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Loss of neurons from the external cuneate nucleus of the ageing mouse brain

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INTRODUCTION

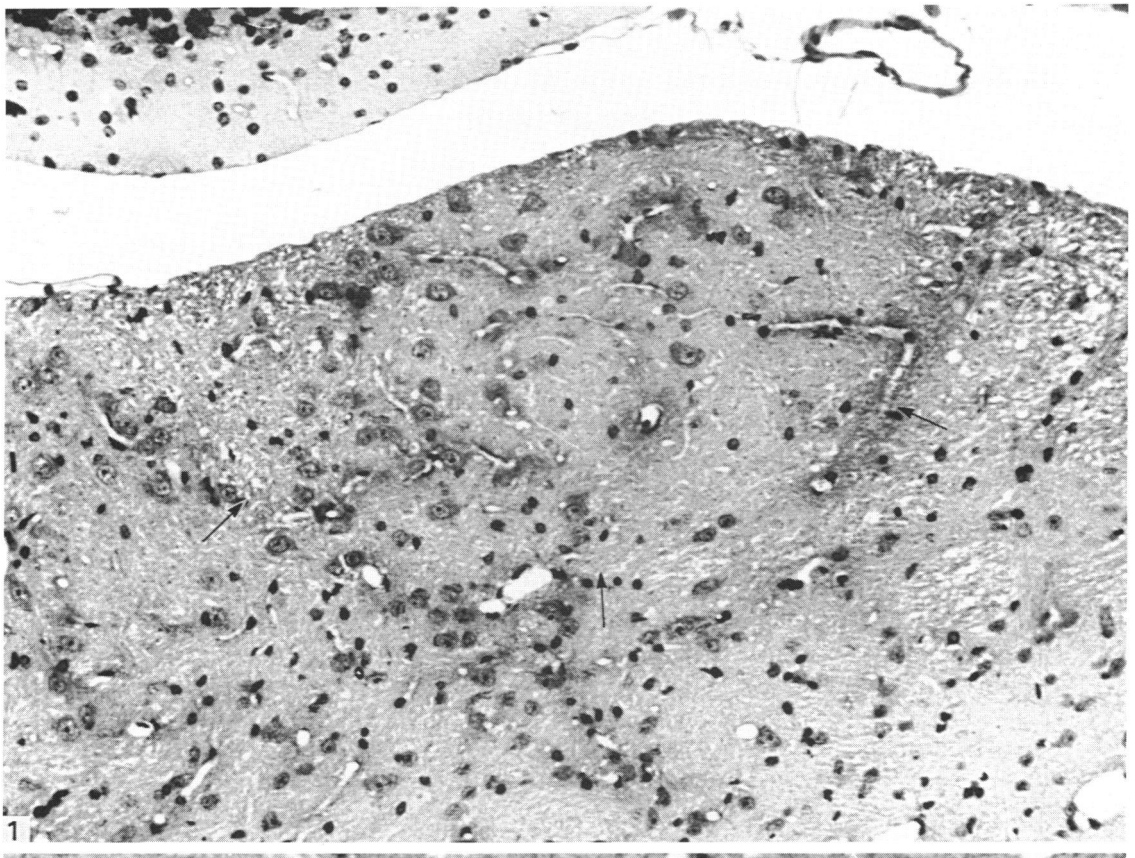
The external cuneate nucleus receives dorsal root afferent fibres from the cervical and upper thoracic roots (Hollis, 1884; Sherrington, 1893; Liu, 1956; Rustioni & Macchi, 1968; Basbaum & Hand, 1973) and also sensory fibres from several cranial nerve centres (Kerr, 1962; Rhoton, O'Leary & Ferguson, 1966; Sobusiak, Zimny & Zabel, 1972). It is considered to be the upper limb, neck and cranial equivalent of Clarke's column, being a proprioceptive relay nucleus to the cerebellum (Oscarsson, 1973). Investigation of the mesencephalic and motor nuclei of the trigeminal nerve (Sturrock, 1987) indicated that loss of proprioceptive neurons preceded loss of motor neurons. Both branchiomotor neurons of the facial nucleus (Sturrock, 1988*a*) and preganglionic parasympathetic neurons of the retrofacial nucleus of the vagus nerve (Sturrock, 1988*b*) decrease in number after 25 months of age in the mouse brain. There is also a loss of anterior horn motor neurons from the mouse spinal cord between 12 and 25 months of age (Wright & Spink, 1959). If loss of these motor neurons is preceded or accompanied by a loss of primary proprioceptive fibres the external cuneate nucleus might be adversely affected by transneuronal degeneration and for this reason the number of neurons in the external cuneate nucleus was estimated in ASH/TO strain mice aged 6, 15, 22, 25, 28 and 31 months of age.

MATERIALS AND METHODS

The material consisted of sets of 6 μm parasagittal serial sections of right halves of mice brains stained with Lapham's stain and 6 μm coronal serial sections of the left halves of the same brains stained with haematoxylin and eosin. The details of fixation and preparation of these sets of sections have already been described (Sturrock, 1987, 1988*a*, *b*). Three sets of serial sections were examined at each of the following ages: 6, 15, 22, 25, 28 and 31 months.

The methods of counting cells and measuring neuronal diameter were identical to those used in earlier investigations of other nuclei in the same brains (Sturrock, 1987, 1988*a*, *b*). Estimates of neuron number were carried out in the parasagittal sections and neuronal nuclear diameter measurements were carried out in the corresponding sets of coronal serial sections to eliminate any orientation bias (Sturrock, 1983, 1987). As noted in the study of the trigeminal nuclei (Sturrock, 1987) in one set of 6 months coronal sections the cerebellum and brainstem had not been sectioned so that the mean diameter was estimated from only two sets of sections at this age.

From 15 months of age degenerating neurons were found in the external cuneate nucleus. The number present was recorded in the ten sections in each set of



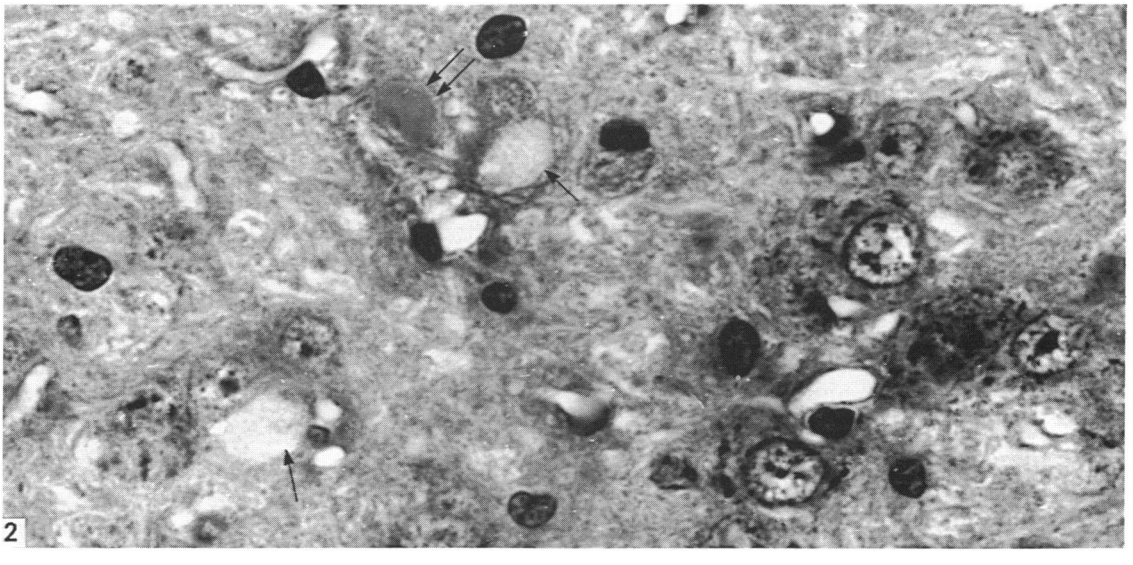


Fig. 1. Parasagittal 6 μm section stained with Lapham's stain illustrating the medial part of the external cuneate nucleus (arrows) which lies beneath the IVth ventricle. $\times$ 250.

Fig. 2. Parasagittal 6 μm section, 28 months mouse external cuneate nucleus containing the remains of three degenerating neurons. Two (single arrows) are pale staining and consist of fine fibrillar cottonwool-like material, whilst the third (double arrows) has a colloid-like appearance. $\times$ 1250.

parasagittal sections used for neuron number estimations. The percentage of degenerating neurons was calculated by dividing the number of degenerating neurons by the crude number of neurons counted. No correction factor was applied to take differences in diameter into account because of the difficulty of determining a mean diameter for the widely varied forms of degenerating neurons. The estimated number of neurons, mean neuronal nuclear diameter and percentage of degenerating neurons at different ages were subjected to analyses of variance.

RESULTS

The external cuneate nucleus consists of widely scattered large neurons and extends from a small triangle of neurons beneath the inferior cerebellar peduncle laterally (Sidman, Angevine & Taber Pierce, 1971), to a larger ovoid structure in the floor of the fourth cerebral ventricle medially (Fig. 1). There is some loss of Nissl substance from the neurons with age but this is not as great as that observed in motor neurons of the trigeminal (Sturrock, 1987) or facial nuclei (Sturrock, 1988*a*). There is a moderate accumulation of lipofuscin in neurons of the external cuneate nucleus from 25 months of age. The most striking morphological feature of ageing, however, is the presence of degenerating neurons in the nucleus from 15 months of age. A variety of different forms of degeneration can be found (Figs. 2–5) and these probably represent different stages of the degeneration process. The first stage consists of fragmentation of the nucleus (Figs. 3, 4) followed by condensation of the nuclear debris (Figs. 3, 5) with the cytoplasm becoming filled with fine fibres. Finally the remains may consist of either an amorphous colloid-like mass (Figs. 2, 3) or bundles of filaments (Fig. 2).

There is a statistically significant decrease in the number of neurons in the external cuneate nucleus between 6 and 31 months of age ($F(5, 12) = 9{\cdot}05$; $P < 0{\cdot}001$) (Fig. 6) but no significant variation in the mean neuronal nuclear diameter over the same period ($F(5, 11) = 0{\cdot}57$; NS) (Table 1). The percentage of degenerating neurons (Table 1) does not vary significantly from 15 to 31 months of age ($F(4, 10) = 0{\cdot}88$; NS). The higher percentages of degenerating neurons found at 22 and 31 months of age were in each case due to one set of sections containing a very large number of degenerating neurons.

DISCUSSION

None of the nuclei previously examined in the same sets of sections showed any evidence of neuronal loss prior to 28 months of age. The mesencephalic nucleus of the trigeminal (Sturrock, 1987), the facial nucleus (Sturrock, 1988*a*) and the retrofacial nucleus (Sturrock, 1988*b*) all lost neurons between 25 and 31 months and neuron number in the motor nucleus of the trigeminal began to fall between 28 and 31 months of age (Sturrock, 1987). In the indusium griseum (Sturrock, 1986), the lateral mamillary nucleus and the anterodorsal nucleus of the thalamus (Sturrock, 1989), all components of the limbic system, neuron number remained constant up to 31 months of age. There is evidence in the external cuneate nucleus of neuronal degeneration as early as 15 months of age. The neurons in the nuclei previously examined which lost neurons were either motor neurons or primary sensory neurons and the loss of neurons was thought to be secondary to degeneration of their peripheral targets (Sturrock, 1988*a*, *b*). The small number of degenerating neurons found in these nuclei were structurally different from the pyknotic neurons found in the developing brain (Sturrock, 1979) and were similar to some stages of the degenerating neurons in the

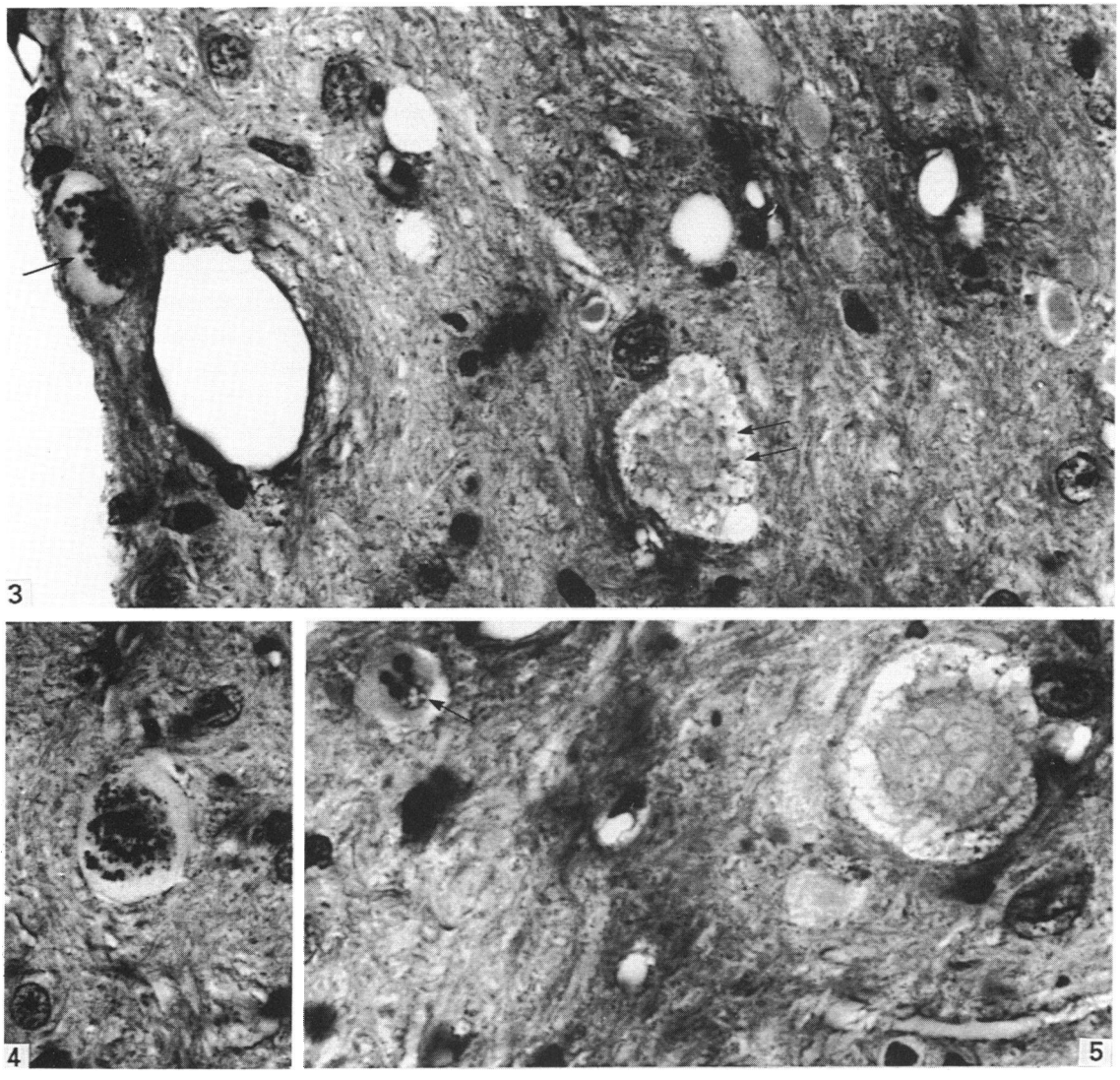


Fig. 3. Parasagittal 6 μm section, 28 months mouse. There is a neuron with a disintegrating nucleus lying adjacent to the IVth ventricle (arrow). A number of small colloid-like degenerating neurons are visible, as is a larger degenerating neuron containing a green staining central mass (double arrows). $\times$ 1000.

Fig. 4. Parasagittal, 6 μm section. Neuron with a degenerating nucleus from a 28 months mouse. $\times$ 1000.

Fig. 5. Parasagittal, 6 μm section. The degenerating neuron on the right has a green-staining mass containing circular inclusions surrounding by sparsely scattered, red-staining filaments. The degenerating neuron on the left (arrow) has an amorphous, pink staining background containing small, dark blue staining spherical bodies. $\times$ 1250.

external cuneate nucleus. It was suggested (Sturrock, 1988*a*, *b*) that the difference in appearance between degenerating neurons in the developing and ageing brain represents the difference between programmed cell death or apoptosis (Wyllie, Kerr & Currie, 1980) and cell death due to changes in the environment referred to as necrosis by Wyllie *et al.* (1980).

The percentage of degenerating neurons should be treated with some caution since

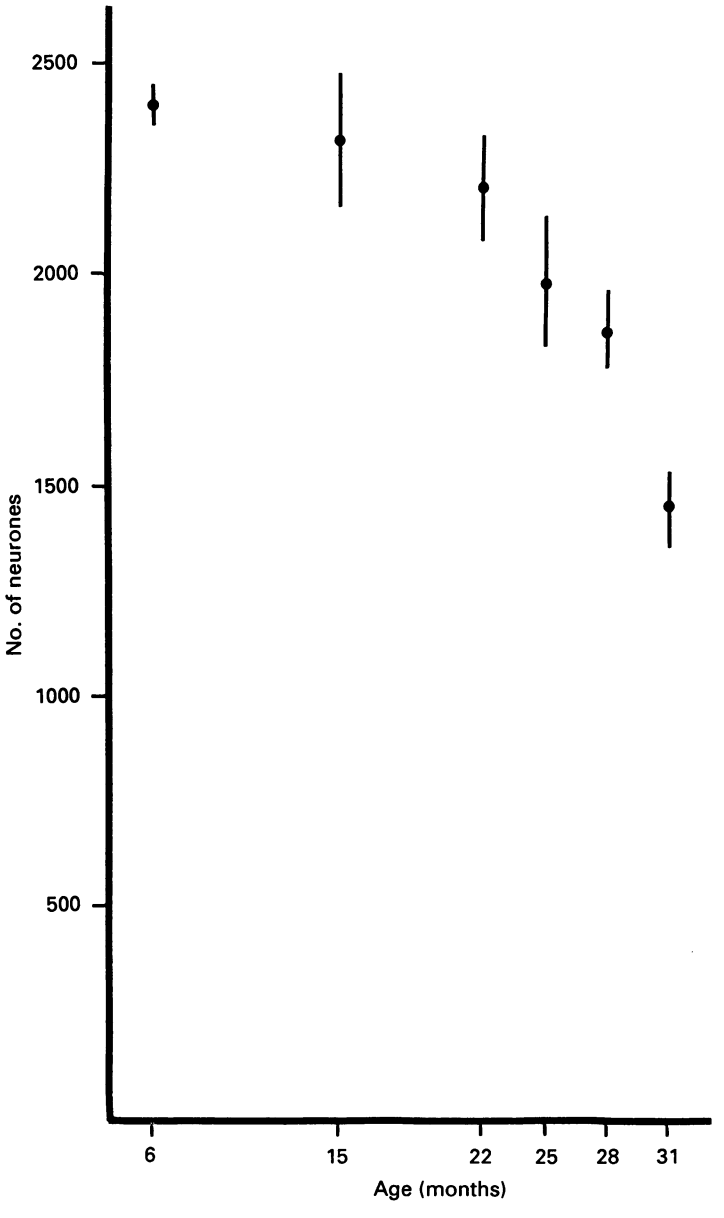


Fig. 6. Estimated changes in neuron number in the external cuneate nucleus (±S.E.M.) between 6 and 31 months of age.

degenerating neurons vary widely in size and whole degenerating neurons were counted but the percentage was calculated without any correction factor being applied to the number of neurons counted. The variation in size of degenerating neurons is probably similar at each age examined and any resultant error is therefore likely to be similar at each age. The fact that there is no variation in the percentage of degenerating neurons with age indicates that removal of neuronal debris by phagocytosis keeps pace with neuronal degeneration.

The external cuneate nucleus receives proprioceptive input from the cervical and

Table 1. *Estimated mean neuronal nuclear diameter and the percentage of degenerating neurons* (S.E.M.) *in the external cuneate nucleus between* 6 *and* 31 *months of age*

	Age (months)					
	6	15	22	25	28	31
Nuclear diameter (μm)	10·5±0·1	10·6±0·1	10·2±2·2	10·4±0·6	10·9±0·3	10·7±0·1
Degenerating neurons (%)	—	1·3±0·2	2·0±0·6	1·2±0·4	1·3±0·3	4·4±3·0

upper thoracic spinal cord (Rustioni & Macchi, 1968; Basbaum & Hand, 1973) and from several cranial nerve nuclei (Sobusiak *et al.* 1972). If the pattern of loss of primary proprioceptive neurons preceding loss of associated motor neurons, which is found in the trigeminal system (Sturrock, 1987), is repeated throughout the rest of the nervous system, then proprioceptive neurons in the dorsal root ganglia should decrease in number between 12 and 25 months in concert with loss of anterior horn neurons (Wright & Spink, 1959). During normal ageing, loss of muscle tissue is known to precede loss of motor neurons (Gutmann & Hanzlikova, 1975) and spontaneous motor activity in mice declines by 23 % between 6 and 12 months and by 40 % between 6 and 30 months (Strong *et al.* 1980). It seems reasonable to suppose that such a diminution in motor function would be accompanied by a loss of primary proprioceptive neurons and that the subsequent loss of neurons from the external cuneate nucleus is due to anterograde transneuronal degeneration.

Since a decrease in number of motor neurons is found in the spinal cord between 12 and 25 months (Wright & Spink, 1959) but not in the brainstem until after 25 months of age (Sturrock, 1987, 1988*a*, *b*) it is possible that the initial loss of external cuneate neurons is due in the first instance to loss of spinal input and the later, more rapid, fall in neuron number is due to loss of brainstem input added to a continuing fall in spinal input.

The results of the present study are consistent with the hypothesis that loss of neurons from nuclei of the central nervous system is secondary to degeneration of peripheral targets, such as muscle, since in regions without direct peripheral input, such as the indusium griseum (Sturrock, 1986), lateral mamillary nucleus and anterodorsal nucleus (Sturrock, 1989) neuron number remains stable even in extreme old age.

SUMMARY

The number of neurons in the ASH/TO mouse external cuneate nucleus was examined at 6, 15, 22, 25, 28 and 31 months of age. There is a highly significant decrease in the number of neurons between 6 and 31 months of age. Neuron loss begins at 15 months when degenerating neurons are first found in the nucleus and becomes more rapid with increasing age. It is suggested that neuronal loss is due to anterograde transneuronal degeneration following a loss of primary proprioceptive neurons, first from the spinal roots and later from the brainstem.

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