

A CRITICAL REVIEW OF THE FUNCTION OF NEUROMELANIN AND
AN ATTEMPT TO PROVIDE A UNIFIED THEORY ¹

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ABSTRACT

In this paper we provide a critical review of the numerous and various biological functions so far attributed to neuromelanin and 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. We believe these two conditions 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, in our view, fundamental to this process because there is a common bioelectric mechanism. The latter is worth of further investigation. It is also proposed to check how ocular melanin dissipates the excessive absorbed

¹ Published at MEDICAL HYPOTHESES (2005) 65, 791-796

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light (as heat or as current?). It has been suggested that inner ear melanin mutes acoustic waves.

We suggest 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. In our view, such irreversible and generic binding mechanisms have no physiological foundation; we suggest 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 .

Introduction. Many biologically active, variously coloured organic pigments can be found all over our planet [1]. The black or brown pigments of the living world are called **melanin** (Greek, *melanos* = black) [2,3], some being located at different sites of our body, where they are biologically active [4]. Melanin's are substituted derivatives of acetylene and contain a polyacetylene spine [5]. They are stable free radicals (radical polarones), closely related to polypyrrole and polyindole, holding positive charges balanced by counter anions and exhibit typical broad IR and EPR spectra [6].

In nature the black particles are formed by the oxidation of o.diphenols or other oxygenated precursors (decarboxycyclodopa, dihydroxyindole(DHI), serotonin, catecholamines,

dihydroxytryptamines, dopamine, adrenaline, dihydroxyquinolines, or generally from polyphenols). All natural and synthetic melanin's include a three dimensional multi-layer graphite-like aromatic backbone, substituted by aliphatic chains and all are composed of amorphous microparticles of different shapes and sizes [7-10], which are believed to be fundamental to their chemical and biological behaviour. All melanin's, especially in the doped state, show a variety of physical properties, like absorption and dissipation of light and sound, binding of metals and organic compounds, storing of liquids and gases, conduction of electrical current and transformation of light into electric energy. These properties have been related to disparate and sometimes conflicting biological functions, for instance, playing a role in camouflage and sexual display, acting as a sun screen, scavenging active chemical species, producing active radicals able to damage DNA and binding to drugs and other molecules in ways that can be either beneficial or deleterious.

Nearly 30 years ago, Proctor and Mc Guinness attempted to unify most of these roles, envisaging melanin as an energy transducer with the properties of an amorphous semiconductor [11]; however, no *in vivo* evidence has been provided to date.

In this paper, we provide a critical review of the biological functions so far attributed to melanin's, as well as attempting to provide a unified and integrated theory on their physiological role.

Biological functions attributed to neuromelanin's. The neuromelanin [12,13] found in the Central Nervous System (CNS) of humans and vertebrates is not an homogeneous product, as commonly accepted, consisting, rather, of a group of black pigments with similar but different chemical structures, having different functions at different sites (*substantia nigra* melanin (*snM*), *Locus coeruleus melanin*, ocular melanin, inner ear melanin, and so on).

The amount and location of *snM* varies among animal species, being greatest in humans. It predominates in the catecholaminergic neurons of *substantia nigra* and *locus coeruleus*, being formed by oxidation of catecholamines. According to oxidative degradation studies, *snM* is a mixed-type indole polymer, consisting of benzothiazine and dopamine units in equal proportions

and is produced in the absence of visible light [14,15]. *snM* is chemically similar but not identical to skin melanin, which is synthesized in the epidermal melanocytes under the influence of light [16]. Light absorption/protection are the predominant physiological functions of skin and eye melanin's while any optical role for *snM* and inner ear melanin may be excluded and their real roles remain controversial.

All melanin's are able to absorb and dissipate light as heat. If the energy input is too great, however, the output can activate chemical species able to damage cellular macromolecules, resulting in cell death, mutations and cancer. In dark skin, the high pigment concentration protects the skin, shielding the nucleus. In light skin, the radical nature of the melanin's participates in the carcinogenic process, particularly after severe episodes of sunburn [17].

Skin melanin (an indole derivative) acts as a sunscreen while it is assumed that *snM* (a benzene derivative) is involved in the coordination of movement and the protection of neurons against oxidative stress as well as being suspected of binding iron as if it were an exchange resin and of being fundamental to the radical aggression and death of *substantia nigra* neurons (Parkinson's disease, PD) [18 - 21].

It has been claimed that ocular melanin absorbs excessive light and protects the ocular tissues by removing, like a molecular sieve, heavy metals, free radicals and a variety of harmful organic molecules from the cytoplasm. It has also been claimed that in the ear, the inner-ear melanin interacts with acoustic waves, muting them in a beneficial way [22]. Numerous papers describe how easily melanin binds foreign compounds, but they do not explain, however, whether the original pigment is regenerated *in vivo* and how this would come about. We know various physiological mechanisms in the body able to bind and release active compounds (at given times, in given conditions and in given quantities), like, for example, the ferritin iron-pump.

Apparently this is not the case with melanin, where the flushing out of any absorbed material without causing permanent substrate damage appears to be an extremely difficult task. On the contrary melanin strongly binds drugs (arsenic, cocaine, amphetamine, promazine, and so on),

which can be easily traced in the hairs of users or abusers, even a very long time after intake. The best choice for liberating them is by irreversible oxidative cleavage, as in nature.

Based upon the evidence collected so far, neuromelanins fulfil different functions at various sites of the CNS following, in our view, a unitary physiological mechanism which can be correlated to the peculiar physical and chemical properties of the melanin particle. These particles are organized into rigid micro cage-like structures of different shapes and sizes (*<the neuromelanin cage>*) and are also fundamental to the optical [23] and bioelectrical properties of neuromelanin.

Substantia Nigra Melanin (snM) During the course of life, *snM* accumulates in dopaminergic neurons, decreasing in senescence, concurrently with a decrease in the number of neurons and in the synthesised dopamine [24]. The neurons with the highest amounts of black pigment and the smallest quantities of reduced glutathione (GSH) are the first to degenerate.

These facts enforced the hypothesis that neuromelanin originates from dopamine at random, and that these radical accidents, catalysed by iron and monoaminoxidase (MAO), lead to neuronal death. Whence, numerous authors considered *snM* a waste product, which takes precious dopamine away from the life cycle, and acts as a neuron killer.

The main objections to this hypothesis are, presumably, that:

1. Intracellular melanogenesis is genetically controlled. Both skin and eye melanin are fundamental to light absorption and this process is of fundamental physiological relevance;
2. *snM*, skin and eye melanin are chemically and physically similar. It is likely, therefore, that *snM* is involved in a related biological function;
3. Since the *basal nuclei* modulate and transmit nervous impulses and *snM* is formed in the brainstem at a very early age, (being continuously regenerated during life), our view is that its presence at this strategic site is unlikely to be fortuitous, a fact which would suggest the possible involvement of *snM* in nervous transmission;
4. *snM* 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). These facts suggest a possible correlation between *snM* and the coordination of movement;

5. The traditional neuronal theory provides a good model for the transmission of nervous impulses. It is still unclear, nevertheless, whether in the strongly pigmented *basal nuclei*, the information is transmitted by dopamine alone, or also by *snM*.

Substantia nigra melanin (snM) and Parkinson's disease (PD).

The apparent cause of PD is specific pigmented neurons dying-off in the *substantia nigra*.

Dopaminergic neurons may be involved in schizophrenia and in various motor disorders and noradrenergic neurons may participate in endogenous depression and Alzheimer's Dementia (AZD).

The symptomatology of PD consists of the derangement of the function of basal ganglions, with reduced activity of dopaminergic components and deficiency of dopamine, as well as of *snM*. It has been suggested that the disease is triggered by a slow and progressive lack of dopamine, but there is still controversy on what leads the dopaminergic cells – located in the *substantia nigra zona compacta* - to gradually reduce their dopamine production, or on what brings this substance to be transformed into inactive products.

The *substantia nigra* is connected to the *striatum* at the base of the brain through nerve fibres with ends secreting 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. The pathological variation of the different biochemical parameters suggests that during PD the *substantia nigra* is stressed by oxygenated free radicals, which leads to the degeneration of almost 80% of dopaminergic neurons before clinical symptoms arise.

snM concentration varies with age. It is virtually nil during early infancy, when the newborn does not have full control over his/her locomotorial functions. *snM* concentration reaches its peak in

adult age, declines over the years and is at its lowest in the elderly. The decrease goes hand in hand with the degradation of locomotorial coordination and reflex response.

Considering this phenomenology, we suspect that the *substantia nigra* plays a role in nervous transmission, which would be consistent with the electro conductivity of the *snM* particle. 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*.

snM, produced constantly through the oxidation of dopamine and its derivatives, is subsequently metabolised through oxidative degradation, establishing, in normal physiological conditions, a homeostatic balance dopamine/*snM*. A low dopamine concentration means smaller amounts of *snM* and consequently impoverished *substantia nigra* deranged in its electric functions.

On the other hand, an excessive formation of *snM*, through abnormal dopamine oxidation, leads to a lack of this neurotransmitter, which in turn upsets dopaminergic functions, killing the neuron.

It is worth stressing that substantial differences exist between the chemistry and biology of *snM* and that of ocular melanin, and that the extra cellular *snM*-iron complex has been claimed to be the cause of neuronal death.

Ocular and Inner Ear Melanin. The retinal pigmented epithelium (RPE), strategically located between the rod and cone photoreceptors and the vascular bed of the choriocapillaris, contains a black melanin pigment which has photoactive properties, though it is not involved in visual phototransduction. In primates, melanocytes and neuro-epithelial cells are responsible for melanogenesis, melanin appearing in the skin and iris stroma around 6 weeks after delivery [25]. In human embryos retinal pigmentation can already be detected at the 7-week stage.

Ocular melanin behaves like a broadband optical absorber and is generally thought to protect ocular tissues against excess light, reducing intraocular light scatter and thus increasing the contrast of visual images formed on the retina.

Albinism, the most severe genetically determined disorder in ocular pigmentation, is characterized

by congenital hypo-pigmentation of eyes, skin and hair (oculocutaneous albinism) or apparently limited to the eyes (ocular albinism). The lack of pigment in the eye causes various problems (reduced visual acuity; nystagmus; strabismus; sensitivity to bright light and glare). The most severe problem is found in the fovea, the small area of the retina which permits acute vision, which does not develop completely because of the lack of the melanin pigment which is needed for prenatal growth of the intraocular fine structures and a normal pattern of nerves routes from the back of the eye to the brain.

Charles Darwin stressed that *< white cats, if they have blue eyes, are almost always deaf >*, anticipating a possible genetic correlation between albinism and deafness. Albinism and deafness can be concomitant in pets, where a molecular alteration of the eye and/or ear melanins should be detectable. We suggest investigating, in humans, the eye and inner ear pigmentation in normal, albino and deaf individuals, to find possible molecular correlations between the two conditions of oculocutaneous albinism and deafness. Furthermore, it is worth confirming, if inner ear melanin has a muting effect on the acoustic waves and if, and how, the semiconductor properties of inner ear melanin are involved in audiology .

In different cells (RPE and epidermal melanocytes respectively) ocular and skin melanin are synthesized from DOPA by a tyrosinase dependent reaction. *snM* is formed in the *substantia nigra* from CYSDOPA/DOPA, via a mechanism, which is not tyrosinase dependent.

It is not clear, if the light absorbed by ocular melanin is transformed into heat (the most likely way) or other forms of energy. In laboratory experiments, natural and synthetic melanin's in the doped state behave like electric conductors. Further studies are merited, to confirm whether the bioelectric properties of ocular melanin are fundamental to the dissipation of the excessive light absorbed by the RPE, which amounts to approximately 80% of the total solar energy absorbed by the eye.

The pigmented cells in the eye are mostly non-dividing, melanin in the RPE and probably in other pigmented eye tissues, showing very little, if any turnover. This means that once it has been

constituted the ocular pigmentation is for life, in marked contrast to skin pigmentation. Consequently, any structural modification that may occur in eye melanin by environmental stress or aging is potentially much more dangerous than those of skin melanin [26]. According to this view, our body would contain two chemically and functionally similar pigments, with very different turnover: Skin melanin with an average life of approx. 15 days and eye melanin with practically no turnover and an estimated life of approximately 25000 days (almost 1700 times longer). Is such a difference possible? Based upon theoretical considerations, we doubt that the RPE cells are non-dividing and suggest checking whether eye melanin really has no measurable turnover at all..

Analogously to the other melanin's, it has frequently been claimed that ocular melanin protects the eye tissues, binding heavy metals and trapping oxygenated free radicals. This is an unvalidated hypothesis, meriting further physiological confirmation. The binding of ions and molecules by melanin so far detected in laboratory models should be considered as rather a fortuitous irreversible and deleterious molecular accident, which may, at times, lead to severe impairments, *in vivo*.

REFERENCES AND NOTES

- [1] Bruno J.R. Nicolaus <*From butterflies to psychotropics, a chemical odyssey*> in Reflections on twentieth-century psychopharmacology (2004), vol.4, pp.250-253; Editors T.A.Ban, D.Healy, E.Shorter; Animula Publishing House, Budapest Hu.;
- [2] Rodolfo A. Nicolaus <*Melanin's* > (1968) Hermann, Paris.
- [3] Giuseppe Prota <*Melanin's and Melanogenesis* > (1992) Academic Press, Inc.
- [4] Assessing melanins in organic tissues and fluids is not an easy task, due their intrinsic chemical

properties. The EPR signal (paramagnetism) represents, whenever applicable, a specific analytical tool for the detection of melanins, whereas any statement based on colour and reactivity towards silver salts alone, may be misleading. In view of this fact various statements published in the international press, should be reconsidered.

[5] W.A.Little < *Possibility of synthesizing an organic superconductor* > Phys. Rev. 134, A1417 (1964).

[6] WS. Enochs, MJ. Nilges, HM. Swartz < *Purified human neuromelanin, synthetic dopamine melanin as a potential model pigment, and the normal human substantia nigra: characterization by electron paramagnetic resonance spectroscopy* > J. of Neurochem.(1993), 61, 68-79.

[7] L. Zeise, B.L. Murr, M.R. Chedekel < *Melanin standard method : particle description* > Pigment Cell Research (1992), 5,133-142 ; Pigment Cell Research Suppl.2,(1992), 48-53.

[8] J.B. Nofsinger , S.E.Forest, L.M.Eibest , K.A. Gold and J.D.Simon < *Probing the Building Blocks of Eumelanins Using Scanning Electron Microscopy*> Pigment Cell Research (2000) 13: 179-184;

[9] Y. Liu, J.D. Simon < *Isolation and biophysical studies of natural eumelanin's : applications of imaging technologies and ultra fast spectroscopy*> Pigment Cell Research, (2003), 16,608-618; 16,72-80; 16,606-618 .

[10] C.M.R. Clancy, J.D. Simon < *Ultra structural organization of eumelanin from Sepia officinalis measured by Atomic Force Microscopy (AFM)* > Biochemistry, (2001), 40, 133-53 ; J. Phys. Chem. B, (2000), 104, 7871.

[11] Peter H. Proctor < *Free Radicals and Human Disease*> CRC Handbook of Free Radicals and Antioxydants, vol 1 (1989),pp.209-221; www.organicsemiconductors.com

[12] R.Crippa, Q J Wang, M.Eisner < *Structure of human neuromelanin by X-Ray diffraction:*

comparison with synthetics > Pigment Cell Research (1996); 5: 2.

[13] KL.Double, L.Zecca, P.Costi, M.Mauer, C.Griesinger, S.Ito, D. Ben-Shar, G.Bringmann, RG.Fariello, P.Riederer, M.Gerlach < *Structural characteristics of human substantia nigra, neuromelanin and synthetic dopamine melanins* > J.Neurochem.(2000), **75**, 2583-9;

[14] Kazumasa Wakamatsu, Kenichi Fujikawa, Fabio A.Zucca, Luigi Zecca and Shosuke Ito <*The structure of neuromelanin as studied by chemical degradative methods* > J. Neurochem. (2003), 86,1015-1023.

[15] D.Zecca, M.Tampellini,M.Gerlach, P. Riederer, R.G. Fariello, I. Sulzer <*Substantia nigra neuromelanin: structure,synthesis and molecular behaviour*> Mol.Pathol.(2001), 54,414-8;

[16] In the liver tyrosine is synthesized from phenylalanine by the enzyme phenylalanine hydroxylase and then transported to the brain by the large neutral amino acid transporter. 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 norepinephrine are catabolized by a two-step process, involving the enzymes mono amino oxidase (MAO) and catechol-O-methyltransferase (COMT).

[17] HZ Hill <*The function of melanin or six blind people examine an elephant*> Bioessays, 1992 Jan;14(1):49-56.

[18] W.S.Enochs < *The roles of neuromelanin, binding of metal ions, and oxidative cytotoxicity in the pathogenesis of Parkinson's disease: an hypothesis* > J. Neural Transm.(P-D Sect.), (1994) **7**,83-100;

[19] L.Zecca, M.Gallorini, V.Schunemann, AX.Trautwein, M. Gerlach, P.Riederer, P.Vezzoni, D.Tampellini < *Iron ,neuromelanin and ferritin content in the substantia nigra of normal subjects at different ages: consequences for iron storage and neurodegenerative processes*> J.Neurochem.

(2001),76,1766-73;

[20] L. Zecca, D. Tampellini, A. Gatti, R. Crippa, M. Eisner, D. Sulzer, S. Ito, R. Fardello, M. Gallorini < *The neuromelanin of human substantia nigra and its interaction with metals* > J. Neural Transm. (2002) 109,663-72 ;

[21] FD. Marco, C. Foppoli, R. Coccia, C. Blarzino, M. Pierluigi, C. Cini, M.L. Mercante < *Ectopic deposition of melanin pigments as detoxifying mechanism: A paradigm for basal nuclei pigmentation* > Biophys. Res. Commun. (2004), 314 (2), 631-7;

[22] Organic semiconductors span from quantum mechanics to human disease (e.g. melanoma, deafness, Parkinson's). Like Schrodinger's Cat, Darwin's deaf white kitty illustrates a macro *quantum* phenomenon, which is well expressed in the strong electron-phonon coupling in the sound-absorbing inner-ear melanin (Peter H. Proctor).

[23] N. Kollias, R. M. Sayre, L. Zeise, M. R. Chedekel < *New Trends in Photobiology – Photoprotection by Melanin* > J. Photochem. Photobiol. B: Biol. (1991) 9:135-160.

[24] L. Zecca, R. Fariello, P. Riederer, D. Sulzer, A. Gatti, D. Tampellini < *The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease* > FEBS Letters (2002) 510,216-20.

[25] Randolph Glickman, James M. Gallas, Steven L. Jacques, Benjamin A. Rockwell and Dhiray K. Sardar < *The Physical and Photochemical properties of ocular melanin* > Internet Presentation for Saratov Fall Conference, 3-6 October 2000; glickman@uthscsa.edu

[26] Tadeusz Sarna < *Properties and function of the ocular melanin. A photo biophysical view* > J. Photochem. Photobiol. B: Biol. (1992) 12: 215-258.

