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Ultrastructure of interneurons within motor nuclei of the thoracic region of the spinal cord of the adult cat

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INTRODUCTION

Classically, the localisation of spinal motoneurons has relied heavily on their retrograde chromatolytic response to peripheral axotomy. Such studies have revealed that their cell bodies are arranged in discrete columns which, in transverse sections, form the motor nuclei and whose precise location in the ventral horn varies according to which muscles the motoneurons innervate (Goering, 1928; Reed, 1940; Rexed, 1952; Romanes, 1951, 1964; Sprague, 1951; Keswani, Groat & Hollinshead, 1954; Keswani & Hollinshead, 1956; Warwick & Mitchell, 1956; Biljani & Keswani, 1961; Sterling & Kuypers, 1967). Another common finding, however, is that many neurons in an apparent motor nucleus may not exhibit chromatolysis following axotomy. Notwithstanding possible variation in the retrograde response of individual motoneurons to axotomy (Lieberman, 1974), this vagary of the chromatolytic method calls into question the view that all neurons in a motor nucleus are motoneurons.

Neuronal cell bodies of various sizes are seen within the territory of motor nuclei. Under suitable conditions almost all the large cell bodies can be made to exhibit chromatolysis following peripheral nerve injury and they are therefore considered to be motoneurons. Studies of electrophysiologically-identified motoneurons after intracellular labelling with horseradish peroxidase (Brown & Fyffe, 1981; Ulfhake & Kellerth, 1981; Zwaagstra & Kernell, 1981; Lipski & Martin-Body, 1987) have further identified large motoneurons as alpha motoneurons and their morphology is well documented (Conradi, 1969, 1976). Much less is known about the smaller neurons within motor nuclei. Some have been identified as motoneurons by their chromatolytic response to peripheral nerve injury (Nyberg-Hansen, 1965) and combined morphological and electrophysiological studies have further identified some of them as gamma motoneurons (Westbury, 1979; Ulfhake & Cullheim, 1981). More recently, the ultrastructure of gamma motoneurons after retrograde labelling with horseradish peroxidase has been described (Johnson, 1985, 1986; Lagerbäck, 1985), but for the remaining small neurons within motor nuclei there is no comparable morphological data.

On the premise that large motoneurons give rise to large axons, the fibre calibre spectra of deafferented limb (Eccles & Sherrington, 1930) and intercostal (Sears, 1964*a*) nerves indicate that alpha motoneurons outnumber gamma motoneurons by about 3:1, yet anatomical studies of motor nuclei (Balthazar, 1952; Schadé & Van Harreveld, 1961) indicate that small neurons outnumber large ones by a similar amount. One explanation of this paradox is that interneurons are present within motor nuclei and that they are of small diameter, as is the case for Renshaw cells and

Ia inhibitory interneurons located adjacent to motor nuclei (Jankowska & Lindström, 1971, 1972; Lagerbäck & Kellerth, 1985; Van Keulen, 1979). Indeed, there is evidence from Golgi-impregnated material (Balthazar, 1952; Cajal, 1952; Matsushita, 1969; Scheibel & Scheibel, 1966, 1969) that interneurons can be found within limb motor nuclei and similarly located interneurons in the thoracic region of the spinal cord of the cat have recently been demonstrated electrophysiologically (Kirkwood, Munson, Westgaard & Sears, 1987).

From a functional viewpoint, recognition of the interneuronal contribution to motor nuclei is important as there is much electrophysiological evidence, from the work of Lundberg and his school, that interneurons interposed in segmental reflex arcs are major sites of convergence of many central and peripheral afferents (Baldissera, Hultborn & Illert, 1981). Indeed, along with alpha and gamma motoneurons, these interneurons form an integral component of the concept of an 'output stage' in the spinal control of movement (Hultborn, Lindström & Wigström, 1979).

To allow putative interneurons to be identified in this study, horseradish peroxidase has been used as an independent retrograde marker of motoneurons, but with attention directed specifically towards those small neurons in the motor nuclei which were unlabelled. It has been assumed that these unlabelled neurons represent a population which is primarily interneuronal. Transsynaptic retrograde labelling of interneurons (Harrison *et al.* 1984; Jankowska, 1985; Appenteng & Girdlestone, 1987) using horseradish peroxidase conjugated to wheat germ agglutinin would be unsuitable for the present purposes, since this technique requires the labelled interneurons to be located outside the motor nuclei in order for them to be distinguished unambiguously from similarly labelled motoneurons.

The aim of the study is to determine whether morphological criteria can be defined which allow interneurons to be distinguished from motoneurons in the motor nuclei of the thoracic region of the spinal cord in the adult cat.

MATERIALS AND METHODS

Tissue was obtained from 3 adult cats that had formed part of a previous study of alpha and gamma motoneurons after retrograde labelling with horseradish peroxidase (Johnson, 1986). Anaesthesia was induced by the intraperitoneal injection of sodium pentobarbitone (45 mg/kg). Full details of the retrograde labelling procedure, morphometric methods and definitions of organelles have been given (Johnson, 1986) and only a brief description is given here.

Labelling with horseradish peroxidase

In anaesthetised cats, either the levator costae muscle, the proximal 15 mm of the external intercostal muscle, or both these muscles were injected with 2–10 μl of 40% horseradish peroxidase in saline. Animals were perfused 24 hours later with 2% glutaraldehyde–1% paraformaldehyde in 0·1 M sodium phosphate buffer, pH 7·4.

Tissue processing

Segments of fixed spinal cord from T8, T9 or T10 were sectioned in phosphate buffer at 70 μm using a Vibroslice (Campden Instruments, London) and processed to demonstrate peroxidase activity (Adams, 1977). Slices were then treated with 1% osmium tetroxide, dehydrated and flat-embedded in Araldite between two poly-tetrafluoroethylene-coated microscope slides.

Microscopy

Analysis was carried out on tissue blocks which had previously been prepared to enable retrogradely labelled alpha (> 40 μm diameter) and gamma (< 30 μm diameter) motoneurons to be studied in the light and electron microscope (Johnson, 1986). These blocks contained one or more labelled motoneurons in the centre of a mesa of approximately 0·5–0·7 mm sides. In this study, however, attention was directed specifically towards those neurons of < 30 μm diameter which were unlabelled by horseradish peroxidase. The dimensions of the mesas and the fact that complete neuronal profiles were rarely preserved at the edges of the ultrathin sections when uncoated 200-mesh grids were used, allowed the position of the unlabelled, small neurons to be defined operationally as within 200–300 μm of labelled motoneurons in the external intercostal and levator costae motor nuclei.

Using a plotting tablet to measure lengths and areas, a quantitative analysis was carried out on electron micrograph photomontages (final magnification × 19000) of the cell bodies of 10 unlabelled neurons. Those features measured were: The frequency of occurrence of lysosomes, mitochondria, Nissl bodies and profiles of the Golgi apparatus (number/100 μm^2 cytoplasm); the proportion of the cytoplasm occupied by Nissl bodies and Golgi profiles (area organelle/100 μm^2 cytoplasm); the frequency of occurrence of nuclear pores (number/100 μm nuclear membrane); the frequency of occurrence of synaptic terminals with either round or flattened synaptic vesicles (number/100 μm plasma membrane) and the coverage of the cell body by these synaptic terminals (total length of synaptic terminal apposition/100 μm plasma membrane). Values for unlabelled neurons were compared with those previously obtained (Johnson, 1986) for 10 alpha (> 40 μm) and 20 gamma (< 30 μm) motoneurons after retrograde labelling with horseradish peroxidase. The significance of differences between sample means was determined using Student's *t*-test.

RESULTS

Size and location of neurons

Retrogradely labelled motoneurons supplying the levator costae muscle (via the dorsal primary ramus of the spinal nerve) were located in the ventromedial aspect of the ventral horn, while motoneurons supplying the external intercostal muscle (via the ventral primary ramus) were located centrally in the ventral half of the ventral horn, dorsolateral to levator costae motoneurons. These motor nuclei have been described previously in the cat (Coffey, 1972; Fedorko, 1982; Larnicol, Rose, Marlot & Duron, 1982). To provide an indication of the size of all neurons within the motor nuclei, the diameters of the first 200 neurons seen in random 70 μm sections of both the levator costae and external intercostal motor nuclei were measured (Fig. 1).

Only 11·5% of these neurons could be identified, by the presence in their cytoplasm of reaction product, as motoneurons. The mean diameter of labelled motoneurons (35·9 μm) and unlabelled neurons (33·5 μm) was similar. As expected, only few labelled motoneurons were found in the external intercostal motor nucleus, since only a small portion of this muscle has been injected. However, even for the levator costae motor nucleus, supplying a small muscle which was apparently injected completely with horseradish peroxidase, unlabelled neurons of all sizes still predominated. As the density of reaction product varied between labelled motoneurons, a simple explanation of the above finding is that the present chromogen (3,3-diaminobenzidine tetra-

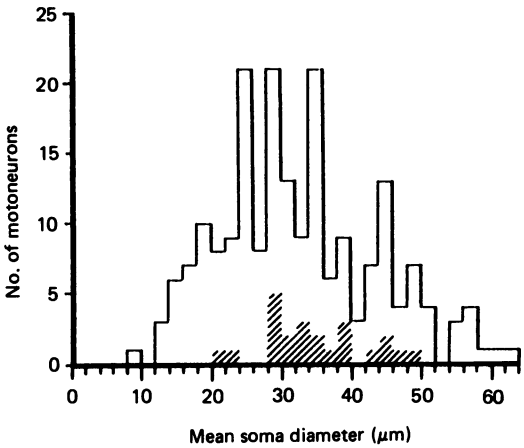


Fig. 1. Frequency histogram (2 μm interval) of mean cell body diameters (max.+min./2) of 200 neurons in the levator costae and external intercostal motor nuclei at T8–10. Measurements were made in the mid-nucleolar plane using an eyepiece graticule. A wide range of neuron sizes is present and retrogradely labelled motoneurons (hatched) account for only a small portion of the total.

hydrochloride) was not sufficiently sensitive to reveal the lightest of retrograde labelling (cf. Mesulam, 1978).

Morphology of neurons

In 70 μm sections, both labelled and unlabelled small neurons had oval, multipolar cell bodies (Fig. 2). Slender dendrites containing reaction product could occasionally be seen extending up to 100 μm from the cell bodies of labelled neurons, whereas the dendrites of unlabelled neurons were much more difficult to detect.

With both the light and the electron microscope, two types of unlabelled small neuron were seen. One type was indistinguishable on morphological grounds from gamma motoneurons (Johnson, 1986), whereas the other was distinct from both alpha and gamma motoneurons. This latter type was the most commonly encountered and is referred to here as an interneuron. The following account focusses on the differences between gamma motoneurons and interneurons.

In 0·5 μm sections (Fig. 3), gamma motoneurons had round, smooth-contoured nuclei, prominent nucleoli and large discrete Nissl bodies. Interneurons, in contrast, had wrinkled nuclei, inconspicuous nucleoli and Nissl bodies which were either small and indistinct, or in the form of an amorphous, perinuclear band. Proximal dendrites were more frequently seen extending from gamma motoneurons compared to interneurons but in all other respects gamma motoneurons and interneurons were similar.

With the electron microscope, gamma motoneurons (Figs. 4, 5) had smooth-contoured nuclear membranes with occasional slight infoldings and Nissl bodies which showed a high degree of order, comprising several lamellae of granular endoplasmic reticulum, with linear arrays of polyribosomes arranged between individual cisternae. Only a few synaptic terminals contacted the cell body and these were of the S-type (round synaptic vesicles) and the F-type (flattened synaptic vesicles); the other synaptic types (C-, T-, M- and P-types) described on alpha motoneurons (e.g. Conradi, 1969) were not seen. In contrast to gamma motoneurons, interneurons (Figs. 6, 7) had convoluted nuclear membranes and Nissl bodies which lacked orderliness, being composed primarily of aggregates of randomly-sited polyribosomes, within which short fragments of granular endoplasmic reticulum were

distributed. Nucleoli were smaller and more irregularly shaped in interneurons compared to gamma motoneurons. Qualitative ultrastructural analyses did not reveal any other differences between gamma motoneurons and interneurons, either in the arrangement of their organelles, their synaptic terminals or their relationships with neuroglia.

Quantitative data for various aspects of the ultrastructure of interneurons is given in Table 1, along with data previously obtained (Johnson, 1986) for retrogradely labelled alpha and gamma motoneurons. For this quantitative analysis, the first 10 unlabelled neurons of $< 30\ \mu m$ diameter possessing a large nucleus were selected. Three of the 10 neurons so selected were tentatively classed on morphological grounds as unlabelled gamma motoneurons. However, subtraction of the values obtained for these 3 neurons made little difference to the final mean values for the whole sample and they are therefore included in Table 1. Interneurons differed significantly from alpha and gamma motoneurons with respect to their Nissl bodies, nuclear pores, lysosomes and synaptic terminals. Particular note was made, however, of the similar values for mitochondrial frequency obtained for all three neuronal types.

Nissl bodies

Within alpha and gamma motoneurons, Nissl bodies were defined for quantitation by the presence of three or more parallel cisternae of granular endoplasmic reticulum, an arrangement which conferred a degree of ultrastructural orderliness on such Nissl bodies. Adhering to this definition, it was found that interneurons had little organised Nissl, their values for Nissl frequency and area being 48·1 and 30·9 % of the respective values for gamma motoneurons and 20·5 and 27·9 % of the respective values for alpha motoneurons.

Nuclear pores

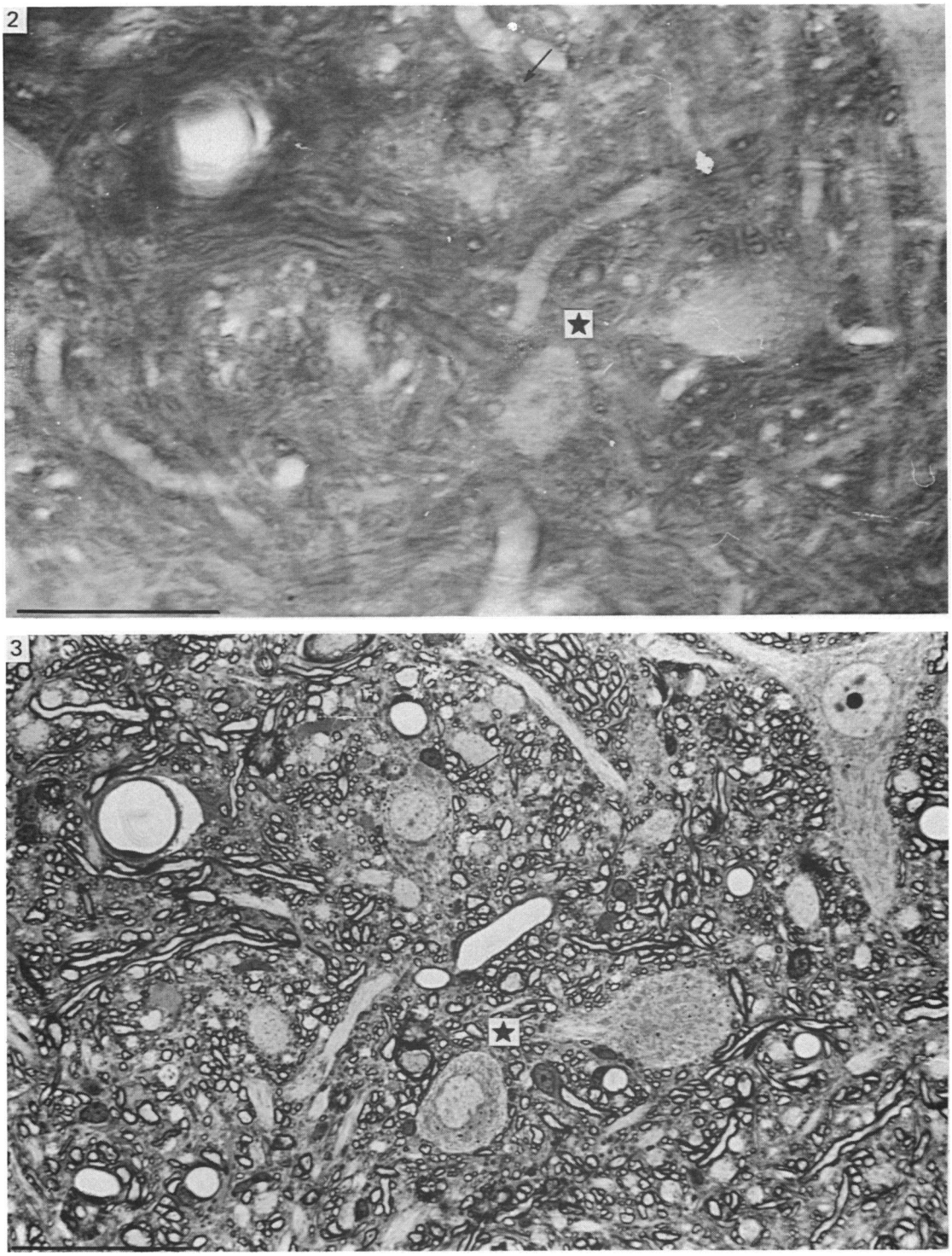
Interneuronal nuclear pore frequency was only 57·0 and 53·3 % of the respective values obtained for alpha and gamma motoneurons.

Lysosomes

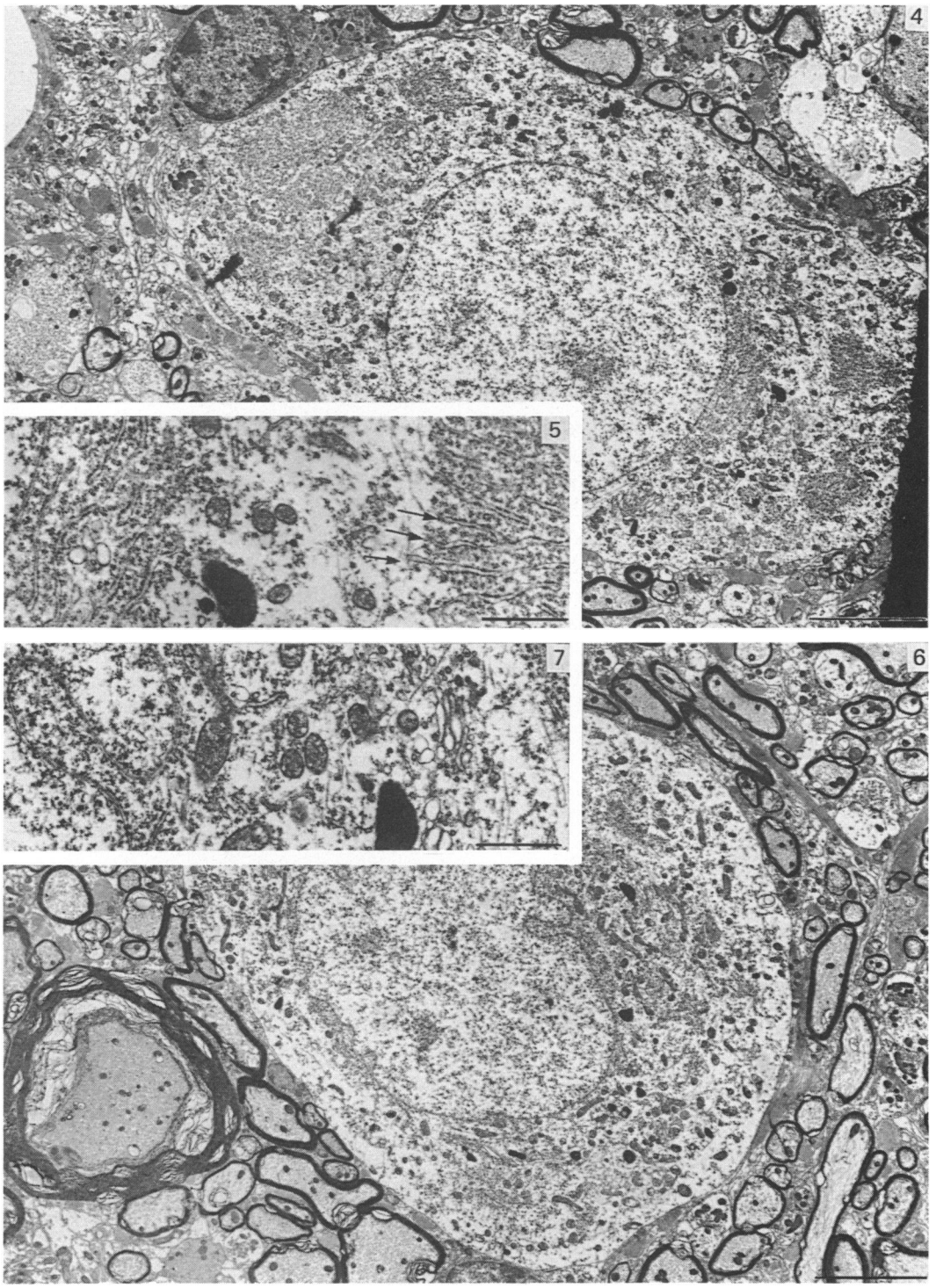
Interneuronal lysosomal frequency was only 57 % of the values obtained for alpha and gamma motoneurons. However, this value for interneurons is not significantly different from that previously obtained from *un*labelled alpha motoneurons ($15{\cdot}1/100\ \mu m^2$), identified in normal material on purely morphological grounds (Johnson, 1986). Low lysosomal frequency is therefore not considered a distinguishing feature of interneurons.

Synaptic terminals

A total length of $597\ \mu m$ of cell body plasma membrane was measured for 10 interneurons and found to be contacted by 46 S-type and 30 F-type synaptic terminals. The total lengths of apposition of these synaptic terminals were $65\ \mu m$ and $51\ \mu m$, respectively. Total synaptic frequency and cover lay between the low values obtained for gamma motoneurons and the high values obtained for alpha motoneurons. Although there were twice as many S-type terminals on interneurons compared to gamma motoneurons ($P < 0{\cdot}01$), this was not significantly different from alpha motoneuron values. Both S- and F-type synaptic terminals were observed on those occasional proximal dendrites which could be traced to their origin from an interneuron but too few dendrites were seen to allow firm comments to be made. As for alpha and gamma motoneurons, those portions of the interneuronal plasma



Figs. 2, 3. For legends see p. 178.



Figs. 4–7. **For legends see p. 178.**

membrane not occupied by synaptic terminals were covered by astroglial processes and the occasional oligodendrocyte.

Neither scatter diagrams nor calculation of the linear regression coefficient revealed any relationship between those features quantified and the cell body diameters of interneurons.

DISCUSSION

On the premise that small (< 30 μm) unlabelled neurons within those regions of the ventral horn of the spinal cord containing retrogradely labelled motoneurons are primarily interneurons, a quantitative ultrastructural analysis of interneurons of the motor nuclei of the thoracic region of the spinal cord of the adult cat has been undertaken. By comparing these data with those previously obtained for alpha and gamma motoneurons (Johnson, 1986), several morphological features have been identified which serve to distinguish these three neuronal types.

Identity of interneurons

Small size and lack of retrograde labelling with horseradish peroxidase are only indirect indicators that the neurons studied here are interneurons. Indeed, for 3 of the 10 neurons selected at random for quantitation, the morphological evidence of round, smooth-contoured nuclei and Nissl bodies with a high degree of ultrastructural orderliness indicates that the above criteria are not exact and that some of the small neurons may in fact have been unlabelled gamma motoneurons. Against this, however, is the fact that the majority of small unlabelled neurons were clearly distinct morphologically from both alpha and gamma motoneurons. While opinions as to the proportion of interneurons to motoneurons in motor nuclei vary (e.g. 'high' – Balthazar, 1952; Schadé & Van Harreveld, 1961; or 'low' – Coffey, 1972; Burke *et al.* 1977), the present approach can be considered to have at least increased the bias in favour of selection of interneurons.

Three types of interneuron in the ventral horn have so far been studied in the light microscope after electrophysiological identification and intracellular injection of dyes.

Spinal border cells

The cell bodies of these neurons, which form the ventral spinocerebellar tract, are located at the periphery of the ventral horn, near to motoneurons (Cooper & Sherrington, 1940; Jankowska & Lindström, 1970). Because of their large size and location, they could be mistaken for alpha motoneurons if no other distinguishing criteria were applied. This is ruled out in the present study since retrograde labelling

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Fig. 2. Light micrograph of two small ventral horn neurons in a 70 μm slice of spinal cord, postfixed in osmium and embedded in Araldite. The neuron containing dark granules of reaction product (arrow) is identified as a gamma motoneuron. The adjacent neuron (star) is unlabelled. Bar, 50 μm.

Fig. 3. Light micrograph of the same neurons shown in Figure 2. 0·5 μm toluidine blue-stained section. The gamma motoneuron has a round, smooth-contoured nucleus and discrete Nissl bodies throughout its cytoplasm. The unlabelled neuron has a wrinkled nucleus and Nissl substance which takes the form of a perinuclear band. Bar, 50 μm.

Fig. 4. Electron micrograph of the gamma motoneuron shown in Figures 2 and 3. Bar, 5 μm.

Fig. 5. Enlargement of Figure 4 to show the smooth-contoured nuclear membrane (left) and the ordered lamellae of granular endoplasmic reticulum (arrows) forming a Nissl body. Bar, 1·0 μm.

Fig. 6. Electron micrograph of the unlabelled neuron shown in Figures 2 and 3. Bar, 5 μm.

Fig. 7. Enlargement of Figure 6 to show the irregular nuclear membrane (left) and an aggregate of polyribosomes forming part of the perinuclear band of Nissl. Bar, 1·0 μm.

Table 1. *Quantitative aspects of alpha, gamma and interneuronal ultrastructure*

Feature	(A) Alpha	(B) Gamma	(C) Interneuron	*t* Tests A vs. B	*t* Tests A vs. C	*t* Tests B vs. C
Nissl frequency	1·85 ± 0·78	0·79 ± 0·44	0·38 ± 0·53	***	***	*
Nissl area	7·66 ± 3·06	6·91 ± 5·54	2·14 ± 2·60	N.S.	***	*
Mitochondrial frequency	98·95 ± 21·79	92·42 ± 22·12	94·06 ± 35·88	N.S.	N.S.	N.S.
Lysosomal frequency	23·80 ± 7·45	23·57 ± 9·39	13·53 ± 4·52	N.S.	**	**
Nuclear pore frequency	322·5 ± 87·53	345·1 ± 95·72	184·0 ± 98·17	N.S.	**	***
Golgi frequency	2·62 ± 0·69	2·72 ± 0·87	2·39 ± 1·20	N.S.	N.S.	N.S.
Golgi area	4·46 ± 1·85	5·22 ± 2·57	3·98 ± 1·34	N.S.	N.S.	N.S.
Total synaptic cover	34·65 ± 10·66	16·60 ± 12·98	21·15 ± 15·56	***	*	N.S.
Synaptic cover (round vesicles)	16·71 ± 7·33	6·71 ± 5·88	11·14 ± 6·94	***	N.S.	N.S.
Synaptic cover (flat vesicles)	17·94 ± 8·28	9·89 ± 9·64	9·98 ± 14·50	*	N.S.	N.S.
Total synaptic frequency	16·16 ± 4·85	9·00 ± 7·28	13·54 ± 9·05	**	N.S.	N.S.
Synaptic frequency (round vesicles)	7·28 ± 2·10	3·68 ± 3·16	7·73 ± 4·42	**	N.S.	**
Synaptic frequency (flat vesicles)	8·88 ± 3·63	5·33 ± 5·44	5·81 ± 7·36	N.S.	N.S.	N.S.

$\bar{x} \pm$ S.D., N.S., $P > 0{\cdot}05$; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$; ***, $P < 0{\cdot}001$.

with horseradish peroxidase was used to identify motoneurons. Inclusion of spinal border cells in the interneuronal population is also considered remote, not only because these neurons would probably be too large (> 30 μm), but also because spinal border cells appear to be restricted to the lumbosacral regions of the spinal cord in the cat (Grant, Wiksten, Berkley & Aldskogius, 1982). Although there is electrophysiological evidence for the existence of a forelimb homologue of the ventral spinocerebellar tract (Oscarsson, 1965), such neurons have not been demonstrated anatomically and are therefore not considered further.

Renshaw cells and Ia inhibitory interneurons

In the lumbosacral region of the spinal cord of the cat, electrophysiologically-identified Renshaw cells (Jankowska & Lindström, 1971; Lagerbäck & Kellerth, 1985; Van Keulen, 1979) and Ia inhibitory interneurons (Jankowska & Lindström, 1972) have been localised to the ventral aspects of Rexed's Lamina VII. Although this location is clearly dorsal or dorsomedial to the motor nuclei of the *ventral* primary spinal ramus (Romanes, 1951), it is possible that it may overlap with that of the motor nuclei of the *dorsal* primary spinal ramus (Sprague, 1951; Coffey, 1972; Fedorko, 1982). A survey of the light micrographs presented by Rexed (1952, 1954) to illustrate his scheme of lamination of the spinal gray matter into 10 morphologically distinct regions gives some support to this view. Those areas of the ventral horn demarcated by him as comprising Lamina IX are seen to be composed of motoneurons which are defined primarily by their large Nissl bodies, but these motoneurons are nevertheless intermingled with other neurons displaying morphological characteristics of the adjacent laminae (VII or VIII). In the present study, levator costae motoneurons, whose axons travel in the dorsal primary spinal ramus, were retrogradely labelled with horseradish peroxidase and interneurons in the vicinity of these motoneurons were examined. This, together with the fact that the ventral horn of the thoracic region of the spinal cord is narrower, leaving less spatial separation between its motor pools than is the case in the limb segments, raises the possibility that Renshaw cells and Ia inhibitory interneurons may have been included in the present interneuronal sample.

The present experimental approach gives no indication of the site of termination of the axons of the interneurons studied. Indeed, studies of the location of chromatolytic neurons after hemisections of the thoracic (Coffey, 1972) and lumbosacral (Sterling & Kuypers, 1968) regions of the cat spinal cord have been taken to indicate that there are few if any interneurons within the motor nuclei. An alternative explanation, however, could be that interneurons are present but have short axons which cannot be interrupted by spinal cord lesions without injuring the cell body directly. If this is correct, such interneurons can be considered as local circuit neurons (Rakic, 1975) and presumptively associated closely with motor activity. In the cat, the levator costae (Hilaire, Nicholls & Sears, 1983) and external intercostal muscles (Duron & Marlot, 1987; Sears, 1964*b*) at the segmental levels studied here are concerned with inspiration. From this one may speculate that the interneurons within these motor nuclei are linked particularly to inspiratory activity.

Intracellular features of interneurons

Three main intracellular features distinguished interneurons from motoneurons: low nuclear pore frequencies, crenated nuclei and Nissl bodies which lacked ultrastructural orderliness. These last two features have been described for other small neurons intrinsic to the central nervous system (Blomqvist, 1981; Lagerbäck, 1983;

Sotelo & Angaut, 1973) and for small unlabelled neurons in the abducens nucleus of the cat after injecting the lateral rectus muscle with horseradish peroxidase (Destombes, Gogan & Rouvière, 1979). Interestingly, nuclear crenation and Nissl body disorganisation also characterise cat thoracic motoneurons following axotomy (Johnson, 1983; Johnson, Pullen & Sears, 1985).

The frequency of nuclear pores in interneurons was only half that in motoneurons. This could be due to there being fewer nuclear pores or more nuclear membrane in interneurons compared to motoneurons. The former explanation is considered most likely since there was little difference, irrespective of regularity of contour, in the total length of nuclear membrane measured in randomly orientated sections through the nuclei of all neuronal types (372 μm for 10 interneurons compared to 539 μm for 10 alpha motoneurons and 851 μm for 20 gamma motoneurons). The functional significance of the low nuclear pore frequency of interneurons is unknown. However, it is known that nuclear pores are the portals through which materials, such as ribonucleic acid and ribosomes, pass (Dingwall, 1985) and changes in protein synthesis in a variety of non-neuronal tissues have been associated with changes in nuclear pore frequency (Ghadially, 1975). Nucleoli are the sites of synthesis of ribonucleic acid and ribosomes (Jordan & Cullis, 1982) and in the present study the nucleoli of interneurons were smaller than those of motoneurons. It is tempting to speculate, therefore, that there may be fundamental differences in the rates of protein metabolism of interneurons and motoneurons within the same motor nucleus.

Mitochondrial frequency was not significantly different for interneurons compared to motoneurons. This is difficult to reconcile with the findings of Campa & Engel (1970) that small neurons in the ventral horn of the lumbar region of the spinal cord of the rat showed more intense staining for oxidative enzymes compared to the large neurons, which were presumed to be motoneurons. It also suggests that an alternative mechanism other than asphyxia underlies the greater vulnerability of small as opposed to large neurons in the spinal cords of cats and dogs after periods of spinal cord ischaemia (Van Harreveld & Schadé, 1962; Gelfan, 1964). Further information on the volume fractions of mitochondria in large and small neurons and the relative contribution of mitochondria to total neuronal levels of oxidative enzymes are required to resolve these issues.

Extracellular relationships of interneurons

No qualitative difference in the neuroglial cover of motoneurons and interneurons was noted. As is the case for most regions of the central nervous system, such cover was provided primarily by astrocytic processes with occasional contributions from perineuronal oligodendrocytes. Microglia were observed infrequently and did not contact neuronal plasma membranes.

Synaptic terminals were much more frequently encountered along the cell body plasma membrane of interneurons compared to gamma motoneurons. A similar situation also obtains when the synaptic terminals of Renshaw cells (21–33 μm diameter) are compared with those of gamma motoneurons (21·5–36·5 μm diameter) in the lumbosacral spinal cord of the cat (Lagerbäck, 1983, 1985). Thus, for small neurons in the spinal cord there is little indication of any relationship between numbers of synaptic terminals on the cell body and cell body diameter, although such a relationship has been found for gracile neurons in the cat (Blomqvist, 1981) and trigeminal motoneurons in the rat (Limwongese & DeSantis, 1980). It has been assumed (Burke, 1981) that small neurons have a greater density of synaptic terminals compared to large neurons to account for their greater excitability (Henneman,

Somjen & Carpenter, 1965). From the disparate findings for spinal compared to cranial neurons, which already exist, however, it would appear that analysis of synaptic terminals on the cell body alone may be insufficient to allow generalisations about synaptic features to be made for certain neurons. This approach is *prima facie* made all the more uncertain when it is acknowledged that for most neurons, little is known of either the efficiency of particular types of synaptic terminals or the electrical properties of the plasma membrane. Furthermore, it would appear to be a general finding for the mammalian central nervous system that most of the receptive area for synaptic input is provided not by the neuronal cell body, but by its dendrites.

SUMMARY

Alpha (> 40 μm) and gamma (< 30 μm) motoneurons in inspiratory motor nuclei of the thoracic spinal cord of the adult cat were labelled retrogradely by the intramuscular injection of HRP. Small (< 30 μm) unlabelled neurons within 200–300 μm of labelled motoneurons were analysed qualitatively and quantitatively with both the light and electron microscope. Most of these small unlabelled neurons had inconspicuous nucleoli, wrinkled nuclear membranes, low numbers of nuclear pores, and Nissl bodies which were either small or had the form of an amorphous perinuclear band. Such Nissl bodies were composed primarily of aggregates of polyribosomes within which short fragments of granular endoplasmic reticulum were distributed. Alpha and gamma motoneurons in contrast had prominent nucleoli, smooth-contoured nuclei, more nuclear pores and large, discrete Nissl bodies. Such Nissl bodies were composed primarily of several lamellae of granular endoplasmic reticulum with linear arrays of polyribosomes arranged between individual cisternae. Alpha motoneurons had most synaptic terminals on their cell bodies, gamma motoneurons had least and small unlabelled neurons had intermediate values. Synaptic terminals of the S-, F- T- and C-type were observed on alpha motoneurons, whereas only S- and F-types were observed on gamma motoneurons and small unlabelled neurons.

Since they were unlabelled and differed morphologically from both alpha and gamma motoneurons, but were similar to small interneurons described elsewhere in the spinal cord and brain, it is suggested that the small unlabelled neurons located in the external intercostal and levator costae motor pools are interneurons. The functional significance of some of the morphological features which distinguish interneurons from motoneurons is discussed.

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