

NEURODEGENERATIVE DISORDERS¹ SEARCHING FOR A COMMON PATHOGENETIC FACTOR AND MOLECULAR MECHANISM

MALATTIE NEURODEGENERATIVE ALLA RICERCA DI UN COMUNE FATTORE PATOGENETICO E MECCANISMO MOLECOLARE

LECTURE ONE PRIMA LETTURA

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FORWORD

< Despite the advent of molecular genetics in neurobiology, our understanding of the functional relationships of the components of the central nervous system (CNS) remains in its infancy, particularly in the areas of cellular interaction and synaptic modulation..>⁴

PARKINSON'DISEASE (PD) INTERPRETED AS A DISTURBANCE OF THE DOPAMINE/NEUROMELANIN PUMP

The first description of this serious neurological illness in scientific literature dates back to 1817 and was made by James Parkinson, who called it *< Paralysis Agitans >*.

Doctors still display different views on the disease's historical origins: some claim it is not a modern-age illness but rather a plague that has always caused distress to mankind, as reported in old Ayurvedic texts. [1] The disease, as a matter of fact, is ubiquitously widespread amongst the elderly populations of all countries and amongst the different ethnic groups and socio-economic classes. Furthermore, its distribution does not seem to depend on demographical, climatic, dietary, socio-cultural elements or any factor related to industrialisation. Its prevalence does not vary from country to country, with the exception of China, Japan and Africa, where it is slightly lower. [2]

¹ Parkinson, Huntington, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Alzheimer ,etc.

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⁴ George J.Siegel et al. *< Basic Neurochemistry- molecular, cellular and medical aspects >* sixth edition, Lippencott, Williams and Wilkins Ed.

Other researchers believe what is written in the old Chinese and Indian texts not to refer to PD proper, but rather to mere tremors that are common to several pathologies. The disease's explosion-like diffusion, thus, can be linked to industrialisation, and especially to the presence of an exogenous neurotoxin in the environment and/or to a substantial change in dietary habits. This hypothesis is corroborated by the consequences of the substitution of whole meal with refined white flour in Great Britain in the early 1800s, which led to the loss of the antioxidant vitamin pool. [3]

The damage caused by different kinds of herbicidal pesticides and other chemicals, which can cause diseases similar to PD, provides further evidence in favour of the neuro toxic hypothesis.

The pathogenesis of Parkinson's disease is still controversial. The disorder is largely widespread and develops in a most insidious way in elderly age, causing progressive movement impairment. This process follows four clearly-defined stages: tremors, bradycinesia, rigidity and postural instability. [4]

The cardinal features of PD are:

1. Tremor, mainly at rest;
2. Muscular rigidity, leading to difficulties in walking, writing, speaking and masking of facial
3. expression;
4. Bradycinesia, a slowness in initiating and executing movements;
5. Stooped posture and instability.

Depigmentation of the *substantia nigra* is a constant feature of Parkinson's disease and appears to be caused by greatly reduced dopamine concentrations⁵, to about one-tenth of normal⁶, which is in turn consequence of the concomitant death of dopaminergic neurons, whether as a result of virus infection, exposure to exogenous toxins or other unknown causes.

Parkinson's symptoms are related to low concentration of dopamine and can be reduced by

⁵ The darkly pigmented neurons in the *pars compacta of the substantia nigra* accounts for 80% of the dopamine in the brain. The dark pigmented neuromelanin is a dopamine polymer, that makes the *substantia nigra* appear black. Motor control in the *striatum (caudate nucleus and putamen)* is thought to involve a balance between inhibitory dopaminergic (D2) and excitatory cholinergic neurons.

⁶ The dopamine production and brain concentration in the different animal species are age related, with a marked decline in senescence (onset of senescence: cattle 15-20 years; dog 10-15 years; humans 60-80 years; mouse 2-3 years; rhesus monkey 20-30 years; rat 2-3 years; rabbit 4-6 years).

dopamine replacement therapy, through oral administration of the dopamine precursor DOPA⁷.

PD, after Alzheimer's disease (AZ), is the chronic degenerative neurological disease affecting the largest number of people worldwide. It is estimated that 1.5 million people are affected in the USA, 80,000 in France, 100,000 in England and 120,000 in Italy. In the USA 50,000 new cases are reported every year.

It is exceptional for patients to develop the disease before they reach the age of 30. In rare occasions the illness may occur before 40, but 80% of cases concern people between 40 and 70 years of age. After the age of 80 the disease no longer occurs. These data must be taken carefully, as often PD is not diagnosed at all or is mistakenly diagnosed in patients affected by other neurological pathologies.

Our current knowledge does not allow us to prove any link between PD and other pathologies, and the role of risk factors, such as brain traumas, low-vegetable-income diets and hypertension, should be investigated further. It is interesting to note that the number of smokers affected by PD is extremely low. Such phenomenon has been interpreted as the consequence of nicotine's alleged protective effect on the dopaminergic system, namely on the *substantia nigra* and *striata*.

The symptomatology of PD consists in the derangement of basal ganglions' functions, with a reduced activity of dopaminergic components and deficiency of the neurotransmitter dopamine, as well as of the black pigment neuromelanin⁸. [5]

⁷ Tyrosine is synthesized in the liver from phenylalanine by the enzyme phenylalanine hydroxylase and then transported to the brain, by the large neutral amino acid carrier. Once in the brain, tyrosine is converted to Dihydroxy-phenylalanine (DOPA) by the tyrosine hydroxylase enzyme using oxygen, iron and tetrahydrobiopterin (THB) as co-factors. DOPA is converted to dopamine by the aromatic amino acid decarboxylase using pyridoxal phosphate (PLP) as a co-factor. Both dopamine and nor epinephrine are catabolized by a two-step process involving the enzymes mono amino oxidase (MAO) and catechol-O-methyltransferase (COMT).

⁸ Neuromelanin, a granular dark brown pigment, mainly found in the nigrostriatal neurons of the human brain stem, is chemically similar but not identical to skin melanin. Their biogenetic site is also different: skin melanin is synthesized in the epidermal melanocytes of the skin under the influence of light, whereas neuromelanin is formed in the darkness by the catecholaminergic neurons of the CNS, which are moreover mostly deprived of melanocytes.

Different chemical structure, different biogenetic site and biochemical mode of synthesis are necessarily leading to different biological roles. As a matter of fact, skin melanin (an indole polymer) is supposed to act as sun screen, whereby the biological activities of neuromelanin (mainly a benzene polymer) are manifold, according to the site of deposition. In the substantia nigra neuromelanin is supposed to be involved in movement coordination, in the eye in light absorption, in the ear in deadening acoustic waves.

Surprisingly, the amount of neuromelanin varies among animal species, being greatest in humans and progressively lower in the lower species. As an example, rats, mice, guinea pigs and rabbits

It has been hypothesised that the disease is triggered by a slow and progressive lack of dopamine, but there is still controversy on what leads the brains' dopaminergic cells – located in the *substantia nigra zona compacta* (SNZC), to gradually reduce their dopamine production, or on what brings this substance to be transformed into inactive products. [6]

The *substantia nigra* is connected to the *striatum* at the brain's base through nerve fibres, whose ends secrete the neurotransmitter dopamine. The latter helps the *striatum* in controlling movements. The progressive death of *substantia nigra* cells is followed by a reduction in dopamine availability. Normally, dopamine is stocked in inert form in vesicles. When secreted, it is metabolised by monoamine oxidase (MAO), or is subjected to auto oxidation processes, if need be. In both cases hydrogen peroxide (H₂O₂) is formed, which can have a cytotoxic effect if not neutralised by catalase, reduced glutathione (GSH) and glutathione peroxidase (GPO).

The pathological variation of the different biochemical parameters suggests that during PD the *substantia nigra* is stressed by oxygenated free radicals [7], which leads to the degeneration of

have little or no neuromelanin at all; this fact raising doubts about the effectiveness of its biological role in the CNS. The highest concentration of neuromelanin is found in the neurons of the *substantia nigra* and *locus coeruleus*, which also contain relatively high amounts of dopamine and nor epinephrine, respectively. According to this fact, the chemical structure of dopamelanin and epinephrine-melanin differ substantially, deriving from two different precursors.

Neuromelanin is found in different places of the CNS, namely *the substantia nigra pars compacta*, the retina and the ear, occurring almost exclusively in catecholaminergic neurons, who also show the highest concentration of the three biogenic amines dopamine, epinephrine and serotonin. Based upon its physicochemical properties, several working hypothesis were made about the possible functional role of neuromelanin, among them we quote: inactivation of harmful organic substances or inorganic ions; free radical scavenger; redox buffer against reducing or oxidizing toxic conditions; binding and retaining of foreign potentially toxic substances; modulator of the neuron electric activity, etc. Most black materials of the living world (melanin, from Greek *melanos*, black) are usually synthesized in specific cell systems, called melanocytes and can be grouped in four chemical classes:

- 1) **pheomelanins** (from sulphurated melanogens),
- 2) **allomelanins** (from polyphenols),
- 3) **eumelanins** (from nitrogenous melanogens),
- 4) **neuromelanins** (eumelanin hybrids from catecholamines)

whereby hybrids of these classes are formed by copolymerisation or inclusion of foreign materials. The melanins derive from three fundamental polymers: pyrrole black (PB), indole black (IB) and benzene black (BB). Various pigments of great biological interest belong to these three groups. Namely: black, red biline derivatives and porphyrins are pyrrole polymers; adrenalin black and serotonin black, two main components of neuromelanin, are indole polymers; black graphite, black and red fullerenes, aspergillin from *Aspergillum niger*, humic acids, DOPA-black, again a component of neuromelanin, are all benzene polymers.

approximately 80% of dopaminergic neurons in the *substantia nigra striata*, before clinical symptoms arise. [8,9]

As a matter of fact, two elements can be noted: the increase in water peroxide concentration (the basis of monoamine oxidase B activity), iron, ferritin, neuromelanin-connected iron, lipofuscin⁹, the peroxidation of membrane lipids and a decrease in oxygen peroxide destruction systems such as reduced glutathione (GSH), glutathione peroxidase (GPO), the I complex (NADH co-enzyme Q-reductase), mitochondria electron transport activity and calcium chelating proteins.

As the disease progresses, the iron concentration in the *substantia nigra* increases, while glutathione concentration diminishes. It is common knowledge that Fenton's reagent ionic iron together with oxygen peroxide (H₂O₂) produces the hydroxyl radical (•OH), which destroys reduced glutathione (GSH) and has a cytotoxic action, as do all radicals.

Post-mortem histopathological evidence in PD patients shows that this degenerative process goes hand in hand with: a rise in the malonic dialdehyde and lipid hydro peroxides (which are an index of lipid peroxidation) basal concentration; a decrease in reduced glutathione (GSH) levels; a boost in the mitochondrial superoxide dismutase (SOD) activity; an alteration in the iron metabolism together with a contraction of ferritin amounts and an increase in the number of ferric/ferrous ions; enhanced neuronal phagocyte activity in the microglia. This data supports the hypothesis according to which "oxidative stress" plays a role in the *substantia nigra striata* degenerative processes observed in PD.

Such hypothesis has theoretical grounds, as the central nervous system is especially vulnerable to any kind of oxidative aggression, in particular by oxygenated free radicals. The brain¹⁰ contains

⁹ Besides neuromelanins, we also observe in the myocardium and neurons throughout the body, the accumulation of aging pigments called *lipofuscin*. These intracellular deposits, which mainly include complex non toxic lipids, proteins and lysosome-type hydrolase's, are considered to be chemically and physiologically different from the neuromelanins.

¹⁰ Do to its jellylike consistency, the brain is protectively encased in a very resistant bony skull, where it is floating in the Cerebral Spinal Fluid (CSF), secreted by the choroids at the daily rate of approx. 500 ml. The CSF is circulating slowly through the four ventricles, serving also as a kind of substitute of the lacking brain lymphatic system. The blood-cerebrospinal fluid barrier and the blood-brain barrier protect the brain against undesirable blood substances, being however permeable to small electrolytes and some other specific small organic molecules such as the essential amino acids.

In contrast to the 6-20% of other organs, almost 50% of dry brain weight is made out of lipids. These lipids are building, as myelin or membranes, the basic structure of nervous cells, whereas the free fatty acids and triglycerides are the main fat components of other organs. This fact is outlining, besides the peculiar functional role, the unique chemical composition of the brain. In this well protected environment, simple organic molecules, like the neurotransmitters, can be synthesized and

large amounts of easily oxidisable polyunsaturated fat acids. Through blood, it receives and uses a quantity of oxygen (O_2) disproportionate to other organs. On the other hand, it lacks antioxidative protection mechanisms, which are profusely diffused in other body parts. For instance, in some areas of the brain high ferritin concentrations are to be found, though with low reduced glutathione (GSH) and glutathione peroxidase levels and the complete absence of catalase.

Ferritin levels in the brain of patients with PD shrink, especially in the *substantia nigra* (SNZC), whilst free iron ions multiply becoming available for possible oxidation reactions. Dopamine plays a crucial role as a neurotransmitter and the onset of PD is linked to its decline in the SNZC.

In the meantime, catecholamines such as dopamine are oxidised, and H_2O_2 is released through a radicalic and semiquinonic process leading to the formation of neuromelanin. Neuromelanin, in the presence of H_2O_2 , can react with Fenton's reagent and release more hydroxyl radicals $\bullet OH$. The latter, if not neutralised, can pursue the peroxidation of membrane lipids, which eventually brings to a halt the mitochondrial electron transport system. It has been recently proved that during dopamine oxidation significant amounts of 6-hydroxydopamine form, together with its quinone, Topamine-quinone (TQ). Both are highly cytotoxic.

Melanin can capture consistent amounts of iron, leading to the formation of iron-melanin, a complex that can easily release activated iron, considered to be more neurotoxic than normal free iron.

Such oxidative processes are catalysed by several metals and especially iron, whose highest concentration in the brain is to be found in the *substantia nigra*. In physiological conditions, iron is present in negligible amounts in its free state because it is stocked in ferritin and hemosiderin in an inactive form. However, it becomes readily available according to physiologic (and pathologic) needs. Suffice it to say that a mole of ferritin contains 4,500 atoms of iron, an amount which, even if only partially released, reacting with hydrogen peroxide H_2O_2 , as in Fenton's reagent, could generate a significant number of hydroxyl radicals $\bullet OH$.

It has been shown that 6-hydroxydopamine can release iron from ferritin, triggering a chain of reactions that will lead to the formation of hydroxyl radicals $\bullet OH$, extremely cytotoxic even for dopaminergic neurons. $\bullet OH$ radicals have a short average life span. Thus, they are likely to act close to where they formed.

perform *in situ* functions, independent of the functions they perform in other parts of the body (see f.i. serotonin in the intestine or platelets; nor epinephrine in metabolism or blood pressure).

Knowingly, the three major groups of neurotransmitters are amino acids (primarily glutamic acid, GABA, aspartic acid and glycine), peptides (vasopressin, somatostatin,neurotensin, etc.) and monoamines (the catecholamines: nor epinephrine and dopamine and the indolamin: serotonin) plus acetylcholine.

This hypothesis is confirmed by the fact that the iron chelator desferroxamine – which can break the oxidative chain – displays some activity in Alzheimer patients, as clinically observed.

Nitrogen monoxide NO has been found capable of releasing iron from ferritin, contributing to the formation of highly reactive oxygenated radicals. [10]

Several pharmacological agents, such as neuroleptics of the phenothiazine groups and butyrophenons or reserpine, can induce syndromes that are similar to Parkinson's. Both interfere with the dopamine mechanism: the former block postsynaptic dopaminergic receptors, the latter use reserpine to remove the available dopamine from pre synaptic neurons.

Therefore, it can be reasonably argued that at the root of PD there is a degenerative process of dopaminergic neurons caused by the aggression of the oxygenated radicals that formed *in situ*, with iron as a catalyser. The concentration of neuromelanin in substantia nigra pars compacta (SNPC) has been measured in male and female normal subjects at different ages in the range of 1-97 years old and in SNPC of Parkinson patients. These data demonstrate a continuous neuromelanin accumulation during life and severe depletion during PD. [11]

In later studies neuromelanin was identified as a genuine melanin with a strong chelating ability for iron and affinity for several organic compounds. The affinity for a variety of inorganic and organic toxins is consistent with the postulated protective function for neuromelanin. Moreover, the neuronal accumulation of neuromelanin during aging and the link between its synthesis and a high cytosolic concentration of catechols suggest a protective role. [12]

The iron, ferritin and neuromelanin concentration in *substantia nigra* was measured in normal subjects, aged between 1 and 90 years, dissected *post mortem*. Iron levels in *substantia nigra* were 20 ng/mg in the first year of age, had increased to 200 ng/mg by the fourth decade and remained stable until the eighty decade of life. L-Ferritin also showed an increasing trend during life, although the concentrations were approx. 50% less than that of H-Ferritin at the age point.

Neuromelanin was not detectable during the first year, increased continuously to 3500 ng/mg in the 80th year. Neuromelanin is therefore the major iron storage in *substantia nigra* neurones in normal individuals. [13]

PD is associated with a significant increase in iron in the degenerating *substantia*

nigra and is measurable in living PD patients and in post-mortem brain. This increase, however, occurs only in the advanced stages of the disease, suggesting that this phenomena may be secondary, rather than a primary initiating event, an hypothesis also supported by evidence from animal experiments.[14]

A person's neuromelanin concentration in the *substantia nigra* varies with age. It is virtually nil during early infancy, when the newborn does not have full control over his/her locomotor functions. The neuromelanin concentration reaches its peak in adult age, declines over the years and is at its lowest in the elderly. Its decrease goes hand in hand with the natural degradation of locomotor coordination and reflex response.

Considering the phenomenology, it is argued that the *substantia nigra* plays a role in nervous transmission, consistently with both the conductor and insulator properties of the biopolymer neuromelanin. It must be highlighted that post mortem examination of patients with PD in advanced stages reveals severe depletion of the black pigment in the *substantia nigra*.

Neuromelanin is a dark pigment produced in the dopaminergic neurons of the human *substantia nigra* and has the ability to bind a variety of metal ions, especially iron. Post-mortem analysis of the human brain have established, that oxidative stress and iron content are enhanced in association with neuronal death. A possible consequence of an overloading of neuromelanin with redox-active elements is an increased contribution to the oxidative stress and intraneuronal damage in patients with PD.[15]

The discovery of PD gene has led to the hypothesis that misfolding of proteins and dysfunction of the ubiquitin-proteasome pathway are pivotal to PD pathogenesis.[16]

Quantification of the total iron content in iron-loaded neuromelanin and synthetic melanin demonstrated that the iron-binding capacity of neuromelanin is 10-fold greater than that of the model melanin. These findings are consistent with the hypothesis that neuromelanin may act as an endogenous iron-binding molecule in dopaminergic neurons, whereby an increased iron in the CNS is associated with increased indices of oxidative stress.[17]

Neuromelanin is produced constantly by dopaminergic neurons through the oxidation of dopamine and its derivatives. It is subsequently metabolised through oxidative degradation.

It can therefore be claimed that, in normal physiological conditions, a homeostatic balance dopamine/neuromelanin is established.

A low dopamine concentration means smaller amounts of neuromelanin are produced and, consequently, the *substantia nigra* is impoverished and its electric functions are deranged.

On the other hand, an excessive formation of neuromelanin, through an abnormal dopamine oxidation, leads to a lack of this neurotransmitter, which in turn upsets dopaminergic functions and eventually kills the neuron.

The overloading of neuromelanin and other metals may trigger inflammatory and degenerative processes aggravating underlying pathologic conditions. [18]

The dopamine/neuromelanin dynamic balance (**dopamine/neuromelanin pump**)is critical for the system to perform correctly.

However, it is also quite unstable as its components are sensitive to and react to oxidation.

Yet, it must be said that doubts have arisen about the definitive pathogenesis and about whether the factors that trigger the degenerative process leading to Parkinson's disease are of endogenous or exogenous origin. [19,20]

Enzymatic reactions follow fixed patterns aimed at synthesising structures with biological properties for specific functions. The new compounds are not an end in themselves: they return into the life cycle thanks to appropriate, and sometimes simple, structure modifications. The picture is more complex when it comes to radicalic reactions. They can, as a matter of fact, form high-molecular-weight structures that are hard and slow to biodegrade and, apparently, have no biological purpose.

At first glance it may seem as though nature got stuck in a cul-de-sac. This is what we believe happens to the dopamine-neuromelanin system during Parkinson's. Whilst it is commonly accepted that oxidative stress plays a role in PD's pathogenesis, there is disagreement on the causal relationship between the two issues: which one is the cause and which one the effect? Maybe, there is no real difference as both are cause and effect in an endless vicious circle.

Neuromelanin is found in the catecholaminergic neurons of men and primates, which also contain high concentrations of dopamine. Based on current experimental evidence, neuromelanin is a copolymer composed of indole residues and residues derived from S-cysteinildopamine. Its composition is different from that of melanin formed in the skin's melanocytes, when stimulated by

sunlight. [21]

Electron paramagnetic resonance spectroscopy showed unambiguously that neuromelanin is a melanin, however it is not well modelled by synthetic dopamine melanin and thus it has to be considered an atypical melanin. This study shows ,moreover, that homogenizing tissues, during purification of any natural pigment, may cause contamination by extraneous metal ions and that subsequent incubation in hot acid leads to degradation of melanin. A relevant purification procedure at low pH and room temperature is thereof described and recommended.[22]

Oxidative degradation of neuromelanin from human *substantia nigra*, in comparison to model melanin, showed that the pigment is a mixed-type melanin, consisting of units derived from benzothiazines and indole derivatives, in about equal amounts.[23]

Certain drugs and chemicals are bound to melanin and retained in pigment cells for long periods. This specific retention can cause adverse effects in the skin, eye, inner ear and pigmented nerve cells of the *substantia nigra* of the brain. The toxicological risks of melanin-related adverse effects are also applicable to persons with a high content of pheomelanin in the skin and hair.[24]

Human neuromelanin differs structurally from synthetic dopamine melanin, both pigments including an aromatic backbone and a large proportion of aliphatic structures. Neuromelanin appears to be a more complex pigment than synthetic dopamine melanin formed via dopamine autoxidation alone. Neuromelanin derives mostly from dopamine with 25% incorporation of cysteine in the form of a benzothiazine structure. The involvement of dopamine and cysteine-dopamine, as building blocks of neuromelanin, was interpreted in favour of an alleged detoxifying role, aiming at preventing the accumulation of potentially toxic dopamine and cysteine-dopamine.[25]

Further comparative chemical degradation studies of neuromelanin, from the *substantia nigra* of normal individuals, and synthetic melanins confirmed that neuromelanin is derived mostly from dopamine with 25% incorporation of cysteine in the form of benzothiazine structure.[26]

A new structure, consistent with the results of oxidative degradation studies was initially proposed [27] and further developed by other authors into a radical polaronic model, with the aim of explaining the electric properties of the biopolymer. [28]

According to most recent views expressed by Paul Meredith (Meredith@physics.uq.edu.au), melanins cannot be considered mesoscopic conductors anymore, deriving all their electrical properties from absorbed water and the redox cycling of the 5,6-phenol ketone equilibrium. According to the same author, an experimental verification of this hypothesis is under way¹¹

During the course of a person's life, neuromelanin is accumulated in the SNZC dopaminergic neurons. Its levels decrease during senescence, in parallel with the decline in the number of neurons and in synthesised dopamine. The neurons with the highest amounts of the black pigment and the smallest quantities of reduced glutathione (GSH) are the first to degenerate and die, leading to the debatable assumption that neuromelanin accumulation equals neuron death¹².

According to this hypothesis, neuromelanin would be a waste product whose formation and pathologic accumulation takes away precious dopamine from the life cycle, thus becoming a neuron killer. Whilst the epidermis' melanocytes and melanosomes are equipped with an apparatus specifically conceived for the production and distribution of melanin from tyrosine, the brain lacks such a system, as well as tyrosinase, and is not directly exposed to solar radiation. These facts corroborate the hypothesis according to which neuromelanin is formed from dopamine following random radicalic accidents (Fe/ H₂O₂), both in normal and pathological conditions, e.g. PD. Monoamine oxidase (MAO), present in large quantities in the substrata, could be responsible for helping trigger such reactions.

The 'neuromelanin = waste product' hypothesis does not take into account the fact that this pigment, like other melanins, has enhanced conductor and insulator properties, that allow it to play a role in transmission and modulation of nervous impulses and in regulating the locomotor system. To this respect, it is interesting to observe that **neuromelanin is absent or significantly scarce in two stages of life – one physiological and the other pathologic – in which locomotor coordination is not yet present (newborn babies) or has been strongly compromised (Parkinson's patients).**

Other theories have been put forward trying to establish neuromelanin's physiological role. Some claim it deactivates hyperactive toxic chemicals, or that it captures heavy-metals that catalyse oxidation-reduction reactions; others consider it to be a radicalic trap or a bio cybernetic operator.

¹¹ Personal communication by Paul Meredith.

¹² <Melanins, Biological Garbage or Jewel > Personal communication presented at the 8th.Meeting of the European Society for Pigment Cell Research Sept. 23-26 (1998), Prague;

We recommend, future studies to focus on bioelectric properties and on transmission and modulation of nervous impulses, regulating the locomotor system.

As to Parkinson pathogenesis, it seems likely to us, that oxygenated radicals may attack the dopamine-neuromelanin system, thus breaking the homeostatic balance and leading to the degeneration and death of dopaminergic neurons. When enough neurons have irreparably degenerated, the first symptoms arise, then the disease follows its course until it reaches its fatal end. Neuromelanin itself, which is linked to highly reactive iron (neuromelanin-Fe³⁺ complex), might act as a catalyst in the formation of hydroxyl radicals •OH that, in turn, can attack other dopamine molecules.

<Defective sequestration of dopamine into vesicles, leading to the generation of reactive oxygen species in the cytoplasm, is a key event in the demise of dopaminergic neurons in Parkinson's disease, and might represent a common pathway that underlies both genetic and sporadic forms of the disorder...increased levels of cytoplasm dopamine in nigral neurons in Parkinson's disease patients might result in dopamine oxidation and the generation of reactive oxygen species that can damage and eventually kill these neurons. Dopamine-induced oxidative stress, impaired synaptic vesicle function and misfolding of α -synuclein, due to mutations or oxidative damage to this protein, might be components in a self-perpetuating vicious cycle that eventually leads to the demise of dopaminergic neurons >.[29]

It therefore seems reasonable to assume that the neurodegenerative process, once started, proceeds by auto catalysis until an unlikely event (radical scavenger, antioxidant) breaks the chain. [30]

The incidence of PD increases with age, as the body's defences weaken. The greater the vulnerability to radicalic aggression, the less the body can fight back through appropriate enzymatic mechanisms. These facts are also in line with what has been put forward¹³.

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<A CRITICAL REVIEW OF THE FUNCTION OF NEUROMELANIN AND AN ATTEMPT TO PROVIDE A UNIFIED THEORY> [6] ABSTRACT from : Medical Hypotheses (2005) 65, 791-796; <http://intl.elsevierhealth.com/journals/mehy>

This paper provides a critical review of the numerous and various biological functions so far attributed to neuromelanin and an attempt to provide a unified theory based on the peculiar physical and chemical properties of the black particle (the neuromelanin cage).

It is stressed that neuromelanin is not homogeneous, as is commonly accepted, but is made up of different substrate specific black pigments formed by the oxidation of o.diphenols or other oxygenated precursors (*substantia nigra* melanin, *locus coeruleus* melanin, retinal pigmented

epithelium or ocular melanin, inner ear melanin, and so on).

Ocular melanin is believed to protect the eye by trapping metals and free radicals. The paper shows that this unconfirmed mechanism is a rather fortuitous irreversible molecular accident, which at times may prove itself deleterious.

Albinism often leads to deafness in animals, indicating a genetic correlation. These two conditions appear to be correlated at a molecular level to eye/ ear pigmentation and suggest verifying this hypothesis in normal and albino human individuals.

Skin and ocular melanin are chemically different. However, they are both involved in light absorption/dissipation. The black particle structure (melanin cage) is believed to be fundamental to this process because there is a common bioelectric mechanism. The latter is worth of further investigation. It is also proposed checking how ocular melanin dissipates the excessive absorbed light (as heat or as current?). It has been claimed that inner ear melanin mutes acoustic waves. This paper suggests investigating the underlying mechanism and also studying whether this pigment is bio-electrically involved in audiology.

According to numerous authors, *Substantia nigra* melanin is only biological garbage. This view is rejected, and it is stressed that intracellular melanogenesis is a fundamental and genetically controlled physiological process.

It has been repeatedly claimed that the binding of iron, heavy metals, free radicals and harmful chemicals by *substantia nigra* melanin is fundamental to body detoxification/protection.

Presumably, such irreversible and generic binding mechanisms have no physiological foundation; it is suggested the alternative that, *substantia nigra* melanin acts as semiconductor, transmitting and modulating nervous impulses, in a reversible way. In fact, *substantia nigra* melanin is absent or significantly scarce in two conditions of life in which the coordination of movement is either inefficient (newborn babies) or strongly compromised (Parkinson).

To check this assumption, further investigation of *nucleus caudatus*, *putamen*, *globus pallidus*, *substantia nigra pars compacta and reticulata*, *nucleus hypothalamicus* is recommended .

HUNTINGTON'S DISEASE INTERPRETED AS A DISTURBANCE OF THE HUNTINGTIN PUMP

Huntington's disease (**HD**) is a progressive and fatal autosomal-dominant inherited neurodegenerative disorder, occurring worldwide in all races and ethnic groups without ethnic prevalence, characterised by progressive motor dysfunction, emotional disturbances, dementia and weight loss. The disease was firstly described by George Huntington in 1872, who outlined the main features: dystonia with tendency to choreiform uncontrollable movements, progressive psychiatric impairment leading to cognitive decline and insanity, hereditary character [31,32]. The illness, detected in approx. 5-10 cases/100,000 individuals, is fast in disabling after arousal of symptoms, but rather slow in killing (15-25 years).

According to neuropathological findings, besides a general neuronal loss in various brain regions, a selective neuronal degeneration of the neostriatum is observed, with a prevalence for the medium-sized spiny striatal projection neurons, while the large and medium-sized aspiny interneurons are mostly spared. [33,34]

The exact cause of neuronal death remains unknown, although mutant Huntingtin is supposed to play some not yet defined role in the progressive degeneration, leading to neuronal impairment and death. [35,36]. Among the possible stresses caused by mutant Huntingtin or its fragments, the followings were cited: oxidative injury, excitotoxic stress, disordered neurophysiology, potentiation of inflammatory signals, pro-apoptotic signals, malfunctioning proteolysis, increased transglutaminase activity and energy depletion, whereby each one of these processes might be able of influencing neuronal degeneration and death, representing potential therapeutic targets. Pharmacological experiments (HD mice model) and clinical trials on HD patients are in progress with a variety of potential therapeutic agents, whereby following selection criteria were mainly adopted:

-scientific rationale, proven efficacy in other models or diseases with common pathogenesis mechanism, prior evidence of neuroprotective effect, lack of toxicity, brain penetration, pharmacokinetics.

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