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The Effects of Ageing on the Pigmented Nerve Cells of the Human Locus Caeruleus and Substantia Nigra

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Summary. The nucleolar volume and melanin content of the human locus caeruleus and substantia nigra has been measured in 70 persons of age range new born to 91 years, all of whom were at death free from overt neurological illness. Both cell types show a reduction in nucleolar size with advancing age, which becomes disproportionately larger towards old age. At 90 years of age, the change in nucleolar volume in cells of the locus caeruleus amounts to about 5 % whereas cells of the substantia nigra show a loss of 20 %. The greater decrease in nucleolar volume in cells of the substantia nigra is attributed to the higher concentration of melanin pigment occurring in these cells at old age, rather than the absolute amount present. This marked decline in nucleolar volume in cells of substantia nigra indicates a reduced activity in the cell which, in turn, may be reflected in the difficulty in control and co-ordination in muscular activity commonly seen in normal elderly persons.

Key words: Ageing – Substantia nigra – Locus caeruleus – Melanin – Nucleolar volume

Nerve cells of the human substantia nigra and locus caeruleus are rather unusual in as much as they accumulate, during their lifetime, the lipoprotein pigment, neuromelanin, and not the more familiar age pigment, lipofuscin (Mann and Yates, 1974a). It is possible that the excessive amounts of melanin that are present in these nerve cells in old age might interfere with their metabolism and lead eventually to dysfunction and even death.

The volume of the nucleolus in nerve cells has been shown to respond appropriately, in various pathological (Mann and Yates, 1974b) and experimental (Watson, 1968) situations to alterations in the demand for protein synthesis and is a useful indicator of cell activity. In this report, therefore, nucleolar volume is related to melanin content in cells of the human locus caeruleus and substantia nigra so that the relevance of melanin accumulation to ageing might be clarified.

Materials and Methods

Blocks of human substantia nigra and locus caeruleus were obtained at necropsy from the brains of 70 patients of age range new born to 91 years, all of whom were at death free from overt neurological illness. There were no significant neuropathological findings. Blocks were fixed in 10 % neutral formalin and paraffin sections cut at 5 and 15 μm thickness. The sections cut at 5 μm were stained with haematoxylin-eosin for routine cytological inspection, whereas those cut at 16 μm were stained with Azure B for RNA (Shea, 1970) and by Schmorls technique (Golodetz and Unna, 1909) for melanin. Measurements of melanin content of nerve cells were made as described previously (Mann and Yates, 1974a). A minimum of 100 cells of each type were measured in every case. Measurements of nucleolar diameter were made using a Leitz ocular micrometer at a magnification of × 1,250. A minimum of 30 cells of each type in each case was measured from which the mean nucleolar volume was calculated.

Cytological Observations

At birth, cells of the locus caeruleus and substantia nigra are small; the amount of RNA (Nissl substance) in the perikaryon is small and melanin granules few. As age progresses, the cell body in both types increases in size and large amounts of rod-like RNA masses are present, which are maximal in number at about 20 years of age. The intensity of melanin pigmentation increases with age making changes in RNA content in many cells in cases of old age difficult to assess. However, despite this, in those cases aged 80 years or above the nucleolus in most cells of the substantia nigra is obviously reduced in size (Fig. 2) when compared to cells in younger cases (Fig. 1). By contrast, there is no such change in cells of the locus caeruleus.

From middle age, and especially so from 80 years onwards, increasing numbers of cells in both the locus caeruleus and substantia nigra show atrophic changes. These changes are characterised by apparent dissolution of RNA, nuclear and nucleolar shrinkage, and clumping of melanin granules (Fig. 3). Final heterolysis of cell contents, by macrophages, results in aggregates of residual melanin being freely deposited outside the cell.

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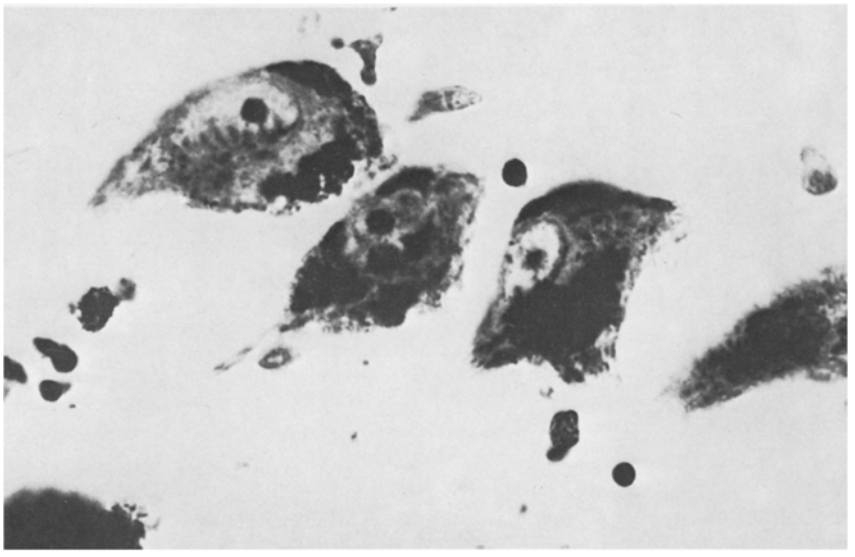

Fig. 1. The human substantia nigra at 24 years of age showing nerve to contain large amounts of rod-like masses of cytoplasmic RNA and a large prominent nucleolus. (Azure B × 550)

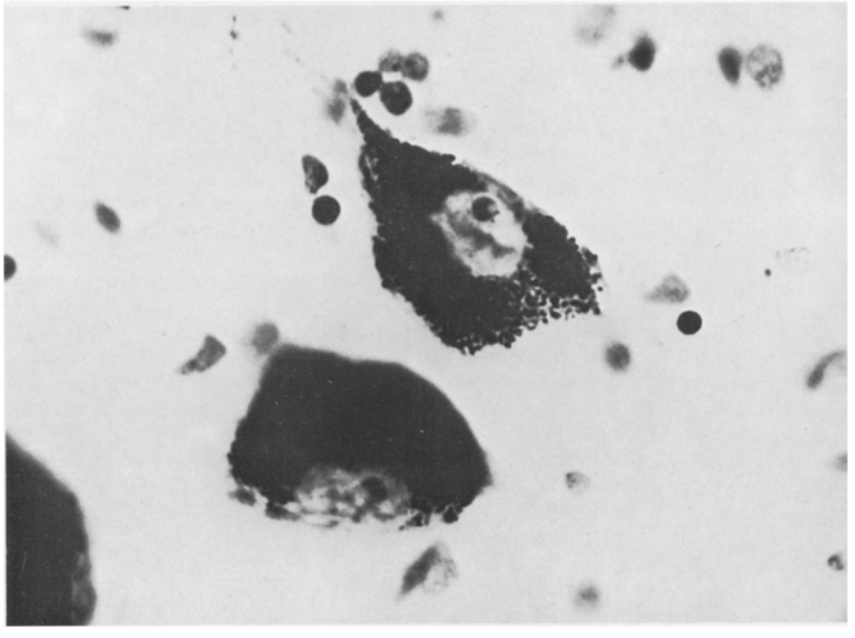

Fig. 2. The human substantia nigra at 83 years of age showing the nucleolus to be reduced in size in comparison to cells in the 24-year-old case. (Azure B × 550)

Results

No significant difference was found in either nucleolar volume or melanin content between males and females (at any age) in either the locus caeruleus or substantia nigra. Male and female values have, therefore, been considered together in all the below calculations.

Values of mean melanin content in each cell type were expressed as the ratio of melanin content of cells of the locus caeruleus to that of cells of the substantia nigra, for each case. Values of ratio of melanin content were then grouped, by age, into 5- or 10-yearly class intervals from which the pooled mean ratio of melanin contents for each age class was derived and plotted against age, as shown in Fig. 4.

The ratio of melanin content has a value of about 1.6 at birth and decreases in a linear manner ($y = 1.57 - 0.00085\,x$; $r = 0.878$; $P < 0.001$) such that unity is reached at about 65 years of age and a level of 0.8 at 90 years of age.

At birth, cells of the locus caeruleus have a nucleolar volume of about 59 μm^3 which increases to a maximum of 69 μm^3 at 15 years of age, and then declines slowly to 66 μm^3 at 80 years of age. By contrast, in cells of

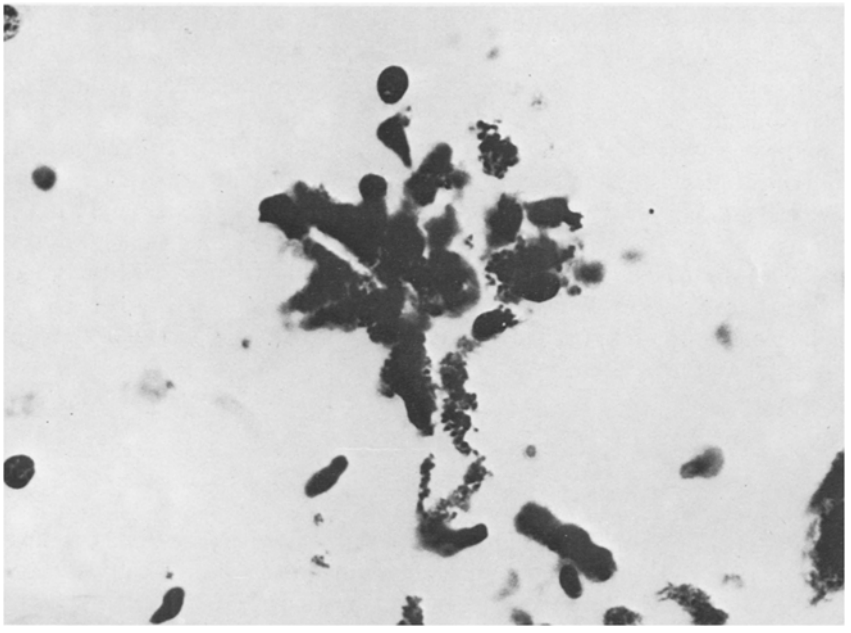

Fig. 3. The human substantia nigra at 83 years of age showing an atrophic cell. There is loss of RNA, shrinkage of the nucleus and clumping of the melanin granules. (Azure B × 550)

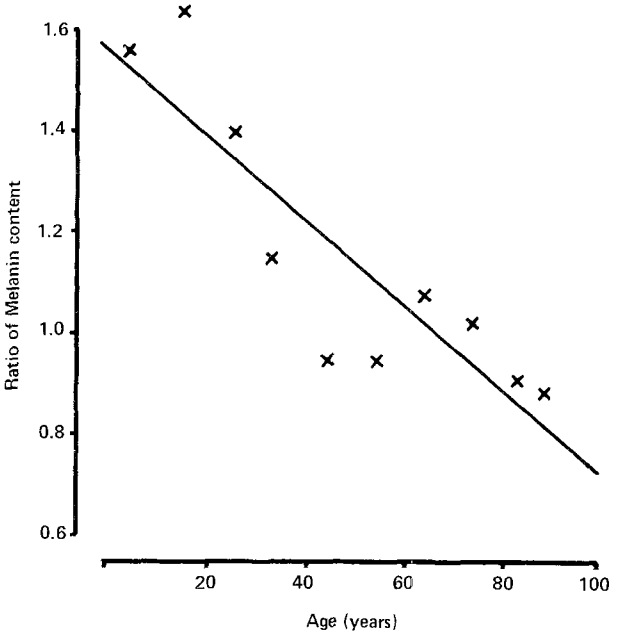

Fig. 4. Graph of ratio of mean melanin content of cells of the locus caeruleus relative to that of substantia nigra as measured in 70 cases of age range new born to 91 years, grouped into 5- or 10-yearly class intervals

substantia nigra the nucleolar volume decreases from a maximum value of 53 μm^3 at 18 months to one of 39 μm^3 at 80 years. For both cell types, the change in volume becomes disproportionately larger towards old age. The mean value of 59 μm^3 at 4.5 years for cells of the locus caeruleus is considered to represent part of the maturing process in these cells and is, therefore, omitted from the following calculations which deal with age related changes of mature cells. A 100% baseline for comparisons was established as the figure for nucleolar volume found in the group whose average age was 15 years. In this way, figures for percentage nucleolar volume are derived and are plotted against mean age, of each class, for both cell types, as shown in Fig. 5.

For both cell types the relationship between percentage volume (y) and age (t) is best described by an exponential equation of the form:

$y = k - Ae^{bt}$ where $k = 100$ (the 100% volume level at 15 years) and A and b are constants. The equations of the lines of best fit, with their respective correlation co-efficients are:

Locus caeruleus: $y = 100 - 0.003e^{0.084t}$; $r = 0.807$

Substantia nigra: $y = 100 - 0.373e^{0.0423t}$; $r = 0.943$.

Both correlation co-efficients exceed the 0.1% significance level.

If the log percentage volume loss [i.e., log (100-percentage volume)] is plotted against age, linear regressions are obtained. Analysis of covariance, on the grouped data for cells of the locus caeruleus and substantia nigra, shows a highly significant difference between the slopes of the two cell types ($F_{1,12} = 37.02$; $P < 0.0001$) and that a single regression line cannot adequately fit the data from both groups ($F_{1,13} = 42.06$; $P < 0.0001$). This finding indicates, therefore, that, although both cell types show a decline in nucleolar volume with advancing age, the rate of decrease is significantly greater in cells of the substantia

nigra. If measurements of nucleolar volume for young and old cases are compared, then this pattern of decrease in elderly cases is more obviously seen.

In Fig. 6, individual values of nucleolar volume are plotted as histograms for cells of the substantia nigra (A and B) and locus caeruleus (C and D), for typical single cases of age 24 years (A and C) and 86 years (B and D).

It is noticeable that the mean nucleolar volume is significantly less for both cell types ($P < 0.0001$) in the 86-year-old case, but the magnitude of this loss differs greatly. Thus, although the mean nucleolar volume in cells of the substantia nigra is reduced by 30%, that in cells of the locus caeruleus is only decreased by about 8%.

Furthermore, the size of the nucleolus is reduced in virtually all the cells of the substantia nigra in the 86-year-old case (B) with few cells having a volume greater than 50 μm^3, whereas in the 24-year-old case (A) most cells have a nucleolar volume exceeding 50 μm^3. Therefore, the reduction in mean nucleolar volume measured in this cell type in the 86-year-old case is due to a decline in value inherent to all cells rather than a pattern involving selective atrophy of certain cells with sparing of others.

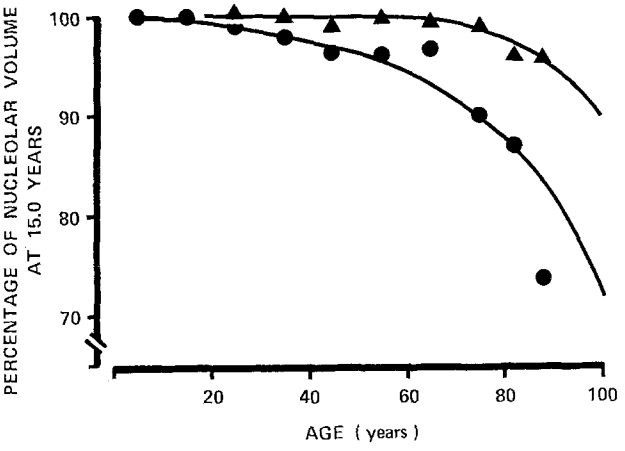

Fig. 5. Graph of mean percentage nucleolar volume plotted against mean age, for cells of the locus caeruleus (▲) and substantia nigra (●) as measured in 70 cases of age range new born to 91 years, grouped into 5- or 10-yearly class intervals

Discussion

Cells of the locus caeruleus give rise to ascending pathways innervating mainly the cerebellum (Olson and Fuxe, 1971), cerebral cortex (Ungerstedt, 1971; Kobayashi et al., 1974) and certain nuclei of the thalamus and hypothalamus (Kobayashi et al., 1974; Versteeg et al., 1976) and their fibres terminate directly upon the small vessels within the brain (Hartman and Udenfriend, 1972) where they have important regulatory functions over the water permeability of the brain microvasculature (Raichle et al., 1976). The substantia nigra regulates the level of motor activity in the cerebral cortex through its influence over the basal ganglia (Hornykiewicz, 1966).

These differing functions are reflected by the different times of maturity of the two cell types. Cells of the substantia nigra attain a maximum nucleolar

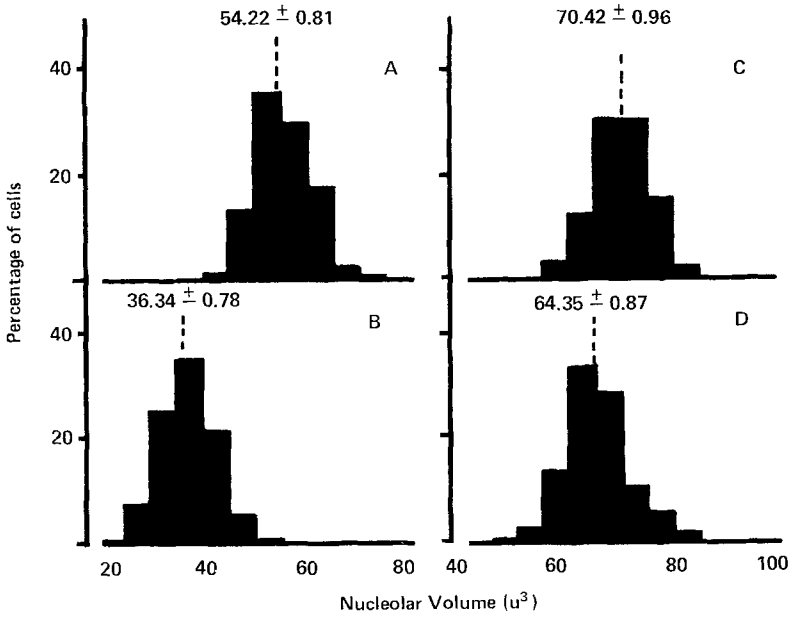

Fig. 6A–D. Histograms showing percentage distribution of nucleolar volume for cells of substantia nigra (**A** and **B**) and locus caeruleus (**C** and **D**) for single cases of 24 (**A** and **C**) and 86 (**B** and **D**) years of age

volume at an early age in accordance with the development of willed co-ordination of muscular ability. However, the rise in nucleolar volume in cells of the locus caeruleus parallels increases in brain weight and with that presumably the extent of the microvasculature and its increased vascular innervation, all reaching a maximum at about 15 to 20 years of age.

Our studies show the reduction in nucleolar volume is of the order of 5 to 10% in aged cells of the locus caeruleus, while in cells of the substantia nigra the change is, on average, 20% and often greater than 30% in many cases. To what might the greater decline in function in these latter cells be attributed?

The pattern of melanin accumulation is not the same in both cell types such that from the age of 65 onwards cells of the substantia nigra contain, on average, greater amounts of melanin than do cells of the locus caeruleus. We have shown elsewhere (Mann et al., 1977) that initially, in individual cells of the substantia nigra, moderate increases in melanin result in no change in nucleolar volume, but when melanin content surpasses a critical point nucleolar volume is decreased with loss of associated cytoplasmic RNA. Also studies of lipofuscin bearing cells (Mann et al., 1978) have shown that it is not the absolute amount of pigment present that determines the extent to which nucleolar volume and cytoplasmic RNA are diminished, but rather the actual concentration of pigment. By this reasoning therefore it is likely that the greater reduction in nucleolar volume measured in neurones of the substantia nigra is a direct consequence of their higher pigment concentration since not only do they contain more pigment than those of the locus caeruleus but they do so in a smaller cell body volume.

How melanin content might bring about these changes in nucleolar volume and RNA content is not clear. Although apparently biochemically inert, it is possible that if the pigment bulk becomes sufficently large it may interfere with protein synthesis through mechanical disruption of the microanatomy of intracellular membranes and neurotubules. This continued situation might eventually lead to an inbalance of those internal control mechanisms regulating the rate of production and transport of proteins which progressively adapt to new and lower levels with consequent effects on the cells metabolic ability and its capacity for function.

It is not unlikely therefore that the difficulties in control and co-ordination of muscular activity commonly seen in many elderly persons arises from loss of cells of the substantia nigra, combined with and probably stemming from the substantial decline in activity occuring in those that remain.

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