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CONDUCTION VELOCITY IS RELATED TO MORPHOLOGICAL CELL TYPE IN RAT DORSAL ROOT GANGLION NEURONES

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SUMMARY

1. Combining intracellular recording and dye-injection techniques permitted direct correlation of neuronal soma size with peripheral nerve conduction velocity in individual neurones of the L4 dorsal root ganglion (d.r.g.) of the anaesthetized 5–8-week-old rat.

2. The conduction velocities fell into two main groups; those > 14 m/s (Aα and β fibres) and those < 8 m/s (Aδ and C fibres).

3. Fibres with conduction velocities in the Aδ range (2·2–8 m/s) in the sciatic nerve between the sciatic notch and the neuronal soma in the d.r.g. often conducted more slowly, that is in the C-fibre range (< 1·4 m/s), in the periphery from the tibial nerve to the sciatic notch.

4. For the fast-conducting myelinated afferents, there was a loose positive correlation between cell size and the conduction velocity of the peripheral axon, whereas a clearer positive correlation existed between neuronal cell size and axonal conduction velocity both for Aδ- and for C-fibre afferents.

5. The relationship of the cell cross-sectional area (measured at the nucleolar level), to the cell volume for each neuronal soma was similar for the different conduction velocity groups.

6. The somata of the fast-conducting myelinated Aα and Aβ fibres had a similar mean and range of cross-sectional areas to those of the large light cell population.

7. The somata with Aδ and C fibres were of a more uniform size and were restricted to the smaller cells within the ganglia. The mean and range of cross-sectional areas of the C cells was similar to those of the small dark cell population. Aδ somata had a larger mean and range of cell sizes than those of the small dark cell population.

8. The relationships of peripheral axon type to the morphological cell types are discussed.

INTRODUCTION

Two main cytologically distinct types of neurones have been described in rat and mouse dorsal root ganglia (d.r.g.s) on the basis of their cytoplasmic appearance both at the light microscope (Andres, 1961; Lawson, 1979) and at the electron microscope

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level (Yamadori, 1971; Lawson, Caddy & Biscoe, 1974; Duce & Keen, 1977; see also review by Lieberman, 1976). These two types of neurones have been called A and B (Andres, 1961) or large light and small dark neurones (see Lieberman, 1976). The large light neurones have an unevenly staining cytoplasm with dense areas of Nissl substance, interspersed with lightly staining regions containing large numbers of neurofilaments. The small dark neurones have, in general, a more evenly staining and intensely basophilic cytoplasm and few or no neurofilaments.

Statistical analysis of the distribution of neuronal sizes in rat and mouse d.r.g.s has consistently revealed two distinct cell populations with partially overlapping normal distributions of size, whose component cells have the cytoplasmic features of the two populations described above. Recently, it has been shown that the large light cell population in rat d.r.g.s can be selectively labelled by an antineurofilament antibody (RT97) which does not label the small dark cell population (Lawson, Harper, Harper, Garson & Anderton, 1984). These two populations have been shown to have different time courses of development, both embryologically and post-natally (Lawson & Biscoe, 1979; Lawson, 1979) and, at least in the rat, they are differentially sensitive to the neurotoxic actions of capsaicin administered neonatally (Lawson, 1981). It therefore seems likely that fundamental differences exist between the two cell types.

As yet, however, there is little direct evidence to link the functional characteristics of the living neurone to the morphological populations. Combining intracellular recording and dye injection provides a powerful technique for examining the relation between structure and function for individual cells. Such a study was performed by Yoshida & Matsuda (1979) using a mouse d.r.g. preparation *in vitro*, to relate axonal conduction velocity to soma size and membrane electrical characteristics. Unfortunately, these results could not be directly related to morphological cell type because at that time the overlap in size distributions of the large light and small dark cell populations (Lawson, 1979; Lawson *et al.* 1984) had not been established, and this overlap means that small sized neurones could belong to either of these morphological groups.

The present study is designed to correlate conduction velocity with neuronal soma size, using intracellular recording *in vivo* followed by dye injection, and to interpret these data in the light of our present knowledge concerning the cell types.

METHODS

Dissection

5–8-week-old female Wistar rats, weighing 120–180 g, were used. Young females were chosen since cell penetration was found to be easier, presumably because there is less collagenous connective tissue in the ganglia than in those of adult males. They were initially anaesthetized with sodium pentobarbitone (50–60 mg/kg intraperitoneally) which was supplemented as required. Core temperature was measured by a rectal thermistor and maintained at 37 ± 0.5 °C by electronic feed-back to a heating pad beneath the ventral surface and additionally by an overhead radiant heat source. The animal was tracheotomized but allowed to respire spontaneously using oxygen-enriched room air. Heart rate was monitored throughout from needle electrodes in the chest wall.

A partial laminectomy exposed the L4 d.r.g. and the dura overlying the ganglion was removed. The preparation was stabilized by clamping the L1, L3 and L6 vertebrae and by applying tension to the base of the tail and the scruff of the neck. The vertebrae were covered with a gel of 5% agar

(w/v) in balanced salt solution (BSS), and the agar overlying the L4 ganglion was removed. In most experiments (forty-eight of sixty-five) a 0·25% solution of collagenase (Sigma 1A) in BSS was applied for 15 min to soften the connective tissue surrounding the ganglion, and this was then washed off over 15 min with a large volume of BSS saturated with oxygen. In later experiments, the connective tissue capsule surrounding the ganglion was removed, but no collagenase was applied. This was found to improve the likelihood of achieving stable recordings from C-fibre cells; these experiments yielded two-thirds of the cells with C-fibre conduction velocity and a quarter of the cells with A-fibre conduction velocity. To maximize the frequency response of the recording micro-electrode only a thin film of BSS covered the ganglion during the experiment. This was changed at frequent intervals during the experiment. The composition of the BSS was (in mM): NaCl, 140; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; glucose, 5; Tris HCl, 5; pH 7·4.

The ipsilateral sciatic and tibial nerve trunks were exposed and dissected free from the surrounding tissue in the lateral aspect of the thigh. Stimulating platinum bipolar electrodes were placed under the sciatic nerve as near to the sciatic notch as possible, and also under the tibial branch of the sciatic nerve just above the knee joint. The temperature in the BSS surrounding the ganglion and the liquid paraffin over the nerve trunks was measured using a small thermocouple probe and was within the range $37{\cdot}0 \pm 0{\cdot}5$ °C.

Intracellular recording

The tips of the intracellular micro-electrodes were filled with either a 4% (w/v) horseradish peroxidase (HRP Sigma type VI) in 0·2 M-KCl and 0·05 M-Tris solution (pH 8·6), or a 3% Lucifer Yellow CH in 0·1 M-LiCl solution.

The micro-electrodes were connected to the recording system by Ag–AgCl wire. An Ag–AgCl pellet embedded in the spinal agar pool served to ground the recording system. The micro-electrodes used were typically found to have d.c. resistances of > 30 MΩ when filled with 3 M-KCl and tested in BSS solution and > 80 MΩ when filled with dye. An active bridge circuit (Model M 707, WP Instruments Ltd., U.S.A.) was used both for recording and for current injection through the recording micro-electrode. Membrane potential and injected current were displayed on an oscilloscope and simultaneously fed to an FM tape recorder (band width 0–10 kHz). Taped signals were subsequently recorded on photographic film for analysis.

Conduction velocity measurements

Cells were impaled using a Microcontrole (France) stepping motor drive (step size 1 μm). Recordings were obtained from neural somata located within 100 μm of the dorsolateral surface of the ganglion. Somatic action potentials evoked by stimulating the tibial and sciatic nerves were recorded, and the conduction time was determined as the time between peripheral nerve stimulation and the generation of a somatic action potential (see Fig. 1). Conduction velocity was calculated directly from the conduction distance, d (see Fig. 1*A*) divided by the conduction time, t (see Fig. 1*B*) for the following nerve lengths: (1) the sciatic nerve to the d.r.g. (d_1/t_1), this was done for sixty-five labelled and subsequently identified neurones; (2) the tibial nerve to the d.r.g., $[(d_1+d_2)/(t_1+t_2)]$, and indirectly (3) tibial to sciatic nerve cathodal stimulating electrodes (d_2/t_2, i.e. the difference between (1) and (2)). Values for (2) and (3) were only available for thirty-four of sixty-five identified neurones, since many neurones could only be stimulated from the sciatic and not the tibial nerve. The sciatic to d.r.g. conduction distance (d_1) was about 45 mm, and was measured in twenty animals of ages spanning those used in this study, and plotted against age. The data points approximated to a linear regression ($r = 0{\cdot}81$) and it was possible to use the line of best fit to calculate this distance in animals where no direct measurement was made, since the electrodes were always placed in the same position on the nerves relative to the available anatomical landmarks. The tibial to sciatic conduction distance was always measured directly. It was approximately 12 mm and varied very little. In all instances the conduction velocity was calculated using twice threshold current intensities for evoking propagated action potentials.

Intracellular dye injection

Following the recording of electrophysiological characteristics of the neurone, injection of an intracellular dye was made by electrophoresis, using outward current pulses for HRP and inward pulses for Lucifer Yellow, both at a pulse frequency of 0·5 Hz. The pulse duration was 500 ms for HRP and 1 s for Lucifer Yellow and the current intensity was 1–5 nA, that is, as high as was

compatible with maintaining the stable electrical characteristics of the neurone. The minimum current–time product required for subsequent identification of the neurone was 2 nA min. Normally one cell was injected with HRP and one was injected with Lucifer Yellow in each ganglion. This was possible since Lucifer fluorescence survives the processing for the HRP reaction product.

Lucifer Yellow proved to be a more satisfactory marker in these experiments, since fifty-four of eighty of the injected cells could be subsequently identified. Only eleven of thirty-four of the

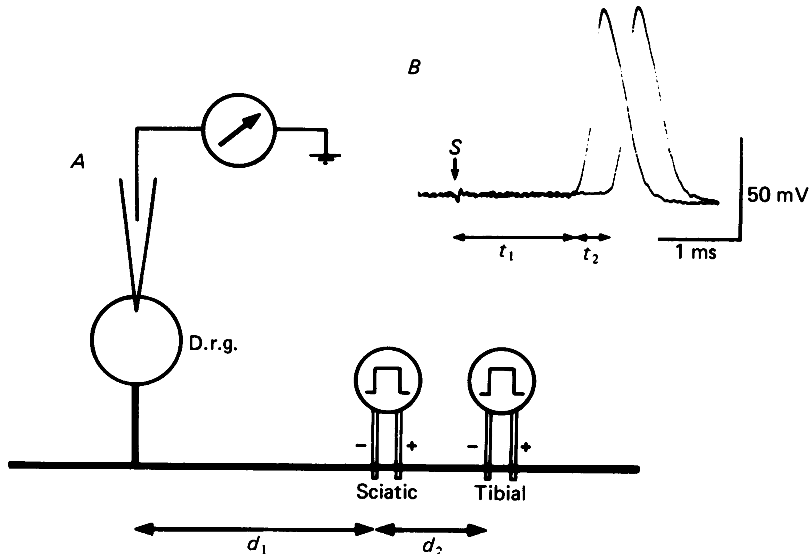

Fig. 1. *A*, schematic diagram of the recording and nerve stimulation arrangement. A dye-filled intracellular micro-electrode in the soma permitted electrophysiological characterization and subsequent examination of the morphology of the individual neurones being studied. The sciatic nerve to d.r.g. conduction distance (usually 45 mm) is called d_1. The tibial to sciatic conduction distance (usually 12 mm), is called d_2. *B*, examples of action potentials recorded in the soma evoked by stimulation at time *S* of the sciatic and tibial nerve trunks. The conduction velocities in the sciatic to d.r.g. and tibial to sciatic lengths of nerve were calculated from d_1/t_1 and d_2/t_2 respectively. In this example d_1 and d_2 were measured as 41 and 11 mm, and t_1 and t_2 were 1·48 and 0·40 ms respectively, yielding conduction velocities of 27·7 m/s in the sciatic to d.r.g. and 27·5 m/s in the tibial to sciatic nerve lengths.

HRP-labelled cells were subsequently identified, the failure rate being much higher in those cells lying more than 50 μm from the ganglion surface presumably because of the difficulty of penetration of the reagents necessary to produce an HRP reaction product.

Histological processing and cell measurement

The animal was fixed by transcardiac perfusion with 2·5% (v/v) glutaraldehyde in 0·1 M-cacodylate buffer, pH 7·2. The ganglion was removed, post-fixed for approximately 16 h and then washed for 45 min in 0·1 M-cacodylate buffer. Processing to produce an HRP reaction product was carried out in whole ganglia using the diaminobenzidene method. The ganglion was then dehydrated, embedded in Epon resin and serial 5 μm sections were cut so that the roots and peripheral nerve were cut in transverse section.

Lucifer Yellow fluorescence was demonstrated under ultra-violet illumination (Stewart, 1978) and photographed using interference contrast combined with fluorescence (Leitz Ortholux II). Photomicrographs of the sections through labelled cells which had one or more nucleoli were used to measure the neuronal cross-sectional area.

Frequency distribution histograms of cell areas of a sample of neurones in three experimental ganglia were produced as follows: the cross-sectional areas of all neuronal profiles containing a nucleolus in 5 μm Epon sections sampled at 200 μm intervals throughout each ganglion were measured. A computer program was used to find the best-fit solution for each histogram. If the best-fit solution was not significantly different from the histogram at the 5 % level using the χ^2 test, then it provided a possible fit for the histogram. For more details of these methods see Lawson (1979).

The volume of each labelled neurone was calculated as the sum of the volumes of each section through that neurone. The volume in each section was calculated from the cross-sectional area of that profile multiplied by the section thickness (5 μm). Soma volumes were estimated for nearly all the cells with fibres conducting in the C- and Aδ-fibre range, and for half (twenty-three of forty-five) of the cells with faster conducting Aα and Aβ axons.

For all data means $\pm$ S.D. are given.

RESULTS

Results are presented for sixty-five d.r.g. neurones. Most electrophysiological data were from cells which exhibited a stable membrane potential ($E_m > 40$ mV) and overshooting somatic action potentials evoked by stimulation of the sciatic nerve trunk. However, conduction velocity measurements were included from a few cells with $E_m < 40$ mV (since deterioration of the E_m was not found to affect the conduction velocity of the axon), and from a few cells in which the propagated action potential failed consistently or occasionally to invade the soma, but in which an intracellular depolarizing current pulse did evoke an overshooting somatic action potential. All cells included in the analysis were subsequently identified in histological sections.

Conduction velocity

The frequency distribution histograms of conduction velocities from sciatic nerve to d.r.g. (d_1), and also between the tibial and sciatic nerve electrodes (d_2) can be seen

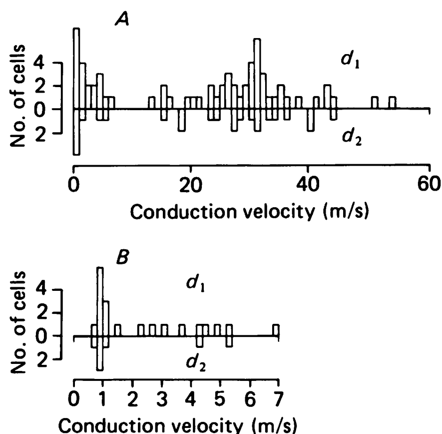

Fig. 2. Frequency histograms of the conduction velocities measured in the sciatic to d.r.g. nerve length, d_1 (sixty-five cells) above the abscissa, and in the tibial to sciatic nerve length, d_2 (thirty-four of sixty-five cells) below the abscissa. All measurements were from individual intracellular recordings in neural somata. Note the absence of fibres conducting in the 8–14 m/s range. In *A*, all conduction velocities were included, bin width 1 m/s. In *B*, conduction velocities of < 8 m/s were plotted using a 0·2 m/s bin width. They are discontinuously distributed.

in Fig. 2. The conduction velocities from the sciatic nerve to the d.r.g. fell clearly into two main groups, those greater than 14 m/s, and those less than 8 m/s. Axons conducting at > 14 m/s had low thresholds for stimulation, typically 0·5–10 V using a 0·02 ms pulse duration. These fibres were classified as Aα (> 30 m/s) and Aβ (14–30 ms). In some cases Aα and Aβ groups will be considered together and designated Aα/β. In contrast the group with sciatic to d.r.g. conduction velocities of < 8 m/s (see in Fig. 2*A* and also on an expanded time scale in Fig. 2*B*) required stimulation pulses of greater duration and strength. The distribution of conduction velocities in this group was discontinuous, ranging from 0·8 to 1·4 m/s, and 2·2 to 8 m/s. The subgroup conducting in the 2·2–8 m/s range typically required stimuli of at least 0·1 ms to attain threshold. These were classified as Aδ fibres. Those conducting at 0·8–1·4 m/s required a 1 ms pulse of > 5 V for threshold stimulation and were classified as C, or non-myelinated, fibres.

Changes in conduction velocity in the periphery

To test whether conduction velocity was constant over two different lengths of the same nerve fibre in the periphery, the ratio of the sciatic to d.r.g.: tibial to sciatic conduction velocities was determined for each of thirty-six neurones, and these two conduction velocities are plotted against each other in Fig. 3. For the axons with sciatic to d.r.g. conduction velocities of > 14 m/s (twenty-six neurones) the ratio was found to be $0{\cdot}950 \pm 0{\cdot}145$, $n = 26$, and the graph in Fig. 3*A* shows that the data points fell on or near the line of equality. They were fitted by a linear regression ($r = 0{\cdot}84$, $P < 0{\cdot}001$). In these fast-conducting fibres, therefore, no increase in conduction velocity was found more centrally. In most (eight of ten) of the slower-conducting

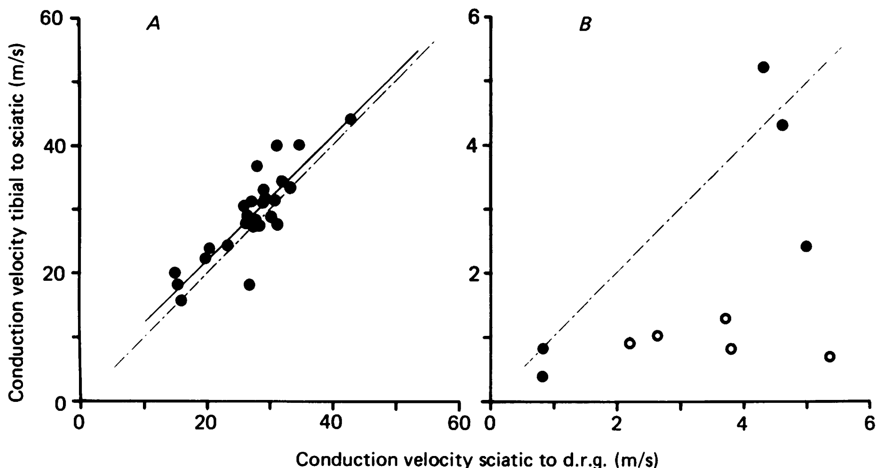

Fig. 3. Conduction velocities measured in the tibial to sciatic nerve length plotted as a function of the conduction velocity measured in the sciatic to d.r.g. nerve length for afferents conducting at > 14 m/s in *A*, and < 8 m/s in *B*. For each plot the line of equality, indicating equal conduction velocities in each nerve length, is shown by the interrupted line. For axons conducting at > 14 m/s the data points are fitted by a linear regression (continuous line, $r = 0{\cdot}84$). In *B*, the open circles represent fibres conducting in the Aδ range from sciatic to d.r.g., and in the C-fibre range from tibial to sciatic.

(< 8 m/s) group of axons with double conduction velocity measurements, the conduction velocity increased along the fibre in the central direction (Fig. 3*B*). Of those with sciatic to d.r.g. velocities in the Aδ range, seven of eight showed some slowing in the more peripheral tibial to sciatic nerve length, five of eight (shown with open circles on the graph) slowed so much that they fell in the C-fibre velocity range. Two fibres had sciatic to d.r.g. conduction velocities in the C-fibre range and of these one showed some slowing in the periphery.

Relationship of conduction velocity to cell cross-sectional area

Fig. 4*A* shows that the slope of the relationship of soma cross-sectional area to the conduction velocity of the axon between the sciatic nerve and the d.r.g. cell body is quite different for the fibres conducting at > 14 m/s, and the fibres conducting at < 8 m/s. For the group conducting at > 14 m/s (the Aα/β-fibre cells) a loose correlation was found ($r = 0{\cdot}26$, $P < 0{\cdot}05$) between cell cross-sectional areas and conduction velocity. Similar relationships have been described for motoneurones and retinal ganglion cells by Culheim (1978) and Wässle, Levick, Kirk & Cleland (1975).

For cells with fibres conducting at < 8 m/s (the Aδ and C-fibre cells) a positive linear relation with a greater correlation coefficient ($r = 0{\cdot}62$, $P < 0{\cdot}01$) was found between cell cross-sectional area and conduction velocity (Fig. 4*B*). A much steeper slope is shown for the Aδ group than the C-fibre group. Within the Aδ group there

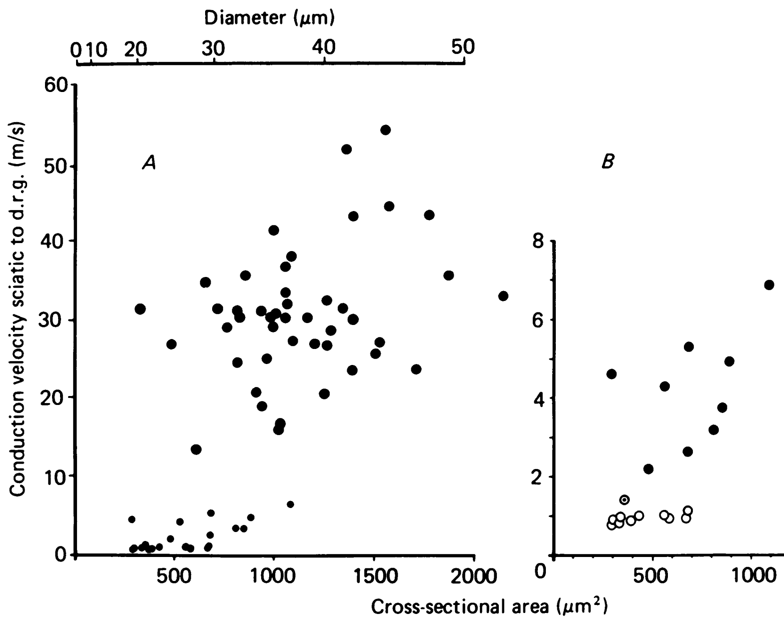

Fig. 4. *A*, relationships of neuronal soma size to conduction velocity of the peripheral axon measured in the sciatic to d.r.g. nerve length. The smaller symbols indicate neurones conducting at < 8 m/s. *B*, enlarged plot of *A* for cells with fibres conducting at < 8 m/s showing the positive linear relationship between cell cross-sectional area and conduction velocity. (●), cells with sciatic to d.r.g. conduction velocities in the Aδ-fibre range (2·2–8 m/s); (○), those in the C-fibre range (0·8–1·4 m/s); (⊙), this is the fastest conducting cell in the C-fibre range (1·4 m/s), but because of its position on the graph it is included with the Aδ cells for the calculation of *r*.

was a tendency for the larger Aδ somata to have faster conducting axons than the smaller somata, the correlation coefficient being $r = 0{\cdot}57$, $P > 0{\cdot}1$. Within the C-fibre group the conduction velocity increased slightly with increase in cell size ($r = 0{\cdot}67$, $P = 0{\cdot}05$).

Over-all cell size distribution

Frequency distribution histograms of neuronal sizes in L4 ganglia from three rats were constructed to show the over-all cell size distributions in these ganglia. The rats were from near the upper age limit, the mean age and near the lower age limit of the experimental series, that is 54, 47 and 39 days old respectively. In all three ganglia the distribution of cell sizes was fitted by two overlapping normal distributions, a consistent finding for all mouse and rat d.r.g.s previously analysed in this way. These two populations have been shown to correspond to the two main morphological populations of the d.r.g., that is, the small dark and the large light cell populations (Lawson, 1979; Lawson *et al.* 1984). The parameters defining these distributions for the three ganglia are given in Table 1. The histogram of cell sizes from the 47-day-old rat (mean age and weight for the series) is shown in Fig. 5*B*. Superimposed on this are shown the two normal distributions which represent the small dark and large light cell distributions for that ganglion and whose sum provided a fit for the histogram. The size ranges of the two populations overlap considerably.

Relationship of conduction velocity to cell type

The relationship between soma size and peripheral axonal conduction velocity was examined further by segregating the electrophysiologically studied cells into the three groups described above on the basis of conduction velocity from sciatic nerve to the d.r.g., namely into $A\alpha/\beta$, Aδ- and C-fibre cells. The somatic cross-sectional areas of neurones belonging to each of these groups were plotted as frequency distribution histograms and are shown in Fig. 5*A*, and given in Table 1. The mean cell sizes of the C-, Aδ- and $A\alpha/\beta$- fibre cells (see Table 1) were compared using the Student's *t* test, making the assumption that these cells were randomly sampled from normally distributed populations. The *F* test was used to compare the variances of the two populations in each case. Where the variances were significantly different (as was found in the comparison of the $A\alpha/\beta$ with the C cell population), a modification of Student's *t* test was used (Bailey, 1959, p. 51). The mean size of $A\alpha/\beta$ cells was significantly larger than both the Aδ cells ($P < 0{\cdot}005$), and the C-fibre cells ($P < 0{\cdot}001$). Furthermore, the mean size of the Aδ-fibre cells was significantly greater than that of C-fibre cells ($P < 0{\cdot}05$).

The mean sizes of these three groups of neurones were then compared to the mean sizes of the small dark and large light cell populations of an L4 ganglion from the 47-day-old rat (see Table 1). This ganglion was chosen since the rat was the mean age and weight for the experimental series, and since it was a collagenase-treated experimental ganglion, and would therefore have been subjected to the same swelling or shrinkage that these procedures might have caused.

The mean size and S.D. of the $A\alpha/\beta$-fibre cells (1142 ± 366 μm^2) were very close to the mean size and S.D. (1095 ± 442 μm^2) of the large light cell population of the d.r.g. from the 47-day-old rat (see also Fig. 5). The mean size and S.D. of the group of C-fibre

cells ($449 \pm 148\ \mu m^2$) were very close to those of the small dark cell population ($420 \pm 125\ \mu m^2$) of the ganglion of the 47-day-old rat (see Fig. 5). In view of the probable bias of micro-electrode sampling towards the large cells in any group, these relationships are suprisingly good. The mean size of Aδ-fibre cells was $702 \pm 448\ \mu m^2$ and the size range of Aδ cells extended considerably above the size range of the small dark cell population.

TABLE 1. *A*. The mean neuronal cross-sectional areas and standard deviations of the two computer-derived normal distributions, corresponding to the large light and small dark cell types. For each ganglion the sum of these distributions was not significantly different, at the 5% level, from the observed values (χ^2 test). One example is plotted out in Fig. 5*B*. % indicates the percentage of all the cells measured which fell into each distribution. * indicates that ganglia were used for electrophysiology, and had collagenase treatment (see Methods). The three rats were female, their ages are given. *B*. Means and standard deviations of the three groups of cells classified according to conduction velocity from the sciatic nerve of the d.r.g. cell body. Histograms of these sizes are plotted in Fig. 5*A*.

A. Cell size distributions in whole ganglia

	Small dark cell distribution			Large light cell distribution			
Ganglion	Mean (μm^2)	S.D.	% of all cells	Mean (μm^2)	S.D.	% of all cells	No. of cells
L4* 39 days	284	97	55	886	364	45	319
L4* 47 days	420	125	59	1095	442	41	330
L4 54 days	336	108	66	899	345	34	212

B. Sizes of cells with different conduction velocities

Conduction velocity (m/s)	Mean cell size (μm^2)	S.D.	No. of cells
$A\alpha > 30$	1171·6	410·1	25
$A\beta$ 14–30	1104·4	309·5	20
$A\alpha/\beta > 14$	1141·7	366·3	45
$A\delta$ 2·2–8	702	448	9
$C < 1·4$	449	148	11

Relationship of neuronal cross-sectional area to neuronal volume

Fig. 6 shows the neuronal cross-sectional areas of labelled neurones measured at the nucleolar level, plotted against the cross-sectional areas calculated from the neuronal volumes, assuming them to be spheres. The line shows where the points would fall if the cell was spherical and if the cross-sectional area through the nucleolar level fell exactly at its mid-point. Points falling above this line show a cell to be elongated in a direction perpendicular to the plane of section. Most points fall slightly above the line, either due to a very slight elongation of the neurones perpendicular to the section, or due to a consistent over-estimation of the volume using this rather approximate but very direct method. The cross-sectional area measurement used is therefore a good indication of cell volume and is equally good for all the cell groups

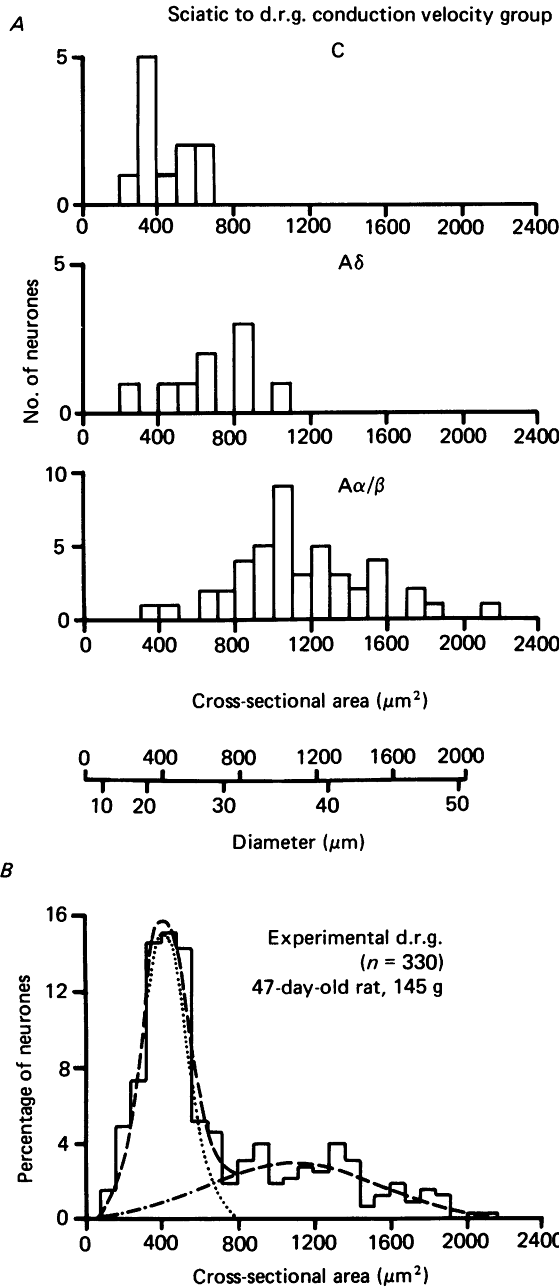

Fig. 5. *A*, frequency distribution histograms of cell sizes of neurones with peripheral axons of C-, Aδ- and Aα/β-fibre conduction velocities (measured in the sciatic to d.r.g. nerve length). *B*, percentage frequency distribution of the cross-sectional areas of all neuronal profiles containing nucleoli ($n = 330$) in 5 μm Epon sections taken every 200 μm throughout an experimental d.r.g.. The d.r.g. was taken from an animal of approximately the mean weight and age of those used in this study. Superimposed is the computer-derived best fit comprised of two overlapping normal distributions, the sum of which (shown as a dashed line, -----) is not significantly different from the observed histogram at the 5 % level, using the χ^2 test. The component normal distributions of the small dark (...) and large light (–·–·–·) cell populations were calculated from the best-fit parameters provided by the computer program. For methods see Lawson (1979).

regardless of their conduction velocity. There was, therefore, no tendency for one group of cells to be more elongated than the others.

Examples of labelled neurones and their electrophysiology

Plate 1 shows examples of the labelled neurones used in this study with their intracellularly recorded action potentials. This Plate clearly demonstrates that small

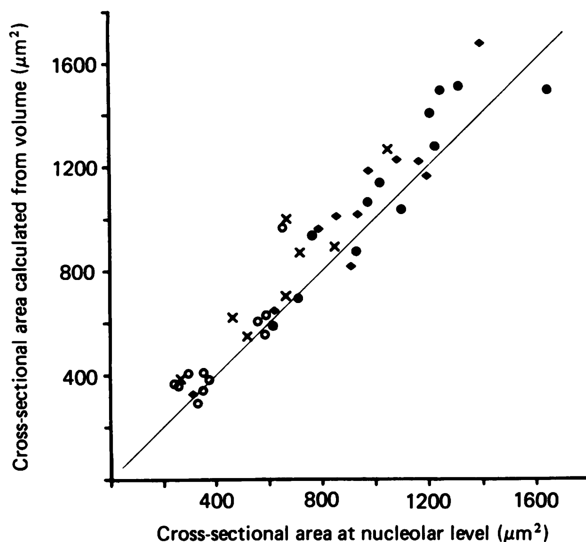

Fig. 6. The cross-sectional area of each labelled neurone directly measured at the nucleolar level is plotted against the cross-sectional area calculated (assuming the neurone to be spherical) from the volume (V) of that neurone using the equations $r = 3\sqrt{\left(\frac{3V}{4\pi}\right)}$ and area $= \pi r^2$. The volume was measured using serial sections of known thickness. The conduction velocity grouping to which each neurone belongs is indicated by the symbols: C-fibre cells (○); Aδ-fibre cells (×), Aβ cells with conduction velocity 14–30 m/s (◆); Aα cells with conduction velocity > 30 m/s (●). The line of equality indicates where the points would fall if the cross-sectional area through the nucleolus was always exactly through the middle of a spherical cell body.

neurones of similar sizes (*A*, *B* and *C*) can have very different conduction velocities (0·87, 4·6 and 31 m/s respectively for these examples). As explained above, this is probably because in this lower size range there are small neurones of the large light cell population with fast conduction velocities and also neurones of the small dark cell population, whose fibres have slow conduction velocities. Furthermore, the fastest conducting cell shown (*E*) is not the largest. Examples of the intracellularly recorded action potentials from each of these cells are included to show the type of record from which the conduction velocity was measured. The C-fibre cell had a very slow action potential, the other action potentials displayed are all quite fast.

DISCUSSION

Conduction velocities

Calculating conduction velocity from the delay between stimulating the peripheral nerve and the arrival of an action potential in the cell soma has the disadvantage of including the conduction velocity through the T junction, and along the unipolar process between the T junction and the cell body. Since there is evidence to suggest that conduction velocity along this process may be slower than in the peripheral nerve (Dun, 1955; Spencer, Raine & Wisniewski, 1973; Zenker & Hogl, 1976), the conduction velocity measurements from sciatic to d.r.g. in the present study may be an underestimate of the true peripheral nerve conduction velocity.

It was to overcome the problems of possible slowing in this unipolar process that we used two stimulus sites in the periphery. The difference in conduction time from the two sites was used to obtain a true estimate of the velocity between the tibial and sciatic stimulating electrodes, since the unipolar process delay is presumably the same from each site and is therefore cancelled out. It was therefore surprising to find that in the $A\alpha/\beta$-fibre group the velocity from tibial to sciatic was the same as that from the sciatic to the d.r.g., despite the latter possibly including such a delay in the ganglion. In contrast, many (five of eight) of the fibres with $A\delta$ sciatic nerve to d.r.g. conduction velocities conducted more slowly, often in the C-fibre range, between the tibial and sciatic stimulating electrodes. If there is a conduction delay in the T junction and/or unipolar process in the slower conducting (C and $A\delta$) fibres, then the slowing of conduction seen in these fibres in the periphery must be even more marked than our data indicate since the sciatic to d.r.g. value would have been an underestimate, and the tibial to sciatic value a true estimate of conduction velocity. A similar decrease in conduction velocity in slowly conducting afferent fibres towards the periphery has been reported for slowly conducting vagal afferent fibres in the cat (Iggo, 1958; Duclaux, Mei & Ranieri, 1976) and cutaneous fibres in the saphenous nerves of the monkey (Iggo & Ogawa, 1971). Duclaux *et al.* (1976) suggested that this was probably due to the fibres becoming progressively more myelinated centrally.

Effect of age on conduction velocity

The conduction velocities within each grouping are lower in this study than those observed by other workers in adult rats, e.g. $A\delta$ fibres have been reported as having conduction velocities in the 5–15 m/s range (Lynn & Carpenter, 1982) whereas the $A\delta$ range in the present study was 2·2–8 m/s. Although, as described above, some delay in conduction may occur in our experiments in the d.r.g., almost certainly a large part of this difference is due to the immature age (5–8 weeks) of the rats used in the present study. The data presented by Birren & Wall (1956) for the fast-conducting myelinated afferent fibres in mixed sciatic nerve show that the myelinated afferents in rats of 6–8 weeks of age were conducting at approximately half the velocity seen in the adult animal. Similar increases in conduction velocity with age have been observed by Hopkins & Lambert (1973) in non-myelinated fibres of the cervical sympathetic trunk of the rat. It is therefore possible that afferent fibres over the entire conduction velocity range, conduct more slowly at 5–8 weeks than they do in the adult rat, possibly at a half to two-thirds of the adult conduction velocity.

The grouping of afferent fibre conduction velocities observed in this study (over both tibial to sciatic, and sciatic to d.r.g. nerve lengths) is more distinct than has previously been reported. This is probably due to the narrow range of ages and weights of the rats used in this study, minimizing age-related changes in conduction velocity in each of the groups. Another possibility is that this distinct grouping may be more apparent in very young rats.

Soma sizes of neurones in different conduction velocity ranges

Cell cross-sectional area at the nucleolar level was shown to give a good indication of cell volume. There was no evidence for any of the groups of neurones defined by conduction velocity having more nearly spherical or more elongated somata than the rest of the neurones. In the subsequent discussion, therefore, the terms 'cell size' and 'cell cross-sectional area' are used synonymously.

The size distributions of the C-fibre and the $A\alpha/\beta$-fibre neuronal cell bodies closely mimic those of the small dark and large light cells in these ganglia (Lawson, 1979), the mean size and range of sizes of the C cell somata being similar to those of the small dark cell population and the mean size and range of the $A\alpha/\beta$ cell somata being similar to those of the large light cell population. This very close correlation in size suggests that the non-myelinated fibres originate from neurones belonging to the small dark population, whilst large calibre myelinated fibres arise from cells belonging to the large light cell population. The overlap between the size distribution histograms of C and $A\alpha/\beta$ cell bodies provides further evidence supporting this view since the cell size ranges of the small dark and large light cell populations overlap in the same way. However, the $A\delta$-fibre cells while being in the smaller size range, do not fall entirely within the range of the small dark cell population and indeed the mean size of the $A\delta$ cells was significantly greater than the mean size of the C-fibre cells.

These data allow several possible interpretations of the relationship of $A\delta$ somata to those of the two main cell types: first, some $A\delta$ cells could be large light neurones and some could be small dark neurones; secondly, the $A\delta$ somata could be an integral part of either the large light cell or the small dark cell population; or thirdly, $A\delta$ somata could comprise a separate (i.e. a third) population of neurones, perhaps with cytological features intermediate between the two main cell types. With respect to cell size, the third possibility seems to us the most likely since the $A\delta$ neurone population seems to have a cell size distribution whose mean and standard deviation differs from those of the large light and small dark cell size distributions. However, it is not clear how the computer analysis could provide a fit comprised of two normal distributions if a third population with respect to size is present. Perhaps the third population becomes obscured by lying in the region of overlap between the two main populations. As yet we have no information on the cytology of these neurones. Indeed it seems likely that these relatively small neurones have often been included in the morphological descriptions of the small dark cell population perhaps because they have cytological features in common with the small dark cell population. For instance, Andres (1961) must have included $A\delta$ neurones in his B (small dark) cell population, since he says that B neurones could have non-myelinated or lightly myelinated processes. Certainly the concept of subgroups within the small dark cell population is well established. For instance, substance P, somatostatin and fluoride-

resistant acid phosphatase (Hökfelt, Elde, Johansson, Luft, Nilsson & Arimura, 1976; Nagy & Hunt, 1982) have been found to exist in different neuronal subpopulations of small neurones in rat d.r.g.s. Thus, although Aδ neurones may often have been included in the small cell population in descriptive studies, clearly their size distribution is not completely consistent with this. It is anticipated that in the near future, cytological differences between Aδ neurones and C-fibre neurones will become apparent.

If the small dark cell population gives rise to peripheral C fibres, and the large light cell population gives rise to Aα/β fibres, then the overlap in cell sizes of these populations provides the explanation for some neurones in the small dark cell size range (e.g. 300 μm^2) having C fibres, while some have Aδ or Aα/β fibres, the latter presumably being very small examples of the large light cell population. This finding therefore demonstrates that the common assumption that large myelinated fibres are attached only to large neuronal somata in the d.r.g., and both the non-myelinated and small calibre myelinated fibres are attached to small diameter cells (e.g. Willis & Coggeshall, 1978), is not correct for the small diameter neurones of the large light cell population in the region of the cell size overlap of the two populations, although it is correct for the majority of cells.

In a similar study to the present one, Yoshida & Matsuda (1979) found that all neurones in mouse d.r.g.s with myelinated fibres had larger cell bodies than those with non-myelinated fibres. However, it is clear from the frequency distributions of cell cross-sectional area of the large light and small dark neurones in mouse (Lawson, 1979) and rat (present paper), that using micro-electrode recording (assuming this to be a random sampling technique), a large number of neurones would have to be studied before obtaining a significant number of small-diameter cells of the large light cell population. The present study includes a larger sample of neurones, particularly those with fast-conducting myelinated fibres than that of Yoshida & Matsuda (1979) which might explain the different interpretations of the relationship of conduction velocity to cell size.

Correlation of conduction velocity with cell size

The poor correlation of conduction velocity with cell soma size in Aα/β and Aδ neurones presumably reflects the dependence of the cell cross-sectional area on a variety of cellular features, and not only on axonal diameter. Such other features probably include the length and extent of branching of processes both peripherally and in the central nervous system, and may also be affected by the metabolic processes of the different types of neurone. The correlation was rather better for Aδ neurones than Aα/β neurones and was particularly good for C neurones. It may be that the narrow size range and closer dependence of cell size on conduction velocity particularly in the C- but also in the Aδ-fibre cells reflects a more uniform and/or limited branching of the processes in these cells as compared with the Aα/β-fibre cells (see Horch, Tuckett & Burgess, 1977; Brown, 1981).

In conclusion, in this study we have provided evidence that peripheral C fibres belong to d.r.g. neuronal somata in the size range of the small dark cell population, that peripheral Aα and Aβ fibres belong to neuronal somata with a similar mean and range of sizes to neurones in the large light cell population, and that the mean size

of somata with peripheral Aδ processes falls between the mean sizes of the two main morphological cell types.

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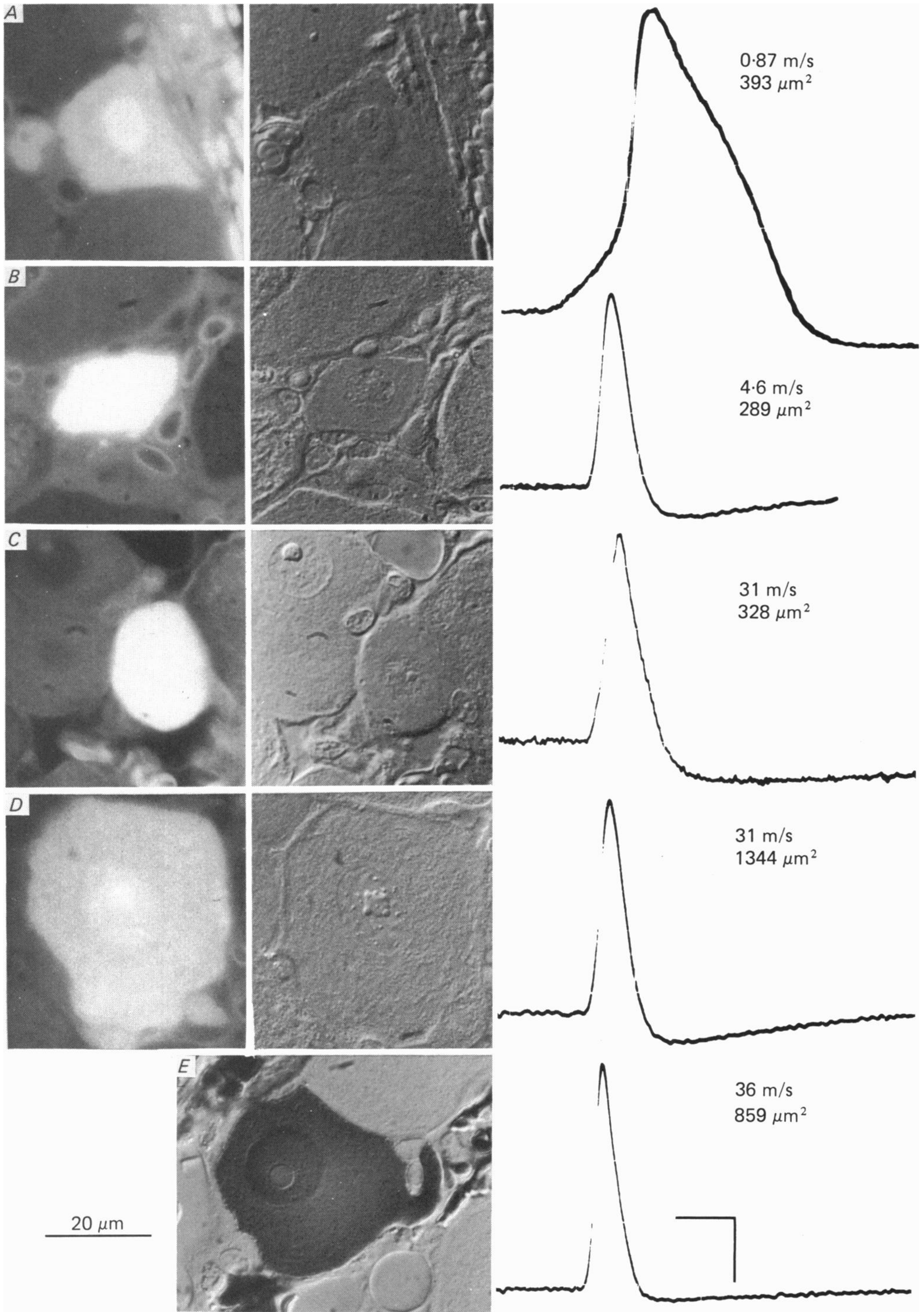
EXPLANATION OF PLATE

Examples of intracellularly labelled neurones with their somatic action potentials evoked by stimulation of the sciatic nerve. In *A*, *B*, *C* and *D* the Lucifer Yellow label is shown by fluorescence microscopy on the left, and the same neurone is photographed with interference contrast microscopy in the middle. In *E*, interference contrast microscopy clearly demonstrates the HRP-labelled neurone. On the right an example of a somatic action potential recorded from each of the cells *A* to *E* is shown. Calibration bars in *E* are 20 mV, 1 ms. The scale is the same for all the cells. Note that although *A*, *B* and *C* have similar sized neurones, the conduction velocities are very different, and also that although *C* and *D* have the same conduction velocity their cell sizes are very different.



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