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New face of neuromelanin

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Summary. The massive, early and relatively circumscribed death of the dopaminergic neurons of the substantia nigra in Parkinson's disease has not yet been adequately explained. The characteristic feature of this brain region is the presence of neuromelanin pigment within the vulnerable neurons. We suggest that neuromelanin in the Parkinson's disease brain differs to that in the normal brain. The interaction of neuromelanin with iron has been shown to differ in the parkinsonian brain in a manner consistent with an increase in oxidative stress. Further, we suggest an interaction between the lipoprotein α-synuclein and lipidated neuromelanin contributes to the aggregation of this protein and cell death in Parkinson's disease. The available data suggest that the melaninisation of the dopaminergic neurons of the substantia nigra is a critical factor to explain the vulnerability of this brain region to early and massive degeneration in Parkinson's disease.

Parkinson's disease (PD) is a progressive neurodegenerative disease which afflicts approximately 1% of the population aged 65 years and older and 4–5% of those over 85 years of age. It is characterised clinically by severe motor dysfunction, including the typical resting tremor, cogwheel rigidity and bradykinesia or poverty of movement. These characteristic symptoms are known to result from the progressive death of dopaminergic neurons in the substantia nigra pars compacta, an important relay region in the brain motor circuit. These clinical symptoms only develop following the death of at least 60% of the dopaminergic neurons in the substantia nigra, leading to a severe reduction of tissue dopamine levels in the caudate and putamen. While a diagnosis of probable PD based upon the typical motor symptoms can be made during life, a definitive diagnosis is possible only post mortem and is based upon two criteria: the marked loss of substantia nigra dopaminergic neurons, and the presence of abnormal inclusions (Lewy bodies and Lewy neurites) in the cell bodies of some surviving neurons. Lewy bodies consist of abnormally aggregated cellular proteins, primarily α-synuclein, a soluble presynaptic protein of uncertain function in the healthy brain. Interestingly Lewy bodies are found in only a small proportion (approximately 5%) of the surviving neurons in PD and are not exclusive to the substantia nigra pars compacta but are found in a number of brain regions in PD patients in a relatively consistent pattern (Braak et al., 2003). Studies of various forms of genetically inherited PD have shown that many forms of inherited PD are not characterised by aggregated α-synuclein (Huang et al., 2004), indicating that the deposition and aggregation of α-synuclein is not a prerequisite for the development of the clinical syndrome of PD. Furthermore, in all forms of PD (genetic and sporadic) neuronal cell loss early in the disease process is restricted to the

dopaminergic neurons of the substantia nigra but does not occur in the nearby dopaminergic nuclei (McRitchie et al., 1997) nor in non-dopaminergic neurons, despite the more widespread Lewy body pathology. From these data two important but often overlooked conclusions must be drawn: firstly, that the dopaminergic neurons of the substantia nigra are selectively vulnerable to degeneration early in PD and secondly, that the abnormal aggregation of proteins such as α-synuclein, while undoubtedly important in the disease process, cannot be the primary mechanism leading to the especial vulnerability of these neurons to cell loss early in the disease. These conclusions thus raise the question of what is it about the dopaminergic neurons of the substantia nigra which results in this early and circumscribed cell loss?

Recent studies on the many inherited forms of PD have identified a number of gene products as abnormal in these genetically determined forms of PD (Huang et al., 2004). These gene products affect several cellular pathways, resulting in α-synuclein deposition and proteosomal dysfunction, oxidative stress and mitochondrial dysfunction (Huang et al., 2004). As a result of these findings, hypotheses regarding each of these mechanisms as the primary mechanism for cell death in PD have developed. While these four mechanisms cannot be assumed to be independent, as each mechanism appears to be capable of impinging upon the other, they are generally discussed as the most likely candidates for degenerative changes within the substantia nigra in PD. While a body of experimental data supports the idea of changes in cellular pathways in the substantia nigra in PD the reason for the especial vulnerability of this region has not been resolved. A search for a feature of the dopaminergic neurons of the human SN which distinguishes them from nearby dopaminergic neurons would find it difficult to avoid the most characteristic feature of these neurons, that is the presence of large amounts of the pigment neuromelanin (NM) which gives the region its characteristic dark appearance and for which the substantia nigra (Latin: dark body) is named.

NM has been a focus of PD research for some time. In 1988 it was reported that the NM-containing cells of the SN are more vulnerable in PD (Hirsch et al., 1988). In fact a direct correlation was reported between cell loss in the individual cell groups which make up the SN and the percentage of NM-positive cells found in them (Hirsch et al., 1988). This suggests that in the normal brain NM may confer an advantage upon the cells in which it is found. The idea that NM may play a protective role in the dopaminergic cells of the SN in the normal brain is attractive and would reflect a corresponding role of melanins in other bodily tissues, such as the skin and the eye. Indeed there are good reasons why such a pigment has evolved within this brain region as the neurochemical environment of the dopaminergic SN is highly oxidative as a result of the catabolism of dopamine, via both enzymatic and non-enzymatic pathways, and a naturally high concentration of tissue iron. While we have recently demonstrated that some published data based upon a synthetic dopamine melanin may be erroneous while applied to the human pigment because of the differing behaviour of these synthetic and native pigments (Li et al., 2005), melanins can exhibit radical scavenging properties and we have shown that human neuromelanin can inhibit iron-induced lipid peroxidation of rat brain in vitro (Double et al., 1999). Further, we have demonstrated that human neuromelanin can attenuate the death of primary mesencephalic cultured cells by an oxidative stimulus (Li et al., 2005).

In view of these findings supporting the hypothesis that NM can play a protective function it is interesting to revisit the early data of Kastner and colleagues (Kastner et al., 1992) who reported that pigmented cells in the PD brain contain less NM than those in control brains. We suggest that NM

in the PD brain may differ to that in the normal brain. The massive loss of pigmented neurons, and therefore NM, in the parkinsonian brain at post mortem means that investigations of NM in the PD brain are technically difficult. Nevertheless a review of the literature reveals some data to support this thesis. It is of interest that one of the primary pathological hallmarks of PD, the Lewy body, is known to form within the boundaries of pigment in the cells in which these are found. As noted above, one of the primary constituents of the Lewy body is α-synuclein, a protein which has been shown to aggregate under conditions of oxidative stress and high iron concentrations (Kaur et al., 2004). In this context it is pertinent that we have shown that NM pigment is an effective binder of iron (Double et al., 2003) in a manner analogous to the iron binding core of the major iron binding protein ferritin (Gerlach et al., 1995). Studies using a variety of sophisticated biophysical techniques have established that the iron signal associated with NM granules in the PD SN is higher than that in the normal brain, suggestive of increased NM-bound iron in this brain region (Götz et al., 2004). Recently Faucheux and colleagues (Faucheux et al., 2003) have demonstrated that the redox activity of NM aggregates, attributed by these authors to Fe^{2+}, is significantly increased in parkinsonian patients, a finding not observed in the surrounding non-melanised tissue. Further redox activity of the NM aggregates was positively correlated with the severity of neuronal loss (Faucheux et al., 2003). These findings suggest that changes in NM precede cell loss in PD.

Structural analyses using nuclear magnetic resonance spectroscopy and electron paramagnetic resonance spectroscopy indicate that NM isolated from the parkinsonian brain may have a decreased ability to bind iron (Aime et al., 2000; Lopiano et al., 2000). Our work has shown that NM appears to maintain a reserve for binding additional iron in the healthy brain (Double et al., 2003), but in the PD brain any increase in iron might result in saturation of NM's iron binding capacity, leading to increased free iron concentrations in the surrounding tissue. These data support the idea that iron concentrations are increased in the parkinsonian SN and that this increase stimulates a localised increase in the oxidative environment of the melaninised neurons. Certainly there is a body of data which indicates that the parkinsonian substantia nigra exists in an environment of oxidative stress (Fasano et al., 2003). A localised increase in iron and/or iron-induced oxidative stress resulting from changes in the amount of iron in the substantia nigra or a reduced ability of NM in the PD to chelate iron could stimulate aggregation of α-synuclein within the boundaries of NM pigment. An early study using nuclear magnetic resonance spectroscopy reported that NM isolated from the parkinsonian brain differed from that in the healthy brain in that it was mainly composed of a highly cross-linked, protease-resistant, lipo-proteic material (Aime et al., 2000). This suggests that the composition of NM may differ in the PD brain, an idea supported by the more recent finding that α-synuclein is covalently bound to NM isolated from the parkinsonian brain but not in NM from the normal brain (Fasano et al., 2003).

In this context it is relevant that α-synuclein can take the structural form of a lipoprotein and we have recently demonstrated that NM granules consist of a significant proportion of lipids, a feature of NM which distinguish it from melanins in peripheral tissues in which no lipids are present (Fedorow et al., 2005). We have recently identified the primary lipid species in NM to be the polyisoprenoid dolichol (Fedorow et al., 2005). Accumulation of dolichol is associated with neurodegeneration in disorders such as the neuronal ceroid lipofuscinoses, but this is the first time that concentrated dolichol has been identified in the normal

brain. Further dolichol is known to increase in grey matter regions of the brain with normal aging, particularly after the sixth decade of life (Fedorow et al., 2005). Given that the greatest risk factor for PD is increasing age it is not unreasonable to suggest that an interaction between α-synuclein and lipidated NM pigment might stimulate the aggregation of this protein and the eventual formation of Lewy bodies within melaninised nigral neurons. This hypothesis would explain the especial vulnerability of the melanised neurons in PD and the formation of the final product of α-synuclein aggregation, the Lewy body, in close association with this cellular pigment. Of course Lewy bodies are not an inevitable consequence of aging, as well as being found in other cellular types, for example in cortical neurons in Dementia with Lewy bodies. It is therefore likely that an additional, as yet unidentified change, early in the parkinsonian SN is required to trigger aggregation of α-synuclein. The limited data available to date however inextricably link changes in NM in PD with two of the most accepted hypotheses regarding the mechanism of cell death in Parkinson's disease; α-synuclein deposition and oxidative stress.

The available data suggest that the melanisation of the dopaminergic neurons of the substantia nigra is a critical factor to explain the vulnerability of this brain region to early and massive degeneration in PD. We hypothesise that changes in NM precede cell death in this disorder, a suggestion that concords with the modest quantity of data gathered to date on changes in NM in PD. Further analyses of the structure and function of this pigment in the parkinsonian brain are required to identify such changes and to characterise their influence on cell survival.

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