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Evidence for specific phases in the development of human neuromelanin

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Abstract

Neuromelanin is a dark-coloured pigment which forms in the dopamine neurons of the human midbrain. The age-related development and regulation of neuromelanin within these dopamine neurons has not been previously described. Optical density and area measurements of unstained neuromelanin in ventral substantia nigra neurons from 29 people spanning the ages of 24 weeks to 95 years old, demonstrated three developmental phases. Neuromelanin was not present at birth and initiation of pigmentation began at approximately 3 years of age, followed by a period of increasing pigment granule number and increasing pigment granule colouration until age 20. In middle and later life the colour of the pigment granules continued to darken but was not associated with any substantial growth in pigment volume. The identification of three phases and changes in the rate of neuromelanin production over time suggests the regulation of neuromelanin production and turnover, possibly through enzymatic processes.

Keywords: Neuromelanin; Development; Human brain; Parkinson's disease; Oxidation; Tyrosine hydroxylase

1. Introduction

Adult dopamine cells in the human substantia nigra contain a unique black–brown pigment known as neuromelanin (NM). The function of NM has yet to be established, but it is known to bind oxidative metals such as iron [42]. Parkinson's disease, a common neurodegenerative disease, is characterised by depigmentation and cell loss within the substantia nigra [16]. It is thought that NM-containing neurons within the ventral region of the substantia nigra are the most vulnerable in Parkinson's disease [14], possibly due to oxidative stress [13].

An early descriptive study demonstrated that most NM-containing neurons do not contain this pigment at birth, although NM pigment is usually visible in the substantia nigra by the age of 5 [14]. NM is traditionally thought to form from oxidation products of dopamine, a process thought to be non-enzymatic. There are several aspects of dopamine cell metabolism that support the synthesis of NM through autoxidation. Firstly, no candidate enzymes for the synthesis of NM have been identified, and the rate limiting enzyme for peripheral melanin synthesis, tyrosinase has not been identified in human brain [23]. In addition, in vitro experiments show that dopamine readily autoxidises to give a black substance [38], although this black substance is not chemically identical to NM [1,11]. These data support the hypothesis that NM formation is not enzymatically regulated.

On the other hand, NM has a similar composition to the peripheral melanins of the skin and eyes, and the production of all peripheral pigments occurs via regulated processes. Even though NM is similar to other melanins, it has a more complex structure with associated proteins and lipids, suggesting that NM is not just autoxidised neurotransmitter. Within the midbrain, not all cells that produce dopamine contain NM [19]. This suggests that either the cells containing NM induce its production, or alternatively dopamine cells that do not contain NM inhibit the production of this pigment. Not

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only is NM absent in some dopamine-producing cells within the substantia nigra, it is also absent in the nigra of human infants [15], and common laboratory animals such as rats, mice, guinea pigs and rabbits [30]. Further, human foetal dopamine neurons, when implanted as a treatment into the striatum of patients with Parkinson's disease, exhibit a precocious production of the pigment [17]. This supports the concept that factors involved in neuronal maturation are important for the production of NM.

Previous quantitative studies on the formation and maturation of NM have measured either pigment area within the cell [20] or optical density of the pigment [29,41]. To date there have been no quantitative studies of changes in both NM volume and optical density. The aim of the current study was thus to quantify changes in NM in the young and maturing brain to further understand the development and possible regulation of this pigment.

2. Materials and methods

2.1. Tissue samples and staining

Formalin-fixed paraffin-embedded transverse midbrain tissue blocks at the level of the exiting third nerve fibres from the brains of 29 people were obtained from three sources (Table 1). All brains were fixed for a period of 2 weeks, thus any fixation-induced changes in the tissue should be comparable. Neuropathological examination of all cases excluded significant neuropathology, particularly nigral pathology or diseases associated with dopamine metabolism. Further, the neuropathological examination of each adult case (20 years or older) included α-synuclein and ubiquitin staining to exclude cases positive for synucleinopathy and ubiquitinopathy disorders. Written consent for autopsy was given for all cases and the project was approved by the Human Ethics Committee of the University of New South Wales under the Human Tissue Act of the State of New South Wales. Ten-micron sections were cut using a Microm HM 325 microtome. One section was mounted unstained, and the adjacent section stained for Nissl substance using 0.5% cresyl violet (Fig. 1).

2.2. Cellular measures

Cell analysis was performed by one investigator blinded to the age of the cases. The positions of the red nucleus, the cerebral peduncle and the substantia nigra were identified in the Nissl-stained section (Fig. 1) using a BH-2 Olympus microscope and the cluster patterns of the pigmented neurons identified. In both the unstained and Nissl-stained sections, high magnification (400×) brightfield light microscopic images of samples from the ventral cell clusters [33] or nigrosome 1 [8]

Table 1
Case details

Case #	Age	Gender (M/F)	Cause of death	Postmortem delay (h)	Source
1	24 weeks	M	Abortion	–	UM
2	2 days	M	Aspiration	70	UM
3	7 months	M	Cerebral trauma	27	UM
4	8 months	M	Dehydration	38	UM
5	1.9 years	M	Cerebral trauma	6	UM
6	1.9 years	M	Stabbed	14	UM
7	1.9 years	M	Aspiration	27	UM
8	2.5 years	F	Pneumonia	6	UM
9	2.7 years	M	Unknown	6	UM
10	2.8 years	M	Unknown	25	UM
11	3.1 years	F	Haemorrhagic shock	24	UM
12	5.3 years	F	Hypoxia	7	UM
13	8 years	M	Sudden death	6	UM
14	8 years	F	Sudden death	27	UM
15	11 years	M	Pneumonia	11	UM
16	14 years	M	Unknown	18	UM
17	15 years	M	Sudden death	40	UM
18	18 years	M	Viral encephalitis	22	UM
19	18 years	F	Unknown	40	UM
20	19 years	M	Sudden death	5	UM
21	20 years	M	Pneumothorax	11	POWMRI
22	24 years	M	Electrocution	24	NSW TRC
23	29 years	M	Cardiovascular disease	22	NSW TRC
24	31 years	F	Pulmonary embolism	94	NSW TRC
25	36 years	M	Drug intoxication	30	NSW TRC
26	70 years	M	Cardiovascular disease	14	POWMRI
27	74 years	M	Lung carcinoma	24	POWMRI
28	94 years	M	Cardiovascular disease	44	POWMRI
29	95 years	F	Pneumonia	73	POWMRI

UM: University of Münster; POWMRI: Prince of Wales Medical Research Institute; NSW TRC: New South Wales Tissue Resource Centre.