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# Loss of neurons from the retrofacial nucleus of the mouse in extreme old age

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## INTRODUCTION

Previous investigations in this series, using the same sets of serial sections, have shown that neuron number remains constant in the mouse indusium griseum up to 31 months of age (Sturrock, 1986), whereas neurons are lost after 25 months of age from the locus ceruleus (Sturrock & Rao, 1985), the mesencephalic nucleus of the trigeminal nerve (Sturrock, 1987) and the facial nerve motor nucleus (Sturrock, 1988). There is also a decrease in neuron number in the trigeminal motor nucleus between 28 and 31 months of age which is not, however, statistically significant (Sturrock, 1987).

The diameter of neuronal nuclei increases significantly after 25 months of age in both the trigeminal nuclei that have been examined (Sturrock, 1987) and in the facial nucleus, but there is no increase in neuronal nuclear diameter in the locus ceruleus. Although motor neurons in the trigeminal motor nucleus and facial nucleus both lose Nissl substance with increasing age, lipofuscin is found in large amounts only in facial nuclei neurons (Sturrock, 1988). Since all the investigations were carried out in the same sets of sections the different responses to ageing in the different groups of neurons are unlikely to have been the result of fixation artefacts.

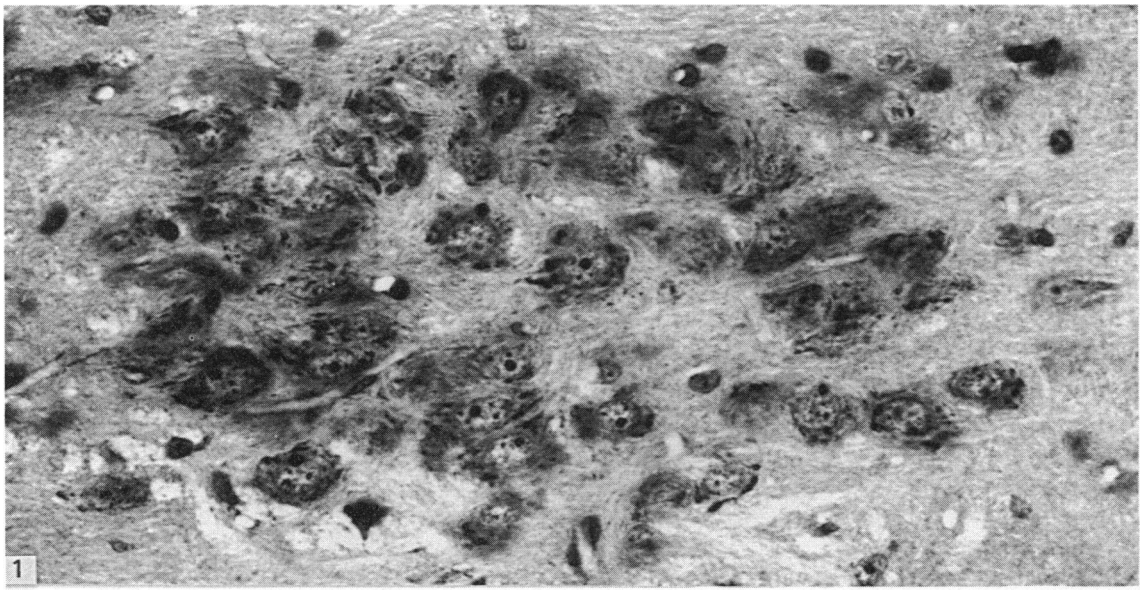
The retrofacial nucleus is a small, but prominent nucleus, situated between the facial nerve nucleus and the nucleus ambiguus (Sidman, Angevine & Taber Pierce, 1971). Degeneration studies (Wohlfahrt, Snorre & Sallstrom, 1939) have shown that the retrofacial nucleus is part of the vagal nuclear complex and physiological investigations have confirmed that, despite its anatomical position, the retrofacial nucleus gives rise to general visceral efferent vagal fibres (Kerr, 1969) as originally proposed by Szentágothai (1952).

The retrofacial nucleus was selected for quantitative histological examination because it is easy to identify, small, and functionally different from the other nuclear groups examined so far in the ageing mouse brain.

## MATERIALS AND METHODS

The material available for this study consisted of sets of perfusion-fixed parasagittal and coronal 6 $\mu$m serial sections of mouse brains at intervals from 6 to 31 months of age. The parasagittal sections, stained with Lapham's stain, were cut from right halves of brains and the coronal sections, stained with haematoxylin and eosin, were cut from left halves of the same brains. The methods of fixation and subsequent processing of these sections have been fully described in earlier papers in this series (Sturrock & Rao, 1985; Sturrock, 1987).

Three sets of sections were used at each age. Since earlier studies indicated that neuron loss did not occur until after 25 months of age only sections from 6, 25, 28 and



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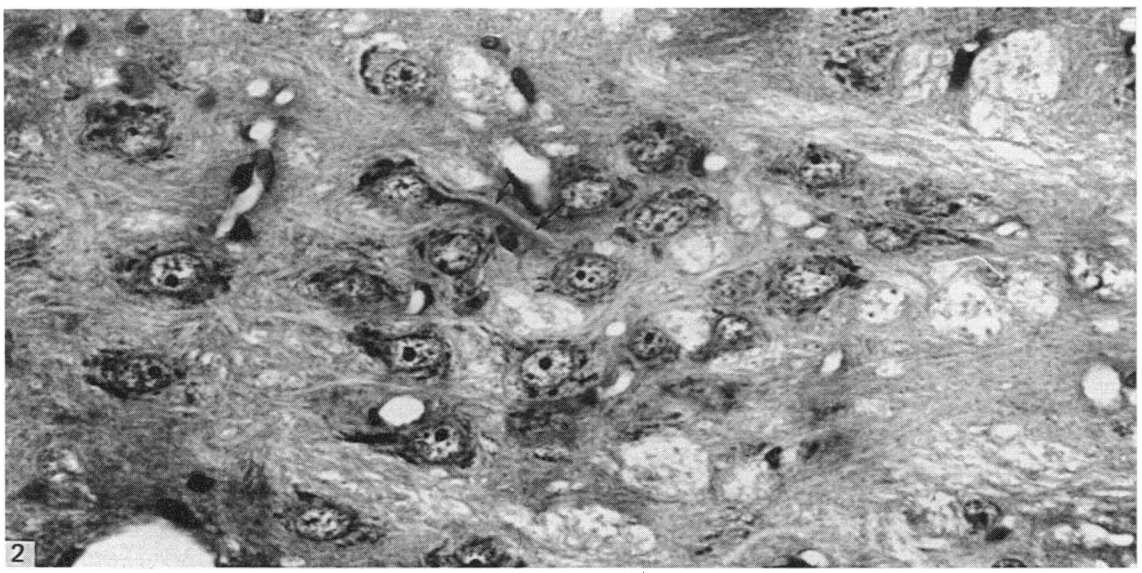

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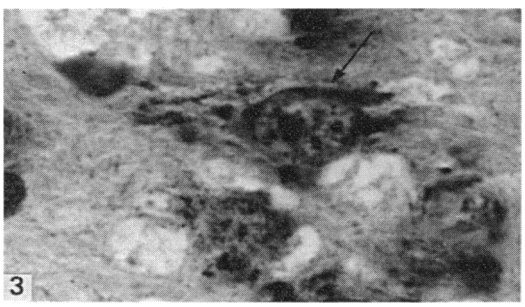

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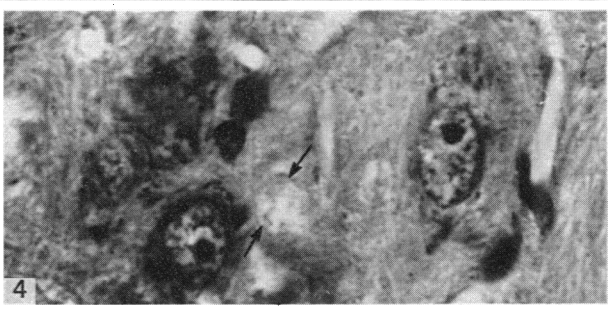

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Table 1. *Number of neurons in the retrofacial nucleus and their mean nuclear diameter* (±S.E.M.)

| Age (months) | Number of neurons | Mean nuclear diameter ($\mu$m) |
|---|---|---|
| 6 | 206±2 | 10·9±0·3 |
| 25 | 209±4 | 11·2±0·2 |
| 28 | 181±11 | 10·8±0·1 |
| 31 | 157±4 | 10·9±0·1 |

31 months were used although, if it had become apparent that changes occurred before 25 months, sets of sections from 9, 12, 15, 18 and 22 months were available for examination.

The counts were carried out in parasagittal sections by scanning the sections at a magnification of ×250 with the aid of an eyepiece graticule. The most medial and most lateral sections containing cells of the retrofacial nucleus were identified and the retrofacial neurons in every fifth section between these two sections were recorded using a haematological counter. Neuronal nuclear diameter was estimated as described previously for neurons of the mesencephalic and motor nuclei of the trigeminal nerve (Sturrock, 1987). The total number of neurons in the retrofacial nucleus was estimated by multiplying the number of neurons counted by the number of sections containing the retrofacial nucleus, dividing by the number of sections examined and the result corrected using Abercrombie's formula (Abercrombie, 1946). Changes in neuron number with age were subjected to an analysis of variance. Every section containing retrofacial neurons at 28 and 31 months of age was scanned to try to find examples of neuronal degeneration.

## RESULTS

The retrofacial nucleus consists of a tightly packed group of neurons (Fig. 1) situated between the facial nucleus and the nucleus ambiguus. There is a loss of Nissl substance from neurons in the ageing brain (Figs. 1–4) and at 28 and 31 months the cells appear less tightly packed than at 6 months of age (contrast Figs. 1 and 2). There is also a substantial amount of lipofuscin accumulation in retrofacial neurons at 25, 28 and 31 months of age. Lipofuscin stains a bright blue colour with Lapham's stain (Sturrock, 1987) but this is impossible to demonstrate convincingly in black and white micrography.

There is no change in neuronal number between 6 and 25 months of age but thereafter there is a substantial, and statistically significant ($F(3, 8) = 14{\cdot}72$ $P < 0{\cdot}01$)

Fig. 1. Sagittal section stained with Lapham's stain. Retrofacial nucleus from a 6 months old mouse. Note the fairly closely packed neurons with perikarya rich in Nissl substance. These cells resemble general visceromotor neurons rather than somatic motor neurons. × 500.

Fig. 2. Sagittal section stained with Lapham's stain. Retrofacial nucleus from a 28 months old mouse. The cells are more loosely scattered and Nissl substance is less prominent than at 6 months. The arrowed dendrite was packed with lipofuscin. × 500.

Fig. 3. Lapham's stain. Retrofacial nucleus neuron from a 6 months old mouse containing a moderate amount of Nissl substance. × 1000.

Fig. 4. Lapham's stain. Retrofacial nucleus neuron from a 28 months old mouse containing very little Nissl substance. The arrowed circular object is a green-staining cell inclusion (more obvious in the stained section than in a black and white micrograph), probably the remains of a degenerating nucleus in a necrotic neuron. × 1000.

decrease in neuron number (Table 1). There is no variation in neuronal nuclear diameter with age (Table 1).

In all the sections scanned at 28 and 31 months of age only one example of a degenerating neuron was detected (Fig. 4).

## DISCUSSION

The results of the present study confirm earlier findings (Sturrock & Rao, 1985; Sturrock, 1988) that neuron loss from mouse brainstem nuclei does not begin until after 25 months of age which is beyond the usual 22 months lifespan of the strain examined (ASH/TO) (Sturrock, 1979). In the mesencephalic and motor nuclei of the trigeminal nerve (Sturrock, 1987) and in the facial nucleus (Sturrock, 1988) the decrease in neuronal number appeared to be related to an increase in neuronal nuclear diameter. No such increase in nuclear diameter was found in the locus ceruleus, whose neuron number also declined after 25 months of age and similarly, in the retrofacial nucleus, neurons were lost after 25 months of age but neuronal nuclear diameter remained constant from 6 to 31 months of age. Changes in nuclear diameter are unlikely to be artefactual since neurons of the mesencephalic nucleus, in which diameter increases, lie adjacent to neurons of the locus ceruleus in which diameter is constant. Similarly, retrofacial neurons are closely related anatomically to facial nerve motor neurons.

There does not appear to be any relation between initial neuronal nuclear diameter in different brainstem nuclei and possible changes in neuronal nuclear diameter in concert with neuron loss. Diameter of neuronal nuclei of the retrofacial nucleus (mean diameter 11·0 $\mu$m) and locus ceruleus (mean diameter 13·7 $\mu$m: Sturrock & Rao, 1985) remains constant despite a substantial loss of neurons after 28 months of age whereas neuronal nuclei of the mesencephalic (mean diameter 10·7 $\mu$m: Sturrock, 1987) and motor nuclei of the trigeminal nerve (mean diameter 13·2 $\mu$m) and of the facial nerve nucleus (mean diameter 11·7 $\mu$m: Sturrock, 1988) all show significant increases in neuronal nuclear diameter after 25 months of age which accompany a loss of neurons. Increase in nuclear diameter occurs in branchiomotor (trigeminal and facial) and primary sensory neurons (mesencephalic) but not in general visceromotor neurons (retrofacial) nor noradrenergic neurons (locus ceruleus). Although functionally different, branchiomotor and general visceromotor neurons are all acetylcholinergic suggesting that the increase in neuronal nuclear diameter is not related to the neurotransmitter released.

Neurons of the retrofacial nucleus show a marked reduction in Nissl substance with age as do motor neurons of the trigeminal (Sturrock, 1987) and facial nuclei (Sturrock, 1988). In the retrofacial and facial nuclei loss of Nissl substance is accompanied by a marked increase in lipofuscin in the perikaryon and dendrites but lipofuscin accumulation is rare in trigeminal motor neurons (Sturrock, 1987). This supports the view (Schlote & Boellaard, 1983) that neuronal degeneration is independent of the lipofuscin content of the neuron. The loss of Nissl substance does, however, suggest a decrease in neuronal activity and this may be a more important consequence of ageing than the actual loss of neurons.

Neuron number in the cerebral cortex of both the mouse (Curcio & Coleman, 1982) and the non-demented human brain (Haug, 1985) remains constant into extreme old age. Neuron number in the indusium griseum of the brains examined in this investigation remains constant up to 31 months of age (Sturrock, 1986) but neurons are lost from a variety of brainstem nuclei after 25 months of age. It is not known

whether this loss of neurons occurs as a result of primary degeneration of the neurons themselves, due to some factor of the ageing process, or whether the neuron loss seen in the brainstem is secondary to degeneration of the muscles or organs supplied by them. The fact that loss of sensory cells precedes loss of motor cells (Sturrock, 1987) suggests that peripheral degeneration, i.e. of muscles etc., is responsible for the loss of neurons. Furthermore the type of neuronal cell death seen in the aged brain (Sturrock, 1988) resembles necrosis which occurs as a result of environmental change, rather than apoptosis, which is the result of programmed cell death (Kerr, Wyllie & Currie, 1972).

## SUMMARY

The retrofacial nucleus, a general visceral efferent component of the vagal nuclei, was examined using quantitative histological techniques in young adult (6 months) and elderly (25, 28 and 31 months) mice.

Neuron number remained constant between 6 months ($206 \pm 2$) and 25 months ($209 \pm 4$) and thereafter declined rapidly at 28 ($181 \pm 11$) and 31 months ($157 \pm 4$). Neuronal nuclear diameter (mean 11·0 $\mu$m) did not vary significantly with age. Neurons of the retrofacial nucleus lost Nissl substance with age and lipofuscin accumulation was marked in the perikaryon and dendrites.

## REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anatomical Record* **94**, 239–247.

CURCIO, C. A. & COLEMAN, P. D. (1982). Stability of neuron number in cortical barrels of aging mice. *Journal of Comparative Neurology* **212**, 158–172.

HAUG, H. (1985). Are neurons of the human cerebral cortex really lost during aging? A morphometric examination. In *Senile Dementia of the Alzheimer Type* (ed. J. Traber & W. H. Gispen), pp. 150–163. Berlin, Heidelberg: Springer-Verlag.

KERR, F. W. L. (1969). Preserved vagal visceromotor function following destruction of the dorsal motor nucleus. *Journal of Physiology* **202**, 755–769.

KERR, J. F. R., WYLLIE, A. M. & CURRIE, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer* **26**, 239–257.

SCHLOTE, W. & BOELLAARD, J. W. (1983). Role of lipopigment during aging of nerve and glial cells in the human central nervous system. In *Brain Aging: Neuropathology and Neuropharmacology, Aging*. Vol. 21 (ed. J. Cervos-Navarro & H. I. Sarkander), pp. 27–74. New York: Raven Press.

SIDMAN, R. L., ANGEVINE, J. B. & TABER PIERCE, E. (1971). *Atlas of the Mouse Brain and Spinal Cord*. Cambridge, Mass.: Harvard University Press.

STURROCK, R. R. (1979). A quantitative lifespan study of changes in cell number, cell division and cell death in various regions of the mouse forebrain. *Neuropathology and Applied Neurobiology* **5**, 433–456.

STURROCK, R. R. (1986). A quantitative histological study of the indusium griseum and neostriatum in elderly mice. *Journal of Anatomy* **149**, 195–203.

STURROCK, R. R. (1987). Changes in the number of neurons in the mesencephalic and motor nuclei of the trigeminal nerve in the ageing mouse. *Journal of Anatomy* **151**, 15–25.

STURROCK, R. R. (1988). Loss of neurons from the motor nucleus of the facial nerve in the ageing mouse brain. *Journal of Anatomy* **160**, 189–194.

STURROCK, R. R. & RAO, K. A. (1985). A quantitative histological study of neuronal loss from the locus coeruleus of ageing mice. *Neuropathology and Applied Neurobiology* **11**, 55–60.

SZENTÁGOTHAI, J. (1952). The general visceral efferent column of the brain stem. *Acta morphologica Academiae scientiarium hungaricae* **2**, 313–328.

WOHLFAHRT, T., SNORRE, A. & SALLSTROM, T. (1939). Einige experimentelle Untersuchungen über retrograde Nervenzellreaktion beim Kaninchen. *Comptes Rendus. Troisième Congrès Neurologique Internationale*, pp. 516–526.