and cortical neurons involve in memory and cognition and caused the dementia phase of ALS-PDC syndrome (Oyanagi, 2005). The lower and upper motor neurons systems that control skeletal muscle contraction also died to cause the amyotrophic lateral sclerosis phase of the disorder. The theory is based on the report that the ALS-PDC or otherwise PDC-ALS is essentially the convergence of three disorders. Patients with PDC showed the signs of rigidity, tremor and bradykinesia (Oyanagi, 2005), the classical signs of Parkinson's disease as well as dementia (Oyanagi, 2005), the main sign of Alzheimer's disease. The ALS phase of the Guam ALS-PDC disorder has been reported to be essentially similar to those of classic ALS. Moreover 5% of the patients with ALS subsequently developed the total clinical symptoms of the ALS-PDC and 38% of the patients with PDC eventually developed the PDC-ALS syndrome (Elizan, et al, 1966; Oyanagi, 2005). So the PDC syndrome may be based on the exposure of the fetus to the cycad toxin during the period of the neurogenesis of both nigrostriatal DA neurons and nucleus basalis neurons. The duration of the toxic exposure of the patients may have been long enough to coincide with the neurogenesis and migration of the nigrostriatal DA neurons as well as the nucleus basalis of Meynert acetylcholinergic neurons. For the ALS patients, it is proposed that the exposure to the prenatal toxin coincides with the birth of upper and lower motor neurons and causing deleterious effects early in life that sensitized them to stress that occurred later in life. The higher 38 percent of patients with ALS may be matching to the longer neurogenesis and proliferation period for the related motor neurons and therefore longer fetal exposure time.

7.1. Proposed fetal basis for the Guam ALS-PDC disorder

The proposition that beta-methylaminoalanine (BMAA), a toxin found in flour produced from the Cycad plant and eaten as food, caused ALS-PDC (Spencer et al, 1987), is of interest.

It was also claimed that the basal ganglia symptoms were produced in monkeys fed BMAA (Spencer 1966), but this claim was disputed on the basis that the dosage used was far too high to represent the amounts that are eaten by human (Ince and Codd, 2005; McGree and Steele, 2011), and the disease produced in the monkeys was a classic acute toxicity model (Ince and Codd, 2005), rather than the progressing model of the ALS-PDC seen in the Guam patients. Moreover, the disease occurred in patients who had not used cycad products for many years (Sacks 1998), again suggesting the fetal basis for this ALS-PDC disorder. The risk of ALS-PDC was carried by migrants who had resided on Guam for the first 18 years of life (Ince and Codd, 2005), suggesting that early exposure is important for those who developed the ALS/PDC disorder, and the disorder takes over 35 years to develop, which is a very long time for a metabolic toxin to cause direct toxicity, and this also deviates from the short-term toxic models that have been presented.

It would be surprising that a major toxin consumed as a major source of food by several families would be so limiting in the number of individual within a family who were affected. In other words, if the ALS-PDC syndrome is due to a single-stage bout of toxic exposure, it would be expected that the toxin, which is ingested regularly as food, would affect a larger proportion of the group. So, it is apparently more reasonable to propose that the individuals that developed the ALS-PDC in Guam were exposed during the period of vulnerability of the nigrostriatal dopaminergic neurons, the nucleus basilis of Meynert acetylcholinergic neurons and the upper and lower motor neurons. They bourne the scar of the early exposure that pair with the changes that occur during aging to precipitate the ALS-PDC syndrome later in life. The sensitization-precipitation concept may be true also for the PD-like toxicity caused by MPTP in the later years of the 70s to the 80s. This may be so because not all individuals who were exposed to intravenous MPTP eventually developed full blown PD symptoms. Those that developed the symptoms of PD were probably predisposed with less resilient nigrostriatal neuronal set, and those that were spared had highly resilient nigrostriatal dopaminergic neurons. It means therefore, that most cases of PD may be caused by encounter made during the stage of neurogenesis and development of the nigrostriatal dopamine neurons, and that aging, the key risk factor for PD, precipitates idiopathic PD. The progressive nature of idiopathic PD may be based on the fact that aging is relenting and progressive in its own right.

8. S-adenosyl-L-methionine (SAM): A model precipitating factor for Parkinson's disease

S-adenosyl-L-methionine (SAM) is presented as a likely precipitating factor for PD. SAM is a naturally occurring and ubiquitous molecule derived from methionine and ATP (Cantoni 1953). It is one of the most reactive and important biochemical (Kotb and Geller, 1993), but its activity seems to be harnessed by the limits and the control placed on its synthesis. SAM is apparently synthesized on demand and rapidly utilized by several enzymes, as the biological methyl donor (Cantoni 1953), for trans-sulfuration reactions and in the synthesis of polyamine (Andres and Cederbaum 2005). As the biological methyl donor, SAM is the co-factor for several methyl transferases, including catechol-O-methyl transferase (COMT) and

indole amine methyl transferase. COMT transfers the methyl of SAM to dopamine (DA) to produce 3-methoxytyramine and to norepinephrine (NE) to produce normetaphrene and by doing so SAM terminates the synaptic activities of DA and NE, via irreversible reactions. SAM also serves to methylate N-acetyl-serotonin, via indoleamine methyltransferase to form melatonin and in the process may deplete serotonin (5-HT). These are major metabolic processes since DA, NE and 5-HT are important in synaptic transmission and in behavior (Agnoli et al, 1976) and are reported to be depleted in PD. So, SAM is a highly reactive endogenous molecule.

The injection of SAM into the cerebral ventricle of rodents produced symptoms that are similar or identical to those described for PD, including hypokinesia, rigidity, tremors (Charlton and Way 1978), the loss of DA, loss of striatal and substantia nigra tyrosine hydroxylase (Charlton, 1990; Charlton and Crowell, 1995; Crowell et al, 1993) and loss of neurons in the substantia nigra (Charlton and Mack, 1994).The PD-like changes that occurred following the cerebral ventricular administration of SAM are based on very logical and mechanistic grounds, since SAM reacts avidly with L-dopa and DA and reduced DA. More importantly, the loss of DA is the hallmark of PD disease, and the methylation of DA at the synapse (Axelrod, 1965) terminates the neurotransmitter activity of DA; a process that irreversibly destroys the dopamine molecule by covalently converting it to 3-methoxytyramine. SAM also drives the synthesis of phosphotidylcholine (PTC) (Hirata et al, 1981) that is accompanied with increases in lyso-PTC (Lee and Charlton 2001), a potent membrane damaging surfactant. It has been shown also, that SAM interacted with and methylated DA receptor protein and inhibited DA receptor binding (Lee and Charlton, 2004). In addition, the carboxylmethylation of protein, including DA receptor protein, by SAM, generates methanol (Axelrod and Daly, 1965), formaldehyde and formic acid (Lee et al 2008), reactive byproducts that can cause irreversible and accumulative damaging changes to cells and cellular constituents. Although the biological role of methanol, formaldehyde and formic acid are not viewed with much significance, these molecules are likely to be of primordial origin, helping to shape the destiny of life. They are produced in the body and are extremely reactive. The activity of SAM is also increased during aging (Mays and Borek 1973; Stramentinoli et al, 1977; Gharib et al, 1982; Sellinger et al, 1988), a critical period for cellular attrition and a stage of life during which the symptoms of idiopathic PD are seen. Today SAM is well studied as the major driver of the epigenetic modification of various genes. The biochemical control that SAM exhibited is remarkable on the basis that SAM is the limiting factor for dozens of methyltransferases, so any increase or decrease in the level of SAM serves as a key driving force for most methylation reactions.

8.1. Common markers exist for methylation and parkinsonism

A review of the results from various laboratories, include our own, shows that various biochemical, functional, anatomical and other markers are common to PD and to the methylation process (Table 3). Metabolites and byproducts of SAM, such as N-methyl dopamine, 3,4-dimethoxy-dopamine, N-methylsalsolinol (Maruyama, et al, 1996; Naoi et al, 2002; Matsubara et al, 2002) and harman and norharman (Kuhn, et al, 1996) are elevated in

Biological Events	PD relevance	Effect on/of SAM
Biochemical changes		
Decreased dopamine	Yes	Yes
Decreased norepinephrine	Yes	Yes
Decreased serotonin	Yes	Yes
Decreased melanin	Yes	Yes
Decreased tyrosine hydroxylase	Yes	Yes
Increased Ach activity	Yes	Yes
Increased HVA/DA	Yes	Yes
Increased DIMPEA	Yes	Yes
Functional defects		
Hypokinesia	Yes	Yes
Tremors	Yes	Yes
Rigidity	Yes	Yes
Abnormal posture	Yes	Yes
Anatomical impairments		
Nigrostriatal damage	Yes	Yes
Loss of DA/TH neurons	Yes	Yes
Other markers		
Older ages	More prevalent	High activity of SAM
L-dopa	Alleviates	Depletes SAM
Methionine	Aggravates	Increased SAM
N-methyl tetrahydroisoquinoline	Causes/in PD brain	SAM metabolite
Methyl beta carboline	Causes	SAM metabolite
MPTP/MPP+	Causes	Enhances methylation
N-methyldopamine	Found in	SAM metabolite
N-methylsalsolinol	Found in	SAM metabolite
Homocysteine	Found in	SAM metabolite
MPP^+	Aggravates	Increased SAM activity
Manganese	Aggravates	Increased SAM activity
Lyso-phosphotidylcholine	PD-like effects	Increased by SAM
Nicotinamide-N-methyl-transferase	High in CSF	SAM is the cofactor

Table 3. Many biological changes seen in PD correspond with the effects of SAM. The table shows the parallel relationship between changes associated with Parkinson's disease and with the effects and biochemical activities of S-adenosyl-L-methionine and its metabolites. A one-one relationship is shown in the activities listed.

the CSF of PD patients and homocysteine (Lee et al, 2005) may cause PD like toxic changes. In addition, methyl-beta-carboline was reported to cause PD-like changes (Collins, et al. 1992; Gearhart et al, 1997). Furthermore, it has been shown that the tissues of PD patients methylate nicotinamide greatly higher than tissues of the control patients (Willams et al, 1993); and that nicotinamide methylation is proposed to be a key factor in the development of degenerative diseases (Williams and Ramsden, 2005). The enzyme, nicotinamide-N-methyltransferase, that transfers the methyl group from SAM to nicotinamide, was shown

to be high in the CSF of PD patients (Aoyama et al, 2001) and N-methyl-nicotinamide was also higher in the brain of PD victims as compared to the control (Williams and Ramsden, 2005). So, as shown, many biological changes seen in PD correspond with the effects of SAM, its enzymes and its metabolites (table 3).

8.2. Actions and effects that support the role of SAM as a precipitation factor in PD

If a secondary precipitating factor is associated with PD, it would more likely fits as a toxic metabolite that is associated with aging. Such a metabolite would be expected to be very reactive. It would show age-related increases in activity, would have a narrow index of safety so that even slight increases would cause toxic reactions. It should react with normal biochemicals that are critically needed on a moment-by-moment basis for the maintenance of essential functions. Moreover, the metabolite should react with biochemical that are found to be modified during the course of PD, for example, DA that is depleted in PD and which is an avid methyl acceptor. In addition, the mode of reactivity of the metabolite should explain others changes that are related to the degenerative disease process, such as the effective therapy for PD and the development of tolerance to the therapeutic agent. So, an evaluation of S-adenosyl-L-methionine (SAM), the biological methyl donor, based on the above criteria, indicates that it fits the role of a precipitating factor for PD. Again, it is an endogenous molecule, its activity is increased during aging, it is very reactive, it has a narrow index of safety, it controls the metabolism of specific chemicals that are modified in PD, the major drug for PD, which is L-dopa, reacts avidly with SAM and L-dopa, in turn, induced methionine adenosyl transferase, the enzyme that produces SAM (Benson et al, 1993; Zhoa et al, 2001). Moreover, as mentioned above, several SAM-induced changes seem to be associated with the neuronal degeneration and many of the biochemical changes that occur in PD.

8.2.1. Age-dependent increases in SAM-dependent methylation

The activities of SAM, denoted by increases in its synthesis and utilization, are increased during aging. This has been reported as, an age-related increase in methionine-adenosyl transferase, the enzyme that produces SAM, increases of various methyl transferases, and the accumulation in products of SAM-dependent methylation reactions, including homocysteine and adenosine (Mays et al 1973; Stramentinoli et al, 1977; Sellinger et al 1988; Gharib et al 1982). It should be noted that a decrease in the absolute concentration of SAM in rats was reported to be related to aging (Baldessarini and Kopin, 1966) but the reduction was apparently due to increases in the turnover of SAM that also occurred during aging (Stramentinoli et al, 1977).

8.2.2. SAM depletion of biogenicamines may occur in PD

In the presence of catechol-O-methyltransferase and other transferases SAM serves as a cofactor in the methylated metabolism of several biogenic amines, including DA and

norepinephrine, by donating its reactive methyl group mainly to receptive hydroxyl of the molecular ring and the nitrogen of the ethylamine side chain (Axelrod, 1965). SAM dependent methylation is the most important mechanism in mammals for the inactivation of catecholamine (Lambrosse et al 1958, Axelrod et al, 1965), consequently SAM is an important factor in controlling the neuronal levels of the biogenic amines. The decreased levels of DA (Hornykiewicz, 1966), norepinephrine (Erhinger and Hornykiewicz, 1960) and serotonin (Bernheimer et al, 1961) observed in PD could be explained by an increase in the methylation of DA, norepinephrine and of N-acetyl-serotonin. The methylation of DA may also explain the increase ratio of homovanillic acid (HVA) to DA (HVA/DA) in PD and the increased level of 3,4-dimethoxyphenylethylamine, the dimethoxy metabolite of DA, that was reported to be contained in the urine of PD patients. More importantly, the DA derived alkaloid, N-methyl-(R)-salsolinol, was shown to occur in the human brain, accumulates in the nigrostriatal system and may play a role in PD (Naoi et al, 2002). An increase SAM-dependent methylation may also help to explain the pharmacology of L-dopa, in treating the symptoms of PD, because L-dopa is not only converted to DA, but it also reacts avidly with SAM, and depletes SAM. SAM dependent regulation of biogenicamines is achieved by methylated catabolism as well as by increasing synthesis, because it has been shown that preincubation with SAM caused activation of tyrosine hydroxylase in the corpus striatum of rats (Mann and Hill, 1983). These and other outcomes suggest that SAM is functioning both intra- and extra-neuronal, therefore its bio-availability at specific sites should be critical in determining the up or down regulation of the activity of biogenicamines. SAM activation of tyrosine hydroxylase (Mann and Hill, 1983) may help to explain the increase in DA turnover that occurs in PD. An increase in the methylation of L-dopa and DA will shunt tyrosine toward the production of L-dopa and L-dopa toward the production of DA, thus, tyrosine will be shunted away from the synthesis of melanin, a process that may help to explain the reduction of melanin in the substantia nigra of PD patients: noting that melanin is a product of tyrosine. Likewise, SAM also methylates phosphotidylethanolamine to produce phosphotidylcholine and phosphotidylcholine, in turn, is metabolized to generates choline molecules for the synthesis of acetylcholine. So, an increase in methylation could conceivable increase the level of acetylcholine and acetylcholinergic activity that occurred in PD, and which may form the basis for the utility of anticholinergic agents in the treatment of PD symptoms.

8.2.3. Mechanisms and selectivity of SAM for the basal ganglia

Conditions that increase the rate of methylation, for example aging (Sellinger et al 1988), may precipitate PD in individuals with susceptible DA neuronal population. In individuals with the normal complement of substantia nigral DA neurons the same level of methylation may represent an age-dependent normal regression of cell population, because the critical cell level that will result in PD would not be reached. Thus, the final effects of an increase in methylation in persons with normal populations of DA neurons would be different degrees of aging. Besides aging, other factors that facilitate an increase in methylation ought to be emplaced. It turns out that (i) the chemistry of the basal ganglia, (ii) the anatomical and physical state of the basal ganglia and (iii) the functions that are controlled by the basal

ganglia coexist in a cooperative way to facilitate the uniqueness of SAM as the methyl donor and as a putative precipitating factor for PD.

For the chemistry of the basal ganglia, the methylation of DA and the methylation of phosphotidylethanolamine may be of major importance. First, the methylation of DA by SAM depletes DA at the synaptic cleft. This is an irreversible reaction that also generates 3-methoxytyramine, a metabolite that has been shown to competes with DA for its receptor binding (Charlton and Crowell, 2000). So, the reaction of SAM with DA and the generation of an competing metabolite will not only depletes DA, but also will interfere with the binding of DA to its receptors, which is consistent with a SAM-induced dopaminolytic state. SAM also methylates phosphotidylethanolamine to produce phosphotidylcholine, and, as mentioned above, to produce choline for the synthesis of acetylcholine. In addition, phosphotidylcholine is readily hydrolyzed to form the toxic surfactant, lyso-phosphotidylcholine (Lee et al, 2001; 2005). The reaction is also relevant on the basis that lyso-phosphotidylcholine is a potent surface-active agent that will damage cellular vesicles and nerve ending, and can contribute to the progression of the degeneration that occurs in PD. The biochemical peculiarity of the basal ganglia, therefore, includes the fact that the neostriatum contains large quantities of L-dopa, DA and norepinephine that are avid methyl acceptors, so they utilize high levels of SAM. SAM is also required for the methylation of phospholipid and the synthesis of acetylcholine, so the neostriatum is a high utility site of SAM, or a chemical 'sink' for, SAM.

The precise functions of the basal ganglia marked it for visible impairments. The basal ganglia dopaminergic system controls precise articulation of the hands, finger, lips and whole body to support emotional expression, gesture and feelings. Therefore in the awaking human the neostriatum is constantly under stress to maintain the delicately balanced and fine-tuned processes that it controls, so slight impairments of the nigrostriatal system will upset the postural balances and precise muscle regulations and will cause visible impairments, that are seen as PD, even when such a degree of impairment or degeneration would not be physically obvious if occurred in other systems. SAM-related age-related changes may also affect vision and hearing, but the changes in the quality of life are not of the same magnitude as seen when the basal ganglia is impacted.

The anatomical or physically states of the basal ganglia also make this structure very accommodative to the effects of an increase in SAM, because SAM, which is very water soluble, will accumulate in the cerebral spinal fluid (CSF). In the CSF SAM is in close proximity to the neostriatum, which courses along and protruded into the lateral ventricle and contains the sensitive dopamine nerve terminals. Studies have shown that the administration of SAM into the lateral ventricle damaged the delicate ependymal cell barrier that separates the CSF from the caudate nucleus neuronal environment. By doing so, SAM gained access to the neostriatum, where it can deplete DA (Crowell et al, 1993), can methylate phospholipids (Lee and Charlton 2001) and DA receptor protein (Lee et al, 2004) and generate methanol, formaldehyde and formic acid (Lee et al, 2008) that are damaging to nigrostriatal dopamine nerve endings. These metabolites, especially formaldehyde will result in permanent changes to the dopaminergic neurons. Interestingly, in a more recent

study, we found that the co-administration of a retrograde neuronal tracer with SAM into the lateral ventricle caused the labeling of cells in the substantia nigra, indicating that molecules placed in the lateral ventricle can gain access to the caudate nucleus DA nerve endings.

The increase in methylation can caused other significant changes, for example, the utilization of SAM imposes a great demand on ATP, because for every mole of DA methylated at the 3-OH and 4-OH positions 2 moles of ATP are utilized to replenish the utilized SAM and for every mole of phosphotidylethanolamine that is methylated to form phosphotidylcholine 3 mole of ATP are required to replenish SAM. Furthermore, the carboxyl methylation of protein by SAM will increase the isoprenylation of the proteins and each farnesyl molecule that is utilized requires 3 moles of ATP for its synthesis and each geranyl-geranyl requires 4 moles of ATP for its synthesis. So, an increased methylation will require increased production of ATP, which increases oxygen utilization and the probability of generating reactive oxygen species. In addition, 1 mole of potentially toxic homocysteine and 1 mole of adenosine may be produced for every mole of SAM utilized, and huge amounts of adenosine will be produced as a result of the metabolism of ATP to replenish SAM. The depletion of ATP may be relevant in this connection, because inhibition of mitochondrial oxidation and ATP reduction are proposed to be involved in the actions of MPTP or MPP^+. It is well understood that SAM-dependent methylation is a normal physiological process, so for one to imagine how SAM may be involved in PD it should be understood that the symptoms of PD are due directly to dopamine biochemical deficiency and indirectly to the neuronal degeneration. This is so because drugs, such as L-dopa and DA receptor agonists relieve the tremors and other symptoms of PD, in spite of the fact that the permanent neuronal degeneration remains. Furthermore, the syndrome of PD wax-and-wane, which, cannot be explained by the existence of a permanent degenerated neuronal set. These examples show that the symptoms of PD, such as tremor and freezing, are striatal biochemical deficiency symptoms, due to the loss of dopamine as a result of the neuronal degeneration.

In spite of the doubts about the methylation concept, it is of interest that most of the other hypotheses concerning the genesis of PD cannot explain many of the changes that are seen in PD. One-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxyl-dopamine (6-OHDA) serve as the most important chemical models for PD. Their efficacies are mostly related to the targeted nigrostriatal cell death, but these agents do not cause changes that reflect the whole spectrum of PD symptoms. For example, MPTP does not cause PD-like symptoms in the rat, which also has a nigrostriatal dopamine system, but SAM does (Crowell et al, 1992; Charlton and Mack, 1994).

9. Conclusion

The abberrations that cause the nigrostriatal degeneration that result in Parkinson's disease are unknown. Since about 90-95% of all cases of PD are not due to genetic changes, it means that the environment plays a major role in the cause of PD. The environment is not restricted to the toxins that might be involved, but includes the biochemical melieu that the

nigrostriatal cells encounter from their origin to the outcome that causes them for die. So, the encounter with inappropriate biochemicals and inappropriate levels of the appropriate biochemicals may occur, and the outcome will vary and will be restricted to the nigrostriatal neurons or will involve other neuronal sets. This type of encounter will produce the syndrome that are eventually expressed and may include symptoms related to nigrostriatal damage only, but may be accompanied with other syndrome. So the expression of symptoms in addition to the classical PD other symptoms, suggests that nigrostriatal neuronal impairment may be accompanied with the impairments of other neuronal groups. These may include the basal nucleus of Meynert acetylcholinergic neurons that are degenerated in Alzheimer's disease (AD) and the upper and lower motor neurons that are involved in the cause of amyotrophic lateral sclerosis (ALS). So, the existence of the Guam amyotrophic lateral sclerosis-parkinsonism dementia complex (ALS-PDC, suggests that the factors that cause PD are not specific for the nigrostriatal neurons, but will affect other neuronal groups, as well.

For PD, it is suggested that the nigrostriatal dopaminergic neurons were exposed by chance encounter during a vulnerable stage of development of the neuronal set. Since aging is the key risk factor for PD, it also means that at least two stages of afflictions are involved in the cause of PD. Evidence and circumstance suggest that the first stage occurs in utero during the neurogenesis and development of cells to form the substantia nigra dopaminergic phenotype. The neuronal set is harmed in a subtle way that does not cause visual symptoms, but the sub-threshold effects weakened the resilience of the neurons so that the stress encounter during the course of living causes further harm to the already affected neurons and precipitates the symptoms of PD. So, the first impairment may occur during the neurogenesis and development of the nigrostriatal dopamine neurons by inappropriate levels of regulatory molecules or by toxins. An increased activity of cyclic-AMP-dependent protein kinase A, for example, may antagonize the signal for sonic hedgehog protein and blocked the induction of dopamine neurons (Hayes et al, 1995). The exposure to alkaloids, such as colchicine or vinblastine may also occur, and these alkaloids may interfere with the development of the cytoskeleton, with long-term and sub-threshold levels of effects. The stress of aging that causes globally deteriorating change will then take a toll on these low resilient neuronal sets to precipitate the symptoms of PD. The prenatal and postnatal effects can also explain the occurrence of juvenile PD, which would involve the substantia nigral dopamine neurons that were affected in ways that make them less resilient and more sensitive to age-related stress, so a short course of living would be enough to precipitate the symptoms of PD in the young individual. The Guam ALS-PDC cases are proposed to be caused by the exposure to the Cycad toxin during the neurogenesis and development of the nigrostriatal dopamine neurons, the basal neucleus of Meynert acetylcholinergic neurons and upper and lower motor neurons. The exposure caused subthreshold harms to those neuronal sets and they failed before other major groups of neurons during the course of aging.

The hypothesis that neurodegenerative disorders, such as PD and others have their origin in the womb is in line with normal physiology, since the lives of all mammals have their origin

in the womb. If the hypothesis is tested to be true further investigation will identify the specific agents and/or the mechanisms that may be involved in the sensitization stage and measures could be adapted to protect the vulnerable neuronal groups during critical stages of fetal development.

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