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ELECTRICAL PROPERTIES OF RAT DORSAL ROOT GANGLION NEURONES WITH DIFFERENT PERIPHERAL NERVE CONDUCTION VELOCITIES

BY A. A. HARPER* AND S. N. LAWSON

From the Department of Physiology, The Medical School, University Walk, Bristol BS8 1TD

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SUMMARY

1. The electrical characteristics of individual rat dorsal root ganglion neurones were studied and related to the peripheral axon conduction velocity and morphological cell type. Neurones were divided into four groups based on the conduction velocity of their peripheral axons (Aα, 30–55 m/s; Aβ, 14–30 m/s; Aδ, 2·2–8 m/s and C < 1·4 m/s).

2. Electrophysiological parameters examined included membrane potential, action potential amplitude and duration, after-potential height and duration, input resistance and the occurrence of time-dependent rectification.

3. The mean duration of the somatic action potentials was found to be characteristic for each of the conduction velocity groupings. However, there was considerable overlap between groups. The fast-conducting (Aα) and slowly conducting (Aδ) myelinated fibres had short-duration action potentials, within the ranges 0·49–1·35 and 0·5–1·7 ms at the base respectively. The Aβ and C cells had somatic action potentials with durations in the ranges of 0·6–2·9 and 0·6–7·4 ms respectively. The longer action potential durations could be related to the presence of an inflexion on the repolarizing phase seen in a third of Aβ neurones (called Aβ_I neurones) and in all C neurones.

4. The action potential overshoot was larger in C neurones and Aβ_I neurones than in the other neurone groups.

5. The mean duration of the after-hyperpolarization was several times greater in C neurones than in A neurones. Aδ neurones displayed the shortest and greatest amplitude after-hyperpolarizations. Large, long-lasting after-hyperpolarizations were not limited to neurones displaying an inflexion.

6. The electrophysiological properties of the soma membrane of Aδ neurones closely resembled those of Aα neurones, while in several respects those of C neurones resembled the Aβ_I neuronal properties.

7. The input resistance was found to be much greater in C than in A cells, although there was no significant difference between specific membrane resistance values calculated for the different groups. A number of A cells exhibited time-dependent rectification.

* Present address: Department of Physiology, The University, Dundee DD1 4HN.

8. The data are related to the size of the cell body and its probable morphological type. The results are discussed with reference to other reports on primary afferent electrophysiology.

INTRODUCTION

Several groups of workers have investigated the electrical properties of the soma membrane of primary afferent neurones in dorsal root ganglia (d.r.g.s) and nodose ganglia in a variety of species (e.g. Sato & Austin, 1961; Bessou, Burgess, Perl & Taylor, 1971; Jaffe & Sampson, 1976; Gallego & Eyzaguirre, 1978; Yoshida & Matsuda, 1979; Görke & Pierau, 1980). To our knowledge, only two of these studies, those of Gallego & Eyzaguirre (1978) and Yoshida & Matsuda (1979), have related the electrophysiological properties of individual neurones to the sizes of their cell bodies in the ganglion. Indeed, relating electrophysiological properties to morphological cell type in the d.r.g. has proved difficult in the past, partly due to a lack of recognition that there is an overlap in cell size distributions between the two main cell types, namely the large light and small dark cell populations (see Lawson, 1979; Lawson, Harper, Harper, Garson & Anderton, 1984 for mouse and rat d.r.g.s respectively).

In the preceding paper (Harper & Lawson, 1985) data were presented indicating subdivisions of rat d.r.g. neurones on the basis of their soma size and the conduction velocities of their peripheral processes. From the distribution of their soma sizes their probable morphological cell type was deduced. The fast-conducting Aα and Aβ neurones had somata which covered the size range of the large light cell population whilst the size distribution of cells with slowly conducting (C) fibres mimicked that of the small dark population. It was not apparent to which of the populations the Aδ cells belonged, since their mean cell size was intermediate between those of the large light and small dark cell populations.

In order to explore further the interrelations of the functional and structural characteristics of these neurones, we have examined a variety of electrophysiological data from intracellular recordings of rat d.r.g. cells *in vivo* and compared these for neurones with peripheral nerve fibres in the different conduction velocity groups. In some cases neuronal soma size was also compared. The results of this study showed that the groupings of neurones based on soma morphology and peripheral axon conduction velocity were also distinguished by distinctly different active and passive membrane properties. Preliminary reports of these results have been published (Harper & Lawson, 1982; Lawson & Harper, 1984).

METHODS

Electrophysiology

The methods of preparation and most of the experimental protocol have been described in the previous paper (Harper & Lawson, 1985). The acceptance criteria for results to be included in the present study were, however, more rigorous. The cells had to exhibit stable electrical characteristics, i.e. a stable membrane potential (E_m) > 40 mV and an overshooting somatic action potential with a stable wave form evoked by stimulation of the sciatic nerve trunk. The majority (forty-eight of seventy) of neurones were successfully labelled with intracellular dyes and the morphological data on these neurones are included in the analysis in the previous paper.

The micro-electrodes were filled with either horseradish peroxidase (HRP) or Lucifer Yellow, and had high tip resistances, > 80 MΩ. To reduce the capacitance of the micro-electrode and recording circuit, the ganglion was covered with only a thin film of balanced salt solution (BSS) during recording periods and recordings were made from somata within 100 μm of the surface of the ganglion. Furthermore, the maximum capacity compensation of the recording system possible without oscillation was used throughout. Since many cells had fast-rising somatic action potentials (> 600 V/s) with amplitudes of > 80 mV, we are confident that any differences in action potential wave form characteristics observed in this study were not due to limitations imposed by the frequency response of the recording system.

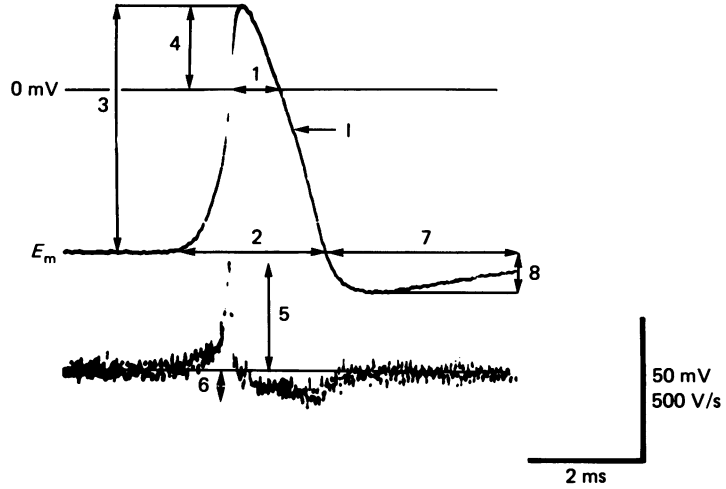

Fig. 1. An example of a somatic action potential evoked by stimulation of the sciatic nerve trunk, demonstrating the wave-form parameters measured. Upper trace: membrane potential. Lower trace: time-differentiated response, dV/dt. The action potential duration was measured both at the 0 mV level (1) and at the resting membrane potential, i.e. across the base (2). The amplitude of the action potential (3) and its overshoot beyond the 0 mV level (4) were also measured. The peak rate of depolarization (5) and repolarization (6) were measured from the lower trace. The after-potential of the action potential was characterized by measuring its amplitude from E_m (8), and also its amplitude from 0 mV ($E_m + 8$), and its time to half-decay to the resting membrane potential (7). The presence or absence of an inflexion on the repolarization phase of the action potential was also noted (I).

Action potential characteristics were measured only on action potentials elicited by stimulation of the sciatic nerve. These characteristics, were measured as shown in Fig. 1. The membrane potentials were differentiated electronically with respect to time (dV/dt). The input resistance was calculated from the maximum membrane potential response to an injected inward 20–50 ms (hyperpolarizing) current pulse the magnitude of which was sufficient to hyperpolarize the cell membrane by up to 10 mV (< 5 nA). In many cells (particularly when Lucifer Yellow filled the electrode) no input resistance measurement or current–voltage relations were available because the micro-electrode tip resistance changed during the passage of current.

All electrophysiological studies on a cell were completed before any dye was passed. For methods of injecting dye intracellularly and measurement of cross-sectional areas of labelled cells see the previous paper (Harper & Lawson, 1985). The Lucifer Yellow dye contained a low concentration (0·1 M) of LiCl while the HRP solution contained 0·2 M-KCl and 0·05 M-Tris buffer. However, since no dye was electrophoresed until electrophysiological recordings were completed and since no evidence of differences in active or passive membrane properties were noted with Lucifer Yellow as compared with HRP-filled electrodes, the results from the two types of electrode are presented together.

Statistical tests

The non-parametric Wilcoxon ranking test for two samples (two-tailed) was used to test whether differences between means were significant. This test was chosen because some groups of data had too few data points to establish whether the distribution was normal while other groups of data were clearly not normally distributed, and therefore Student's *t* test was inappropriate. For all groups of data the mean ± S.D. is given.

RESULTS

Action potentials with an after-depolarization were recorded from only two neurones and these had fast-conducting myelinated axons. Histological examination showed the unipolar process in these two cells to be very strongly stained with the injected dye whilst the soma was only weakly marked. Since the recorded wave form strongly resembled that observed in myelinated axons by Stämpfli & Hille (1976) it is probable that we were recording from a site other than the soma, probably the unipolar process, and these cells were excluded from the data analysis. In all other neurones (seventy), action potentials were followed by an after-hyperpolarization as reported by Görke & Pierau (1980).

Conduction velocity

Neurones were classified on the basis of the conduction velocity measured from the stimulation site on the sciatic nerve trunk to the recording site in the d.r.g., and the classification was supported by the threshold for stimulation. Approximately three-quarters (fifty-four of seventy) of the neurones had axons conducting at 14–55 m/s with thresholds of 0·5–10 V at 0·02 ms duration for peripheral nerve stimulation. We have arbitrarily designated cells with peripheral processes conducting at 30–55 m/s as Aα neurones (twenty-two of fifty-four) whilst those conducting within the range 14–30 m/s are called Aβ neurones (thirty-two of fifty-four). Of the remainder, eleven of seventy conducted in the range 2·2–8 m/s, requiring stimulation pulses of 0·1 ms to attain threshold. These are called Aδ neurones. The neurones which had axons conducting at $< 1{\cdot}4$ m/s and which required stimulation pulses of 1 ms duration are classified as C neurones.

Resting membrane potential

The mean resting membrane potential (E_m) measured in BSS was $50{\cdot}34 \pm 6{\cdot}16$ mV, $n = 70$. E_m was not found to have any simple relation to cell size, which indicates that the small-diameter cells which met the acceptance criteria did not sustain more damage than the large cells during impalement. Furthermore, there was no difference between the mean E_m of the different conduction velocity groups (see Table 1), which is in accordance with previous reports that E_m was not significantly different between A and C cells in d.r.g. or nodose neurones (Görke & Pierau, 1980; Gallego & Eyzaguirre, 1978).

Effect of collagenase

Collagenase digestion (0·25 % at 37 °C for 15 min) of the connective tissue sheaths surrounding and investing the ganglion was used for most experiments (twenty of twenty-two Aα neurones; twenty-two of thirty-two Aβ neurones; nine of eleven Aδ

neurones and none of the C neurones). For the remainder of the neurones, no collagenase was used since in the latter stages of the study replacement of this procedure by mechanical tearing of the connective tissue capsule was found to permit better and more stable recording from C-fibre neuronal somata.

To examine the possibility that collagenase might have affected the properties of the neuronal membrane we compared a variety of electrophysiological parameters of the collagenase-treated with the non-collagenase-treated Aβ neurones. Using the Wilcoxon ranking test to compare means, none of the following were significantly different at the 5% level in the collagenase-treated ($n = 22$) as compared with the non-treated ($n = 10$) group: E_m, action potential amplitude, action potential width at 0 mV, maximum rate of repolarization of the action potential, after-hyperpolarization relative to E_m and input resistance ($n = 16$ and $n = 5$ respectively for input resistance). We have therefore decided to pool data from collagenase-treated ganglia with those from ganglia not treated with collagenase.

Action potential characteristics

For the purposes of analysis, the Aβ neurone group was divided into two groups according to whether the somatic action potentials displayed an inflexion on the repolarization phase. Those with an inflexion are called Aβ_I, while those with no inflexion are called Aβ_0 neurones.

Action potential amplitude and overshoot

The action potential height and overshoot were compared between each of the groups of cells, that is, Aα-, Aβ-, Aδ and C-fibre cells (see Table 1). For both parameters a significant difference at the 5% level was found between C- and Aα-fibre cells but not between any of the other groups.

The action potential amplitude and overshoot was greater in those groups which displayed an inflexion on the repolarization phase of the action potential, namely the C and Aβ_I neurones. Indeed, the values for these two groups were not significantly different from one another ($P > 0{\cdot}1$). The mean overshoot was significantly greater in the Aβ_I group than in all the other A groups (Aβ_0, Aα and Aδ, $P < 0{\cdot}01$ in each case), while the Aβ_0 group had a mean similar to that of the Aδ and Aα groups ($P > 0{\cdot}1$ in both cases).

Action potential height shows no apparent relationship to cell cross-sectional area.

Action potential duration vs. conduction velocity

Since it was a consistent finding that action potential duration at 0 mV was less dependent on E_m than was duration at the base and since other workers in the field often use duration at the base, we have measured all durations both at 0 mV and at the base, (see Fig. 1).

In Table 1 the mean action potential durations in each conduction velocity group are compared with those of each of the other groups. Fig. 2 shows graphs of action potential duration plotted against conduction velocity and Fig. 3 shows examples of action potentials recorded from cells with different conduction velocities. There is considerable overlap in the range of action potential durations recorded in the

TABLE 1. A. Action potential parameters

	E_m	3	4	2	1	I	5	6
Conduction velocity group	E_m (mV)	Amplitude (mV)	Overshoot (mV)	Width (ms)		Inflexion	Peak rate (V/s)	
				Base	0 mV		Depolarization	Repolarization
Aα ($n = 22$)	52·1 ± 5·9	72·5 ± 9·6	20·4 ± 8·2	0·98 ± 0·20	0·25 ± 0·72	1/22	458 ± 187	244 ± 130
Aβ ($n = 32$)	49·4 ± 5·7	74·9 ± 10·2	25·5 ± 8·8	1·29 ± 0·59	0·46 ± 0·28	12/32	369 ± 172	182 ± 84
Aδ ($n = 11$)	51·2 ± 6·6	73·6 ± 13·1	25·5 ± 11·2	0·89 ± 0·4	0·28 ± 0·2	2/11	449 ± 181	282 ± 157
C ($n = 5$)	48·6 ± 9·2 $n = 7$	81·6 ± 6·9	31·8 ± 11·4	4·97 ± 2·16	2·28 ± 1·69	5/5	227 ± 94	73 ± 42·7
$A\beta_0$ ($n = 20$)	49·8 ± 6·2	72·0 ± 10·71	22·25 ± 8·08	1·04 ± 0·29	0·331 ± 0·157	0/20	412 ± 200	206 ± 90
$A\beta_I$ ($n = 12$)	48·3 ± 4·9	79·9 ± 7·22	31·17 ± 7·32	1·71 ± 0·65	0·685 ± 0·306	12/12	298 ± 76	142 ± 56

B. *P* values for Wilcoxon ranking tests

Aα : Aβ	0·1	> 0·1	> 0·1	< 0·05*	< 0·01*	—	> 0·05	> 0·05
Aα : Aδ	> 0·1	> 0·1	> 0·1	> 0·1	> 0·1	—	> 0·1	> 0·1
Aα : C	> 0·1	< 0·05*	< 0·05*	< 0·01*	< 0·01*	—	< 0·02*	< 0·01*
Aβ : Aδ	> 0·1	> 0·1	> 0·1	< 0·01*	< 0·01*	—	> 0·1	> 0·05
Aβ : C	> 0·1	> 0·1	> 0·1	< 0·01*	< 0·01*	—	> 0·05	< 0·01*
Aδ : C	> 0·1	> 0·1	> 0·1	< 0·01*	< 0·01*	—	< 0·05*	< 0·01*
$A\beta_I$: $A\beta_0$	> 0·1	< 0·05*	< 0·01*	< 0·01*	< 0·01*	—	> 0·1	> 0·05

Mean values ± S.D. for the electrical characteristics of neuronal somata with peripheral fibres in the conduction velocity ranges Aα (> 30 m/s), Aβ (14–30 m/s), Aδ (2·2–8 m/s) and C (< 1·4 m/s), and also Aβ neurones with ($A\beta_1$) and without ($A\beta_0$) an inflexion (I) on the repolarization phase of the action potential. Above the Table the letters and numbers refer to Fig. 1, which indicates exactly how each measurement was made. A. Mean values for: the resting membrane potential (E_m), action potential amplitude, action potential overshoot, action potential duration at the base and at 0 mV, occurrence of an inflexion on the repolarization phase and peak rate of depolarization and repolarization of the action potential are given. (Values for E_m of two C neurones without overshooting action potentials are included.) In B, the probability values for the null hypothesis tested by the Wilcoxon ranking two-sample probability test are given. * indicates a significant difference between means (i.e. $P < 0·05$). Column headings are as for A.

different velocity groups. The same over-all pattern emerges in Fig. 2 whether duration at the base or at 0 mV is used, namely that Aα and Aδ neurones had fast action potentials, with mean durations which were not significantly different from each other, but both of which were significantly faster than those of Aβ and C neurones. Some Aβ neurones had fast, and some had relatively slow action potentials, with a general trend for the slower ones to have the slower conducting fibres. The C neurones had a very wide range of action potential durations, the mean being

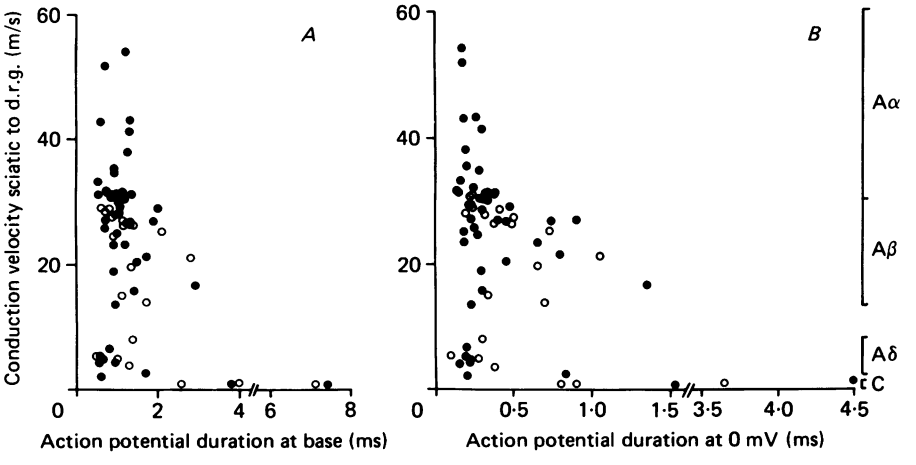

Fig. 2. Duration of somatic action potentials plotted against the conduction velocity from the sciatic nerve to the d.r.g. cell body. Action potentials were evoked by stimulation of the sciatic nerve trunk. In *A* the duration was measured at the base and in *B* it was measured at 0 mV and plotted on an expanded time scale. On the right, the conduction velocity ranges of Aα, Aβ, Aδ and C fibres are indicated. Neurones labelled with intracellular dyes are indicated with filled circles (●), unlabelled neurones are shown with open circles (○).

significantly slower than those of the other groups. For examples of all the above see Fig. 3.

Many more neurones displayed an inflexion on the falling phase of the action potential in the neurone groups with the slower mean action potential durations, i.e. the C and Aβ cells, than in those with faster action potentials, the Aα and Aδ neurone groups (see Table 1 and Fig. 3). Within the Aβ group the mean duration of the action potentials was significantly longer for Aβ_I than Aβ_0 neurones (see Table 1 and Fig. 3). That is, the slower action potentials within the Aβ group were due to the presence of an inflexion on the falling phase. There was no significant difference between action potential durations of Aβ_0 and either Aα or Aδ neurones whether values for 0 mV or the base of the action potential were compared. Thus, the slower action potential durations seen in C and Aβ_I neurones seem to result from a delay or inflexion on the falling phase. Very few (two of thirty-two Aβ and two of five C) neurones displayed an inflexion on the depolarization phase, and this is therefore unlikely to be a major cause of the slower action potentials of Aβ_I and C neurones.

The mean conduction velocity of Aβ_I neurones ($24{\cdot}1 \pm 3{\cdot}6$, $n = 12$) was very close to that of the Aβ_0 neurones ($24{\cdot}0 \pm 5{\cdot}5$ m/s, $n = 20$), and therefore the presence or

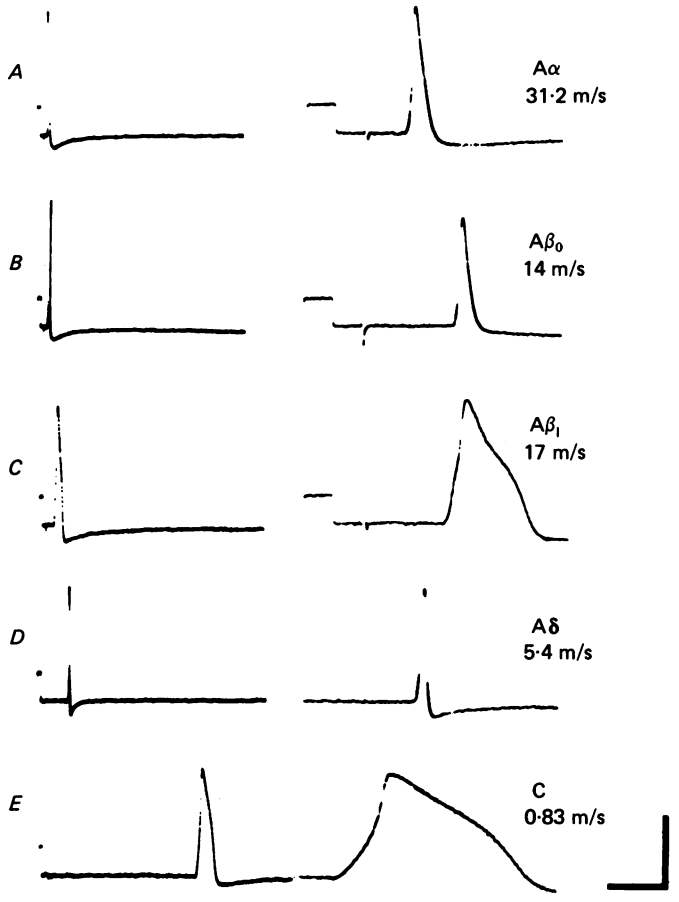

Fig. 3. Examples of somatic action potentials evoked by stimulation of the sciatic nerve trunk and recorded from neurones in the four conduction velocity groups. In *A*, *B* and *C* the nerve stimulation artifact is seen 1 ms after the end of the calibration pulse. For each cell two records are shown, the one on the right is on an expanded time scale. Calibration pulse: vertical 50 mV; horizontal 20 ms on the left and 2 ms on the right. The conduction velocity of the peripheral axon is given on the right.

absence of an inflexion was not related to conduction velocity. Within the $A\beta_I$ group, however, the slowest conducting neurones had the slowest action potentials.

Action potential duration vs. cell size

There is no obvious relation of cell size to action potential duration within the $A\alpha$, $A\beta$ and $A\delta$ conduction velocity groups, but we have too little data to make any comment about the C-fibre group. The mean cross-sectional area of the $A\beta_I$ neurones was very close to and not significantly different from the mean for the $A\beta_0$ neurones ($A\beta_I$: $1084 \pm 316\ \mu m^2$, $n = 6$; $A\beta_0$: $1110 \pm 331\ \mu m^2$, $n = 13$, $P > 0{\cdot}1$).

Peak rates of depolarization and repolarization vs. conduction velocity

Peak rates of depolarization and repolarization of the action potential are listed in Table 1. Not surprisingly the long duration of the action potentials of the Aβ and even more obviously the C cells is reflected in the lower peak rates of repolarization of the action potentials. There is a significantly lower peak rate of repolarization in C cells than in Aα and Aδ neurones. The depolarizing and repolarizing rates in Aα neurones closely resembled those of the Aδ cells, as expected, since their action potential durations were similar.

After-hyperpolarization amplitude and duration

For the three groups of A neurones the mean after-hyperpolarization (measured from E_m) was greatest for Aδ and least for Aα neurones, these two groups showing a significant difference from one another.

The mean duration, measured as time to half-decay (see Fig. 1, 7), varied between the groups as follows, from the shortest to the longest: Aδ, Aα, Aβ_0, Aβ_I and C neurones. For statistics see Table 2B, and as follows: Aβ_0 neurones showed a significant difference from the Aδ and C neurones ($P < 0{\cdot}01$, $P < 0{\cdot}05$) and the Aβ_I neurones had significantly longer durations than Aα and Aδ neurones ($P < 0{\cdot}02$, $P < 0{\cdot}01$ respectively).

Considering all neurones with an inflexion on the falling phase of the action potential, it was found that those with longer action potentials also tended to have a longer after-potential. This was particularly noticeable within the C and Aβ groups. No such relation was apparent in neurones which displayed no inflexion, all of which had fairly fast action potentials, and some of which also displayed long after-potentials.

Frequency-dependent effects on action potential characteristics

For each cell initially a single individual stimulus was applied to the sciatic nerve to evoke an intracellular action potential. Thereafter the stimulus frequency was set at 0·5 Hz. In Aα and Aβ neurones, the stimulation frequency could be increased to several hundred hertz without any measurable effect on conduction velocity or action potential height or duration, and thus 0·5 Hz had no noticeable effect on these parameters. However, stimulation frequencies of 0·5 Hz often caused dramatic changes in the somatic action potential wave form of C cell somata. Two examples of this behaviour are shown in Fig. 4. The somatic action potential became wider with successive stimuli, primarily due to the decrease in rate of repolarization and accentuation of the inflexion on the falling phase (see Fig. 4*A* and *B*). These effects were not attributable to membrane potential changes, they were reversible on cessation of stimulation and they were accompanied by a more slowly developing decrease in action potential amplitude. Another type of behaviour exhibited by C cell somata with frequent stimulation was that the action potential often failed to invade the soma, only on axon spike being recorded in these instances, for example see Fig. 4*C*.

Passive membrane properties

Input resistance

Table 2 lists the mean values of input resistance for each group of neurones. The mean for Aδ neurones was significantly higher than that for Aβ neurones, otherwise no significant differences were seen between the subgroups of A cells. The mean input resistance of C cells, however, was at least double that of each of the A-fibre groups (highly significant), and it was also significantly higher than the mean of all A cells grouped together ($P < 0{\cdot}01$). Over-all, the value ranged from 1 MΩ (in a large Aα cell) to 50 MΩ (in a small C cell).

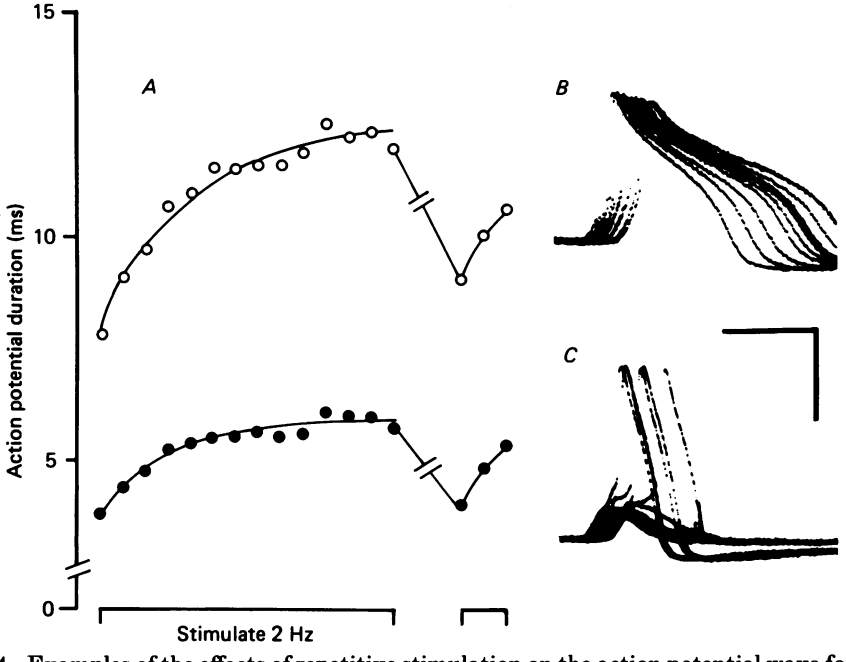

Fig. 4. Examples of the effects of repetitive stimulation on the action potential wave form of C cell somata. *A*, the increase in duration of the action potential, measured both at the 0 mV level (●) and at the base (○) as a function of stimulus number. The break in the abscissa represents a cessation of stimulation for 5 s. *B*, trace showing the increase in latency and duration of the action potential with successive stimuli (stimulation frequency 2 Hz). *C*, another type of behaviour seen in C cells on repetitive stimulation. Stimulation at 2 Hz increased the latency and the conducted action potential often failed to invade the soma, becoming an abortive axon spike. Calibration bar: vertical, 50 mV; horizontal, 5 ms.

Specific resistance

The specific resistance, R_m, of the somatic membrane was calculated from the input resistance and somatic surface area (calculated from cross-sectional area at the nucleolar level, assuming the cell body to be a smooth spheroid). The results are presented in Table 2B. There was no statistical difference in mean R_m values between any of the conduction velocity groups for which adequate data were available for comparison (Aα, Aβ and C groups).

TABLE 2. A. After-potential and input resistance parameters

Conduction velocity group	E_m+8 to 0 mV (mV)	8 to E_m (mV)	7 Dura-tion (ms)	Input resistance (MΩ)	Specific membrane resistance (Ω cm²)	Time-dependent rectification
	After-potential					
Aα	58·2±5·5 $n=22$	5·84±2·97 $n=22$	3·79±3·33 $n=21$	12·5±11·3 $n=15$	609±356 n = 13	4/17
Aβ	57·2±5·7 $n=32$	7·90±4·2 $n=32$	5·79±4·64 $n=32$	8·7±5·0 $n=20$	398±269 $n=13$	8/17
Aδ	60·8±6·84 $n=11$	9·80±2·6 $n=11$	1·99±1·06 $n=11$	15·0±2·1 $n=4$	311±143 $n=3$	2/4
C	58·0±9·7 $n=7$	8·20±5·1 $n=5$	12·87±8·4 $n=4$	37·1±13·9 $n=6$	583±225 $n=4$	0/6
$A\beta_0$	56·3±5·9 $n=20$	6·75±3·19 $n=20$	4·85±4·8 $n=20$	9·3±5·3 $n=14$	362±248 $n=9$	5/12
$A\beta_I$	58·75±5·3 $n=12$	9·92±5·02 $n=12$	7·37±4·08 $n=12$	7·4±4·6 $n=6$	480±336 $n=4$	3/5
			B. P values for Wilcoxon ranking tests			
Aα : Aβ	> 0·1	> 0·05	> 0·1	> 0·1	> 0·1	—
Aα : Aδ	> 0·1	< 0·01*	> 0·05	> 0·1	—	—
Aα : C	> 0·1	> 0·1	< 0·02*	< 0·01*	> 0·1	—
Aβ : Aδ	> 0·1	> 0·1	< 0·01*	< 0·02*	—	—
Aβ : C	> 0·05	> 0·1	> 0·05	< 0·01*	> 0·1	—
Aδ : C	> 0·1	> 0·1	< 0·01*	< 0·01*	—	—
$A\beta_I : A\beta_0$	> 0·1	< 0·05*	> 0·1	> 0·1	> 0·1	—

Mean values ± S.D. for the electrical characteristics of neuronal somata with peripheral fibres in the conduction velocity ranges Aα (> 30 m/s), Aβ (14–30 m/s), Aδ (2·2–8 m/s) and C (< 1·4 m/s), and also Aβ neurones with ($A\beta_I$) and without ($A\beta_0$) an inflexion (I) on the repolarization phase of the action potential. Above the Table the letters and numbers refer to Fig. 1, which indicates exactly how each measurement was made. A. Mean values for: after-potential amplitude to 0 mV and to E_m after-potential duration to half-decay, input resistance, specific membrane resistance and occurrence of time-dependent rectification. B. The probability values for the null hypothesis tested by the Wilcoxon ranking two-sample probability test are given. * indicates a significant difference between means (i.e. $P < 0·05$). Column headings are as for A.

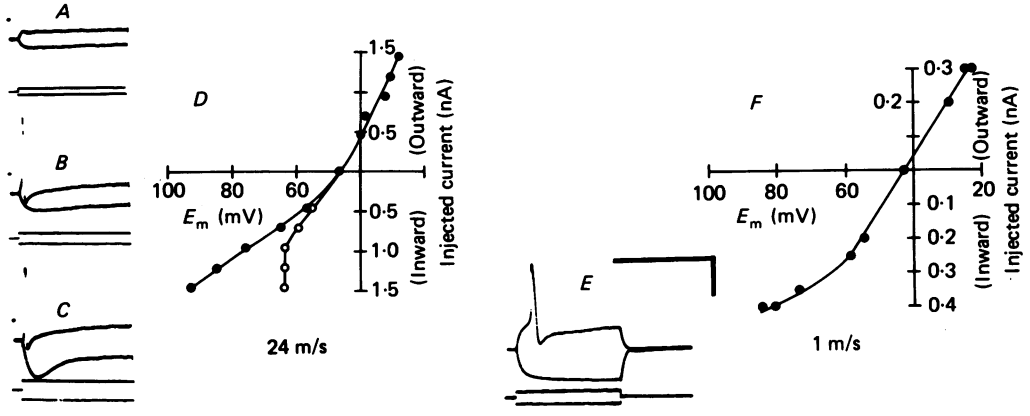

Fig. 5. Current–voltage (*I–E*) characteristics of two neurones whose sciatic to d.r.g. conduction velocities are indicated in m/s. *A–D*, characteristics of an Aβ neurone, showing time-dependent rectification becoming more apparent with increasing hyperpolarization. *E* and *F*, a C-fibre neurone, showing no time-dependent rectification. *A*, *B*, *C* and *E* show examples of oscilloscope tracings. Upper trace: voltage responses, depolarization upwards. Lower trace: injected current. Calibration bars: vertical 40 mV, 10 nA (in *A*, *B* and *C*), 4 nA (in *E*), horizontal 50 ms. In *D* and *F*, The *I–E* curve for each neurone is plotted. Peak hyperpolarized voltage response, (●), steady-state response, measured 50 ms after start of the current pulse (○).

Rectification

In some cells time-dependent rectification was seen with intracellular current injection strong enough to hyperpolarize the cell by 10–20 mV. The peak voltage response decayed to a steady-state value which was largely independent of injected current magnitude (see Fig. 5*A–D*). The time of onset of this decay decreased with increasing current strength, and the extent of the decay varied markedly between individual neurones. Such time-dependent rectification was observed in a small proportion of Aα, Aβ and Aδ neurones (see Table 2), but not in C neurones (see Table 2, and for example see Fig. 5*E* and *F*). It is possible, however, that some of the cells which did not display this behaviour might have done so if stronger hyperpolarizing current had been used. Unfortunately, in many instances the electrode characteristics prevented the injection of current of high intensity.

DISCUSSION

From the sizes of neuronal somata and the conduction velocities of their peripheral fibres it has been proposed that C fibres are the processes of neurones of the small dark cell population in rat d.r.g.s, while the Aα and Aβ fibres are the processes of neurones of the large light cell population (Harper & Lawson, 1985). In the present paper a variety of electrophysiological parameters of neuronal somata with peripheral fibres in the different conduction velocity groups (Aα, Aβ, Aδ and C) were compared, in order to ascertain if the somata of these groups, and hence of the large light and small dark cell populations, have different active and/or passive membrane properties.

One of the most marked differences between somata with different peripheral fibre conduction velocities was the duration of the somatic action potential. Most previous studies have compared action potentials in C fibre somata with those in somata of all other fibres grouped together as A neurones. Using a similar analysis, our results show that C cells have a much slower somatic action potential than A-fibre cells with a greater likelihood of an inflexion on the falling phase. Thus, our results show similar action potential conformations in C and A neurones to those reported for pigeon d.r.g. cells *in vivo* (Görke & Pierau, 1980), and for rat cells *in vitro* (Williams & Zieglgänsberger, 1981). The results of Yoshida & Matsuda (1979) also showed much longer-duration action potentials in their slowest-conducting mouse d.r.g. neurones *in vitro*.

In the present study, the action potential duration was shown to be related not only to whether the peripheral fibres had conduction velocities in the C or A range but also to their specific group (Aα, Aβ, Aδ or C). Thus Aα and Aδ cells had short-duration action potentials, about a third of Aβ cells had action potentials of much greater duration primarily due to an inflexion on the falling phase, and C neurones had a wide range of somatic action potential durations, up to several times the duration of any other group, and these potentials were characterized by a prominent hump on the falling phase of the action potential.

Neurones with short mean duration somatic action potentials (Aα and Aδ neurones) were also those whose neurones rarely displayed an inflexion on the falling phase of the action potential. Furthermore, the mean action potential duration of the Aβ neurones which displayed no inflexion was not significantly different either from the Aα or Aδ means. Fast somatic action potentials have previously been described in somata with fast A-fibre conduction velocities in d.r.g. neurones of the cat, mouse, pigeon and rat (Bessou *et al.* 1971; Yoshida & Matsuda, 1979; Görke & Pierau, 1980; Williams & Zieglgänsberger, 1981). However, it has not to our knowledge previously been demonstrated that Aδ neurones have very fast somatic action potentials, although the data (see their Fig. 3) of Görke & Pierau (1980) indicate that similar characteristics are present in a population of pigeon d.r.g. neurones with a comparable conduction velocity range. Perhaps the relation between action potential duration and conduction velocity is more apparent in our study than in others since conduction velocity increases with age (see Harper & Lawson, 1985) and in our study a very narrow range (5–8 weeks) of rat ages was used.

Long-duration action potentials in Aβ and C neurones were found to be associated with the presence of an inflexion on the repolarization phase. Slow action potentials have previously been reported in slowly conducting (C fibre) d.r.g. neurones in mouse, pigeon and rat (Yoshida & Matsuda, 1979; Görke & Pierau, 1980 and Williams & Zieglgänsberger, 1981). An inward calcium current has been proposed to underlie the presence of the inflexion in cultured d.r.g. neurones of the chick and mouse (Matsuda, Yoshida & Yonezawa, 1976; Dichter & Fischbach, 1977; Yoshida, Matsuda & Samejima, 1978; Heyer & Macdonald, 1982) and in pigeon d.r.g. neurones *in vivo* (Görke & Pierau, 1980). Kostyuk, Veselovsky & Fedulova (1981), using an isolated membrane preparation of immature rat d.r.g. neurones found that a specific calcium component was present to some extent in all investigated neurones, although its magnitude varied from cell to cell. If the presence of an inflexion is indicative of a relatively large inward calcium current in these neurones, then on the basis of our present results the somatic action potentials of most or all C neurones and

approximately a third of the Aβ neurones might be expected to display a marked calcium dependence.

Although we have shown that action potential duration is closely related to the conduction velocity group of the peripheral axon, this is not a simple relation and there is considerable overlap of duration between the groups. Somatic action potential duration cannot therefore be used as a sole indication of the conduction velocity group with the possible exception of the C-fibre cells with very long-duration action potentials.

Previously it has been reported that the amplitude of the somatic action potentials in C neurones is significantly greater than in A neurones in d.r.g. of, for instance, the cat and the pigeon (Bessou *et al.* 1971; Görke & Pierau, 1980). This was confirmed in the present study, since the mean amplitude and overshoot was larger in C cells than in other neurones. However, we also found that the mean overshoot of the $A\beta_I$ neurones was very close to that of the C neurones, while the value for $A\beta_0$ neurones was much closer to the Aα and Aδ neurone value. This is interesting in view of the proposed involvement of a calcium inward current in neurones displaying an inflexion, since the calcium equilibrium potential is considerably more positive than that for sodium (see McBurney & Neering, 1983) and this could thus account for the larger overshoot in such cells. Yoshida *et al.* (1978) also found in mouse d.r.g. neurones *in vitro* that the average spike amplitude was smaller in neurones with fast action potentials than in those with slower action potentials, many of which had a substantial calcium component.

The amplitude of the after-hyperpolarization was slightly smaller in Aα neurones than the other groups, being significantly different from the Aδ group which had the biggest amplitude. These results therefore differ from those of Görke & Pierau (1980) and Yoshida & Matsuda (1979) who found larger after-hyperpolarizations in the slowest conducting (C) neurones in pigeon d.r.g.s *in vivo* and mouse cultured d.r.g. neurones *in vitro* respectively. However, the mean duration of the after-hyperpolarization was found to be longest in C neurones, in accordance with the results of both Görke & Pierau (1980) and Yoshida & Matsuda (1979). Most, but by no means all, of the neurones with the largest or longest after-hyperpolarizations were also neurones which displayed an inflexion on the action potential. There was therefore no clear correlation between presence of an inflexion and magnitude of the after-hyperpolarization.

The input resistance of cells with peripheral C fibres was more than double that of any other group. The mean values for the A and C cell groups are similar to those reported for pigeon d.r.g. neurones (Görke & Pierau, 1980), although Williams & Zieglgänsberger (1981), did not observe any difference in values between A and C cell neurones in the rat d.r.g. *in vitro*.

The specific membrane resistance values calculated showed no significant difference between conduction velocity groups. The values were comparable with those reported by Scott & Edwards (1980) for mouse d.r.g. neurones, but were much lower than those reported for cultured d.r.g. neurones and cat nodose neurones (Brown, Perkel, Norris & Peacock, 1981 and Gallego & Eyzaguirre, 1978, respectively). It seems likely for two reasons that the values calculated in the present study underestimate R_m. First, it was assumed that current flow was limited to the somatic membrane although

it is probable that some current flows through the unipolar process (Brown *et al.* 1981). Secondly, Pannese, Gioia, Caradente & Ventura (1983) have shown that perikaryal projections increase the surface area of d.r.g. somata by approximately 40%, and the surface area value we have used in the calculations is therefore probably an underestimate.

Time-dependent rectification was first observed by Ito (1957) in toad spinal ganglion neurones. Subsequently, this behaviour has been reported in many other types of primary afferent neurone in a variety of species (e.g. Jaffe & Sampson, 1976; Gallego & Eyzaguirre, 1978; Görke & Pierau, 1980; Belmonte & Gallego, 1983; Mayer & Westbrook, 1983). Although several reports agree to the presence of time-dependent rectification in neurones with A-fibre and C-fibre conduction velocities, Gallego & Eyzaguirre (1978) and Görke & Pierau (1980) reported that this behaviour was more marked in A cells, higher currents being required to elicit this behaviour in C cells. In the present study, time-dependent rectification was observed only in A cells. This may have been because, as reported by others, the threshold potential for this behaviour is more negative in C than in A cells. It was not possible to test rigorously for this in the present study since the dye-filled micro-electrodes did not allow the injection of large hyperpolarizing currents in many cells. Mayer & Westbrook (1983) discussed the possibility that the time-dependent rectification they saw in cultured mouse d.r.g. neurones might have the role of counter-balancing the effects on membrane potential of calcium entry during an action potential. The present results, however, do not indicate a clear relation between this type of rectification, and the presence of an inflexion (probably due to substantial calcium current) on the falling phase of the action potential.

It was proposed by Harper & Lawson (1985) that large light neurones have Aα and Aβ peripheral nerve conduction velocities. If this is correct, the majority of large light neurones (Aα and Aβ_0 neurones) display fast somatic action potentials with no hump on the falling phase, fairly short after-hyperpolarizations, and a low input resistance. A small proportion of large light neurones (the Aβ_I neurones) display slower action potentials with an inflexion on the falling phase, more pronounced after-hyperpolarization and a larger action potential overshoot than other large light neurones. Time-dependent rectification exists in a proportion of these Aα and Aβ neurones. The Aβ_I neurones therefore appear to be electrophysiologically rather different from the rest of this population. However, the mean soma cross-sectional area of Aβ_I neurones was almost identical to the means for Aβ_0 neurones, for the over-all A population and for the large light cell population (see Harper & Lawson, 1985). There is therefore no evidence that these Aβ_I neurones might be a distinct subgroup of the large light neurone population with respect to their soma size. Harper & Lawson (1985) proposed that peripheral C fibres are the processes of small dark neurones. If this is the case, small dark neurones display long-lasting action potentials with a pronounced inflexion on the falling phase, a greater overshoot, longer after-hyperpolarization and much greater input resistance than seen in somata of the fast-conducting neurones, and a smaller likelihood of displaying time-dependent rectification than other neurones. There is no clear indication at present about which of the main types of neurone possess peripheral Aδ fibres (Harper & Lawson, 1985). The data on the soma

membrane electrophysiological characteristics of Aδ neurones are very similar in all respects to those of Aα neurones, the means of the electrical parameters of the two groups differing statistically in only one respect, that is the Aδ neurones displayed a larger mean after-hyperpolarization. The clearest differences between the Aα and Aδ neurones remain the conduction velocities and the ranges of their neuronal soma sizes (Harper & Lawson, 1985).

In conclusion, the present study has enabled certain electrical characteristics of d.r.g. neuronal soma membranes to be associated with the peripheral fibre conduction velocities. Furthermore, some indication as to differences in soma membrane properties of large light and small dark neurones are now becoming apparent.

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