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Stability of neuron and glial number in the abducens nerve nucleus of the ageing mouse brain

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INTRODUCTION

Neuron number in the mesencephalic nucleus of the mouse trigeminal nerve declines after 25 months of age and this is followed by a probable decrease in the number of neurons in the trigeminal motor nucleus between 28 and 31 months (Sturrock, 1987). The facial nerve nucleus loses neurons after 25 months of age (Sturrock, 1988*a*) and in both motor nuclei the fall in neuron number is preceded, or accompanied, by a statistically significant increase in diameter of neuronal nuclei and loss of Nissl substance.

Whilst the majority of neurons of the mesencephalic nucleus of the trigeminal nerve synapse on neurons of the trigeminal motor nucleus, a small number may be associated with the nuclei of the cranial nerves controlling eye movements (Cooper, Daniel & Whitteridge, 1953) and because of this it was decided to examine one of these small motor nuclei to determine whether they too showed evidence of neuronal fallout in old age. The abducens nerve nucleus was selected for examination because it is easy to identify due to its close anatomical relationship to the genu of the facial nerve. The abducens nucleus is also probably unique among motor nuclei because a large number of neurons within it send their axons to another motor nucleus, the oculomotor, rather than to muscle (Carpenter & Batton, 1980). These interneurons may comprise up to 50% of the population and it is impossible to distinguish them from motor neurons on morphological grounds (Spencer & Sterling, 1977).

MATERIALS AND METHODS

The material consisted of sets of 6 μm coronal and sagittal serial sections of brain from mice aged 6, 25, 28 and 31 months killed by perfusion fixation with Bouin's solution under sodium pentobarbitone anaesthesia. After fixation the brains were bisected in the midsagittal plane. The right halves were sectioned in the parasagittal plane and stained with Lapham's stain (Lapham, Johnstone & Brundjar, 1964) whilst the left halves were sectioned in the coronal plane and stained with haematoxylin and eosin. Full details of the method of fixation and subsequent processing have already been described (Sturrock, 1987). Three sets of coronal and sagittal sections were available at each age.

The coronal sections were used for neuron and glia counts and the sagittal sections were used to measure the mean nuclear diameter of neurons and glia using an identical method to that previously used to estimate nuclear diameter in the mesencephalic and motor nuclei of the trigeminal nerve (Sturrock, 1987).

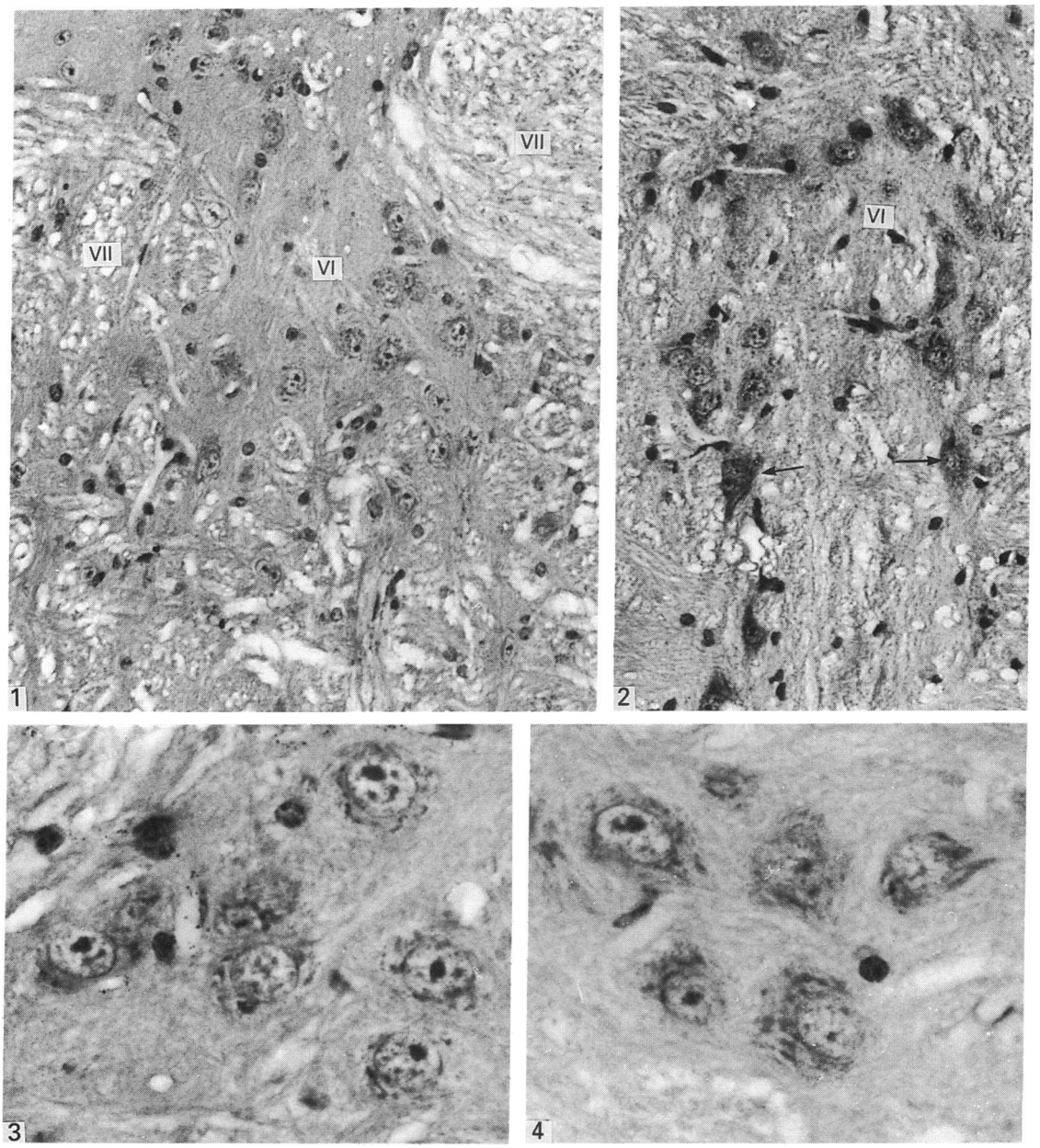

Fig. 1. Coronal section. In sections in this plane the abducens nucleus (VI) consists of loosely scattered motor neurons closely related to fibres of the genu of the facial nerve (VII). H and E. × 315.

Fig. 2. Sagittal section. In the part of the abducens nucleus (VI) where nerve fibres leave it, motor neurons (arrows) lie alongside nerve fibres outside the boundaries of the nucleus. Lapham's stain. × 315.

Fig. 3. Coronal section. Abducens motor neurons from a 6 months old mouse. H and E. × 900.

Fig. 4. Coronal section. Abducens motor neurons from a 31 months old mouse. These neurons are identical to those at 6 months of age. H and E. × 900.

Table 1. *Estimated number of neurons and glia (±S.E.M.) and the glia to neuron ratio in the mouse abducens nucleus at 6, 25, 28 and 31 months of age*

Age in months	Mean number of neurons	Mean number of glia	Glia to neuron ratio
6	131±15	184±12	1·43±0·10
25	104±7	152±10	1·46±0·01
28	113±7	160±11	1·42±0·03
31	122±10	172±10	1·41±0·05

The rostral and caudal boundaries of the abducens nerve nucleus were identified in each set of coronal sections and the number of neuronal and glial nuclei in every fifth section was recorded using a haematological counter. The total number of neurons and the total number of glia was estimated by multiplying the number of each type of cell counted by the number of sections containing the abducens nucleus, dividing by the number of sections examined and correcting the resulting figures using Abercrombie's correction formula (Abercrombie, 1946). The estimated totals at each age were subjected to an analysis of variance, as was the glia to neuron ratio.

RESULTS

The abducens nucleus consists of a small group of scattered motor neurons partially surrounded by the genu of the facial nerve (Fig. 1). Some neurons lie outside the inferior boundary of the nucleus alongside fibres of the abducens nerve (Fig. 2). Unlike motor neurons of the facial and trigeminal nerves, the neurons of the abducens nucleus show no evidence of loss of Nissl substance with age (Figs. 3, 4) but Nissl substance is not such a prominent cytoplasmic constituent in abducens nuclei neurons, even at 6 months of age, as it is in neurons of the facial and trigeminal motor nuclei of the same brains (Sturrock, 1987, 1988*a*). Only a few small lipofuscin granules are present in the perikarya of neurons of the abducens nucleus at 31 months of age and these are usually only apparent when the sections are examined under oil immersion.

The results of the counts of neurons and glia are given in Table 1. There is no statistically significant variation in neuron number ($F(3,8) = 1{\cdot}18$; NS), neuroglial number ($F(3,8) = 1{\cdot}64$; NS), or in the glia to neuron ratio ($F(3,8) = 0{\cdot}12$, NS). There is no difference either in mean neuronal (13·0 μm) or mean glial nuclear diameter (6·2 μm) with age.

DISCUSSION

Unlike the facial nucleus, and to a lesser extent the trigeminal motor nucleus, the mouse abducens nucleus shows no sign of neuronal loss even in extreme old age. Neither is there any evidence of cytological changes with age in abducens neurons at the light microscopic level, except for a very minimal accumulation of lipofuscin. In contrast motor neurons of the facial and trigeminal motor nuclei lose Nissl substance between 6 and 25 months of age and neuronal nuclei in both increase significantly in diameter between 25 and 28 months of age. Motor neurons in the facial nerve accumulate a moderate amount of lipofuscin with age but those of the trigeminal nucleus do not (Sturrock, 1987, 1988*a*). General visceral efferent neurons of the retrofacial nucleus also lose Nissl substance with age and large amounts of lipofuscin are present in their perikarya from 25 months of age. Neuronal nuclei of the retrofacial

nucleus do not increase in diameter with age (Sturrock, 1988*b*) although neuron number falls between 25 and 31 months of age.

Nuclei of the cranial nerves supplying the extrinsic muscles of the eye differ embryologically from those of the facial and trigeminal motor nuclei in that the former are somatic motor nuclei whereas the latter are branchiomotor. It seems unlikely that this embryological difference is responsible for the stability of neuron number in the abducens nucleus since somatic motor neurons are lost from the mouse spinal cord between 12 and 25 months of age (Wright & Spink, 1959).

Previous investigations in this series have shown that a loss of proprioceptive input precedes loss of the corresponding motor neurons (Sturrock, 1987, 1989*a*,*b*) and Gutmann & Hanzlikova (1975) have shown that loss of muscle tissue precedes loss of motor neurons during normal ageing. In the mouse there is a 40% reduction in motor activity between 6 and 30 months of age (Strong *et al.* 1980) and Hooper (1981) has demonstrated a loss of muscle fibres with age in various muscles of the mouse which is most marked in the hind limbs and appears to proceed in a caudorostral direction. This could reflect relative degrees of reduction in motor activity.

The extrinsic muscles of the eye are possibly the most active of all skeletal muscles, since when the animal (or man) is awake, if they are not moving the eyes under conscious control, they are involved in spontaneous saccadic movements (Dodge, 1903) which are probably initiated by the abducens system (Miyoshi, Hinatashi, Kishimoto & Tamada, 1981). For a large proportion of the time during sleep, when other skeletal muscles are totally relaxed, the extrinsic eye muscles are producing rapid eye movements (Jouvet, 1967). This high degree of muscle activity is the result of a corresponding high degree of activity in the motor nuclei, initiated by input to the nuclei from both the frontal eye fields of the cortex and the visual association cortex mediated via the paramedian pontine reticular formation and directly from the vestibular nuclei and the reticular formation (Carpenter & Sutin, 1983). Since all the above movements involve conjugate eye movements, both motor neurons and interneurons in the abducens nucleus will be involved. The high degree of activity of extrinsic eye muscles may prevent loss of muscle fibres, which in turn, prevents loss of motor neurons.

The glia to neuron ratio is much lower in the abducens nucleus than in either the facial or trigeminal nucleus in both of which the glia to neuron ratio is around six to one (Sturrock, 1988*c*). Neurons in the abducens nucleus are less widely scattered than those in the facial and trigeminal motor nuclei, indicating a relatively smaller neuropil. It was postulated that a large number of glia in the latter pair of nuclei might be associated with capillaries and myelin sheaths in the neuropil rather than acting as neuronal satellites. Although the abducens nucleus contains many myelinated axons (Spencer & Sterling, 1977) there appear to be fewer capillaries in the abducens nucleus than in the facial and trigeminal motor nuclei. If glia do play an important part in neuronal metabolism, it is surprising that the glia to neuron ratio is not higher in the abducens nucleus in which the neurons are almost certainly more active than in motor nuclei not supplying extrinsic eye muscles.

SUMMARY

The number of neurons and glia in the mouse abducens nerve nucleus was estimated at 6, 25, 28 and 31 months of age. There was no significant variation in either the number of neurons (mean 118) or glia (mean 168) or in the glia to neuron ratio (mean 1·42) with age. Mean neuronal and glial nuclear diameters also remained constant and

there was no obvious loss of Nissl substance and only a minimal accumulation of lipofuscin in neuronal perikarya with age.

The apparent lack of age-related changes in neurons of the abducens nucleus could be a consequence of the high degree of motor activity in the extrinsic eye muscles during both the waking and sleeping states.

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