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Stability of neuron number in the subthalamic and entopeduncular nuclei of the ageing mouse brain

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INTRODUCTION

A recent series of investigations of age-related changes in neuron number in the cerebellum and in brainstem nuclei, projecting to and receiving projections from the cerebellum, have shown a chronological sequence of neuron loss with age. A decrease in neuron number begins in the external cuneate (Sturrock, 1989*a*) and pontine nuclei (Sturrock, 1990*a*) between 15 and 22 months of age and is accompanied by a loss of Purkinje cells (Sturrock 1989*b*, 1990*b*) and stellate and basket cells (Sturrock 1989*b*) from the cerebellar cortex. This is followed by a decrease in number of neurons from the intracerebellar nuclei beginning between 22 and 25 months of age (Sturrock, 1989*c*) which precedes the loss of neurons from the red nucleus (Sturrock, 1990*c*) and lateral vestibular nucleus (Sturrock, 1989*d*), which begins between 25 and 28 months of age. In both the red nucleus and lateral vestibulospinal nucleus, neuron loss is largely restricted to the large neurons which give rise to the rubrospinal and lateral vestibulospinal tracts. In the cerebellar system, therefore, neuron fallout begins in nuclei projecting to the cerebellum and eventually results in loss of neurons from motor nuclei which receive a large cerebellar input.

Motor activity is also greatly influenced by the activities of the basal ganglia, although in a less direct manner. Quantitative histological investigations of age-related changes in the basal ganglia have tended to concentrate on the substantia nigra because of the importance of this nucleus in the aetiology of parkinsonism, but the results of such studies are the source of controversy. Some authors have reported a decrease in dopaminergic neurons with age (McGeer, McGeer & Suzuki, 1977; Mann & Yates, 1983) whilst others have found no age-related changes in neuron number (Pakkenberg & Brody, 1965; Bogerts, Hantsch & Herzer, 1983; Gibb & Lees, 1987; McNeill, Koek & Haber, 1988). Some of these differences may be species related as there are subtle differences in the connections of the basal ganglia between rodents and primates (Evarts & Wise, 1984) but some are methodological. McGeer *et al.* (1977) did not apply any correction factor to take into account neuronal shrinkage with age, and indeed McGeer, Itagaki, Akiyama & McGeer (1988) recently stated that if a correction had been applied it would decrease any age-related change.

There are some important differences in basal ganglia nomenclature and circuitry between rodents and primates. In primates the globus pallidus consists of a medial and lateral segment, whereas in rodents the primate lateral segment is represented by the globus pallidus and the medial segment by the entopeduncular nucleus (Nauta & Domesick, 1984). Whereas in primates the major output of the medial segment is to the thalamus, in rodents the projection from the entopeduncular nucleus to the

thalamus is much smaller and the major projection is to the lateral habenular nucleus (Herkenham & Nauta, 1977). In both rodents and primates the subthalamic nucleus receives a massive projection from the globus pallidus, or lateral segment, and in turn projects both to the globus pallidus and entopeduncular nucleus (in rodents) or lateral and medial segments (in primates) (Nauta & Domesick, 1984). The subthalamic nucleus also receives somatotopically arranged afferents from the motor cortex (Afsharpour, 1985; Toledano & Crossman, 1991). As well as the projection from the subthalamic nucleus the entopeduncular nucleus also receives fibres from the neostriatum (Nauta & Domesick, 1984).

In a recent study McNeill and Koek (1990) have shown that there is no variation in the number of dopaminergic neurons in the pars compacta of the substantia nigra between 6 and 30 months of age in a strain of mice with a similar lifespan to the ASH/TO strain. The present study set out to examine the effect of ageing on neuron number in the subthalamic and entopeduncular nuclei has in view of their central position in the basal ganglia complex.

MATERIALS AND METHODS

The material used in this study consisted of sets of 6 μm sagittal and 6 μm coronal serial sections of brains from male ASH/TO strain mice aged 6, 15, 25, 28 and 31 months. The methods of fixation and staining have already been described in detail (Sturrock, 1987). Three sets of sections were examined at each age.

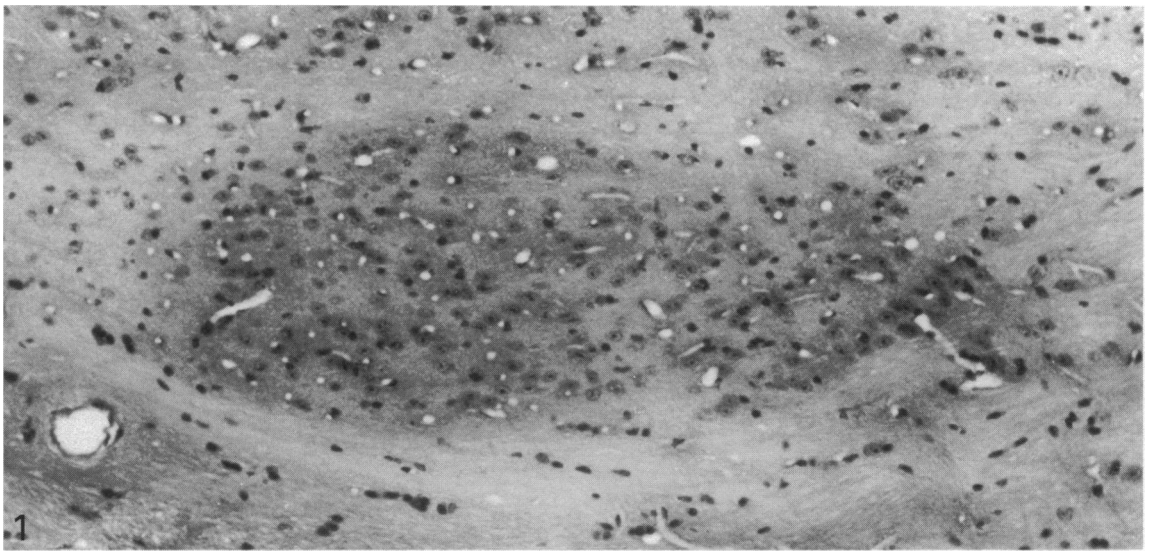
The subthalamic nucleus and entopeduncular nucleus were identified in each set of sagittal sections, the medial and lateral boundaries of each were determined and the number of sections containing each nucleus was recorded. Every 10th section was used for neuron counts. In order to ensure random sampling a number R between 1 and 10 was obtained from a random number table and the number of neurons in the Rth section and every 10th section thereafter was used for counts. The number of neuronal nuclei was counted at $\times 250$ magnification using an eyepiece graticule. Mean transverse neuronal nuclear diameter was estimated as described previously (Sturrock, 1987). The total number of neurons in each subthalamic and entopeduncular nucleus was estimated by multiplying the number counted by the total number of sections containing the nucleus, dividing by the number of sections used for counting and applying the correction formula of Abercrombie (1946).

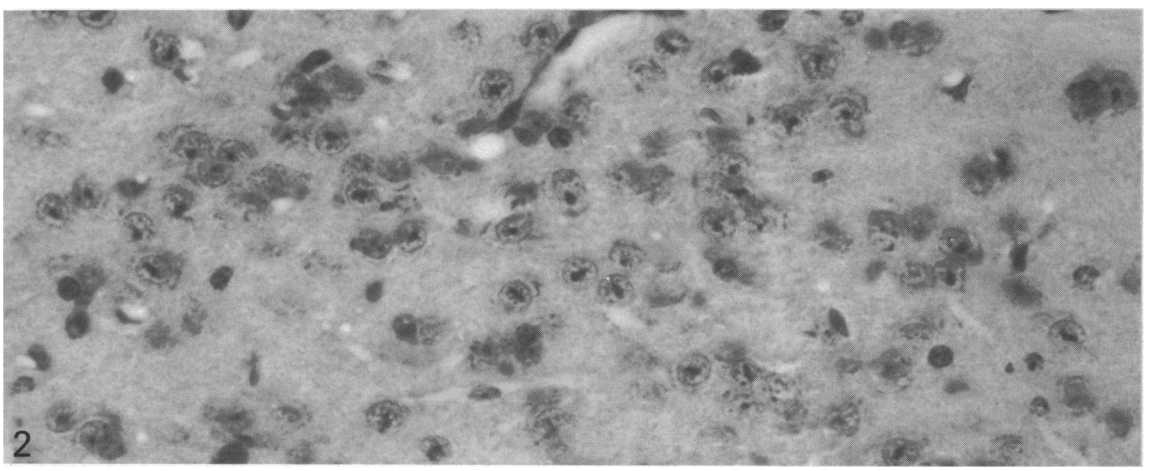
The results of neuron number estimations a different ages and of the number of sections containing each nucleus at different ages were subjected to analyses of variance.

RESULTS

In sagittal sections the lateral part of the subthalamic nucleus is easily recognised due to its lens shaped structure (Fig. 1) which finally merges laterally with the internal capsule. In a medial direction the nucleus becomes progressively more circular in outline and ends as a small tightly packed group of small neurons lying about 200 μm rostral to the pars compacta of the substantia nigra. In material stained with Lapham's stain (Lapham, Johnstone & Brundjar, 1964), the neuropil of the subthalamic nucleus is magenta coloured, indicating the presence of numerous myelinated axons and aiding identification of the nucleus, particularly in the most medial parts of the nucleus which contain relatively few neurons.

Subthalamic neurons are small (mean nuclear diameter 10 μm) with a thin rim of cytoplasm. A peripheral band of Nissl substance is occasionally visible. Neuron





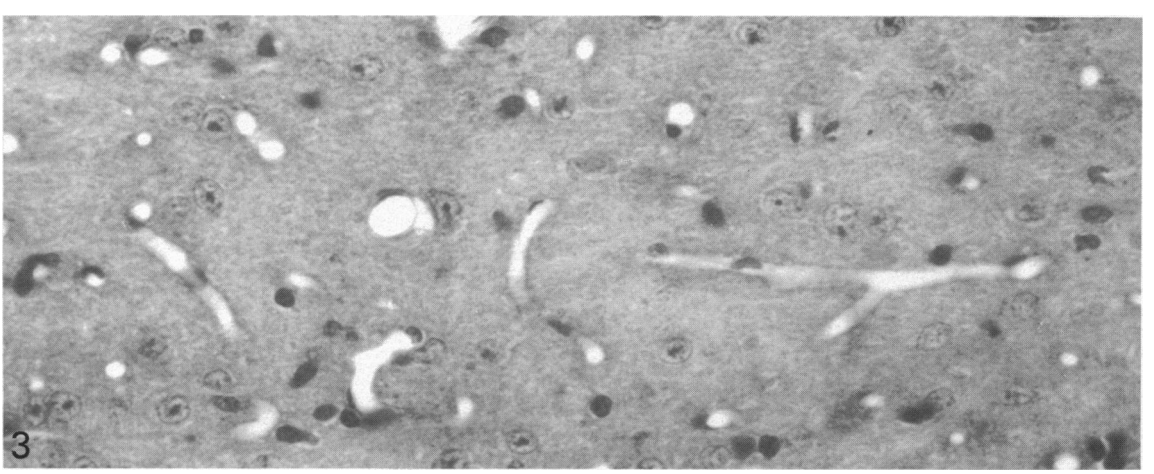


Fig. 1. Laterally the subthalamic nucleus is easily identified as a lens-shaped structure with a darkly staining neuropil. Lapham's stain, × 125.

Fig. 2. At 6 months of age neurons in the subthalamic nucleus are tightly packed. × 500.

Fig. 3. At 31 months of age the neurons appear sparsely scattered throughout the subthalamic nucleus. × 500.

Table 1. *Estimated total number of neurons (±SEM) in the subthalamic and entopeduncular nuclei and the mean number of sections (±SEM) containing each nucleus between 6 and 31 months of age*

Age (months)	Subthalamic nucleus		Entopeduncular nucleus	
	No. of neurons	No. of sections	No. of neurons	No. of sections
6	5288±156	133±6	1120±9	112±9
15	5293±48	138±2	1130±124	98±1
25	5571±292	164±8	1054±44	103±7
28	5427±473	172±7	1101±50	112±1
31	5251±189	190±15	1099±29	115±2

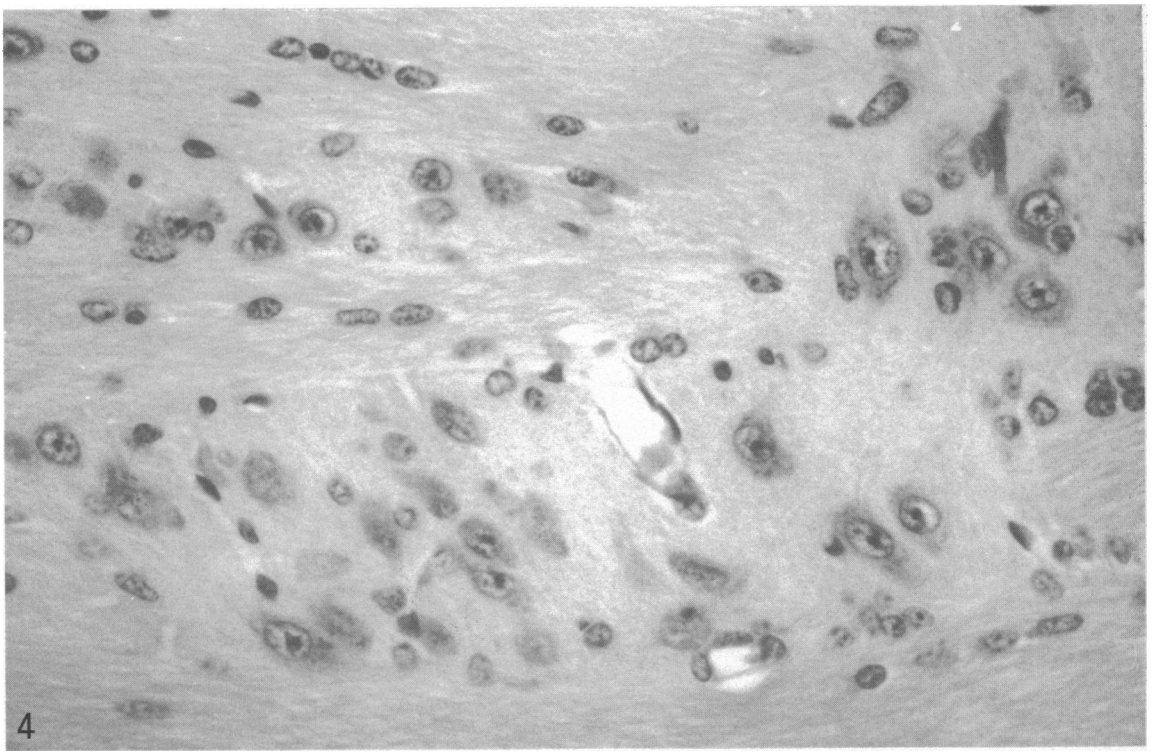


Fig. 4. Entopeduncular neurons are loosely packed within the internal capsule (6-month-old mouse. ×500).

morphology does not change with age. Lipofuscin accumulation is not a feature of ageing subthalamic neurons. At 6 months of age, the neurons are closely packed (Fig. 2). The most striking structural change with age in the subthalamic nucleus is that the neurons become much more widely scattered throughout the nucleus (Fig. 3). This change in packing density is first apparent between 15 and 25 months of age which coincides with a progressive increase in the number of sections containing the nucleus (see Table 1). The increase in the number of sections is statistically significant ($F(4, 10) = 7{\cdot}48$; $P < 0{\cdot}01$).

The entopeduncular nucleus lies within the internal capsule (Fig. 4). Entopeduncular neurons are structurally similar to subthalamic neurons and also exhibit no marked morphological changes with age. Unlike the subthalamic nucleus the number of sections containing the entopeduncular nucleus does not change significantly with age ($F(4, 10) = 1{\cdot}91$; ns) (Table 1), nor is there any obvious decrease in neuronal packing density.

There is no significant variation with age in the number of neurons in either the subthalamic nucleus ($F(4, 10) = 0{\cdot}24$; ns) or entopeduncular nucleus ($F(4, 10) = 0{\cdot}21$; ns).

DISCUSSION

Neither the subthalamic nucleus nor the entopeduncular nucleus shows any evidence of neuron loss with age and morphological changes, such as lipofuscin accumulation are minimal. McNeill & Koek (1990) found no evidence of loss of dopaminergic neurons from the pars compacta of the substantia nigra between 60 and 30 months in a strain of mice with a similar lifespan to the ASH/TO strain. They did, however, report a significant loss of cholinergic neurons from the neostratium between 25 and 30 months of age and a similar loss of neostriatal cholinergic neurons was also found by Altavista *et al.* (1988). The large cholinergic neurons make up only 1–2% of the neostriatal population and function as interneurons (Dray, 1979). McNeill and Koek (1990) suggested that the loss of these neurons leads to proliferation of dendrites of medium spiny I striatal neurons rather than to a decrease in number of any other type of striatal neuron.

From data obtained during an earlier study of neostriatal ageing (Sturrock, 1986) it was possible to make a tentative estimate of total neuron number in that part of the neostriatum lying rostral to the anterior commissure. It should be emphasised that those calculations are very approximate but nevertheless the estimated number of neostriatal neurons at 6 months is $111\,110 \pm 7120$ compared with $126\,970 \pm 10\,130$ at 31 months. Such estimates are obviously not capable of picking up a significant loss of neurons forming only 1–2% of the population but they do suggest that there is no major loss of medium-sized neostriatal projection neurons which make up most of the neostriatal population.

The stability of neuron number in different parts of the basal ganglia contrasts with the sequential loss of neurons from the cerebellar system with age as described in the Introduction. The first parts of the cerebellar system to lose neurons with age are the nuclei which project to the cerebellum. The onset of neuron loss and the subsequent rate of neuron loss appear to be similar in the external cuneate nucleus (Sturrock, 1989*a*) and the pontine nuclei (Sturrock, 1990*a*). The external cuneate nucleus receives input from the cervical and upper thoracic region of the spinal cord and neuronal fallout in the external cuneate nucleus could be a consequence of the loss of peripheral input. The pontine nuclei constitute the most important relay pathway from the cerebral cortex to the cerebellar cortex (Mihailoff, Watt & Burne, 1981), receiving fibres from the ipsilateral sensorimotor cortex and from the visual cortex. As well as cortical afferents the pontine nuclei receive afferents from the deep cerebellar nuclei (Brodal, 1968; Swenson, Kosinski & Castro, 1984), from the dorsal column nuclei (Kosinski, Neafsey & Castro, 1986), from the superior and inferior colliculi (Altman & Carpenter, 1961; Kawamura, 1975) from the spinal cord and from the spinal trigeminal nucleus (Swenson *et al.* 1984).

The subthalamic nucleus receives a large projection from the globus pallidus (Nauta & Domesick, 1984) and a somotatopically arranged input from the sensorimotor

cortex (Afsharpour, 1985) and anterior cingulate cortex (Toledano & Crossman, 1991). If the loss of neurons from the pontine nucleus was due to loss of neurons from the sensorimotor cortex, the subthalamic nucleus might have been expected to have been similarly affected. The stability of neuron number in the subthalamic nucleus could be due to the continuing large input from the globus pallidus protecting subthalamic neurons, or the cortical input could be from different parts of the sensorimotor cortex than those supplying the pontine nuclei. Another possible explanation is that loss of pontine nuclei neurons is the result of loss of input from other regions apart from the cortex. It is known, for example, that in ASH/TO mice neuron degeneration is visible in the superior colliculus from 6 months of age onwards (Sturrock, 1989*e*).

The entopeduncular nucleus receives input from the subthalamic nucleus and neostriatum (Nauta & Domesick, 1984), neither of which show evidence of loss of projection neurons with age. The stability of neuron number in the basal ganglia may be due to their having no direct connections with spinal or medullary centres, unlike the cerebellum which receives a wide variety of input from the spinal cord, vestibular system and reticular formation.

The large increase in the number of sections containing the subthalamic nucleus and the consequent dispersion of neurons within the nucleus is difficult to explain. No such increase in size was found in the entopeduncular nucleus which lies in close proximity to the subthalamic nucleus. This suggests that the increase in size is not an artefact caused by the ageing brain responding differently to fixation. Of the 28 other nuclei counted in the same sets of sections, none showed evidence of a similar progressive increase in size with age which also seems to exclude the increase in size being an artefact. The staining characteristics of the neuropil of the subthalamic nucleus indicate that it contains a large proportion of myelinated axons and it is possible that the increase in size of the nucleus is the result of an increase in the size or number of myelinated axons although neither of these explanations appears likely.

SUMMARY

The subthalamic and entopeduncular nuclei of mice aged 6, 15, 25, 28 and 31 months were examined in parasagittal 6 μm sections using quantitative histological techniques. The mean number of neurons in the subthalamic (overall mean 5366) and entopeduncular (overall mean 1101) nuclei did not vary significantly with age. The number of sections containing the subthalamic nucleus increased progressively in number with age with the increase beginning between 15 and 25 months. The increase in size of the nucleus was accompanied by a decrease in neuron packing density. No similar changes were observed in the entopeduncular nucleus despite its close anatomical and functional relationships to the subthalamic nucleus.

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