J. Physiol. (1984), **357**, *pp.* 453–483
With 11 *text-figures*
Printed in Great Britain

AN INVESTIGATION OF THRESHOLD PROPERTIES AMONG CAT SPINAL α-MOTONEURONES

BY B. GUSTAFSSON AND M. J. PINTER

From the Department of Physiology, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden

(*Received 13 April 1984*)

SUMMARY

1. In anaesthetized cats, thresholds for long (rheobase) and brief duration current pulses have been obtained from spinal motoneurones and compared with other cell parameters and membrane properties.

2. Rheobase showed only weak over-all relationships with conduction velocity and with cell size, estimated as the total capacitance of individual motoneuronal equivalent cylinders. Rheobase showed a clear tendency to vary inversely with after-hyperpolarization (a.h.p.) duration and was strongly correlated with the input conductance and with the inverse of the membrane time constant. However, the range of rheobase current exceeded that of input conductance by almost a factor of 2.

3. Part of this range discrepancy arose because threshold depolarization tended to increase with rheobase current. Thus, among motoneurones grouped according to rheobase magnitude (three groups), those within the lowest rheobase group had threshold depolarizations about 6 mV on average lower than those within the highest rheobase group. Even though this difference was not directly related to resting potential differences between the groups, further analysis suggested that it may have arisen secondarily to impalement-induced depolarization.

4. The finding that experimentally estimated threshold depolarizations in individual motoneurones were generally larger than those predicted by the product of input resistance and rheobase indicated that a subthreshold rectification process also contributed to the range of rheobase. The difference was largest in the low-rheobase group and smallest in the high-rheobase group. Because these differences were proportional to the differences in input resistance between the separate motoneurone groups, it is suggested that the magnitude of the current underlying the rectification process does not differ systematically among motoneurones.

5. Within groups of motoneurones classified on the basis of rheobase or a.h.p. duration, significant correlations existed between rheobase current and input conductance. An analysis of variance indicated that even within such functional subgroups of motoneurones, rheobase was appreciably better correlated with membrane time constant than with estimated cell size.

6. Although showing a range approximately half that of rheobase, the brief current threshold was similar to rheobase in its relations with total cell capacitance, a.h.p. duration and the inverse of membrane time constant. An analysis utilizing compart-

mental models of individual motoneurones showed that the range of brief-pulse threshold could be explained on the basis of variations in neuronal surface area, dendritic geometry and experimentally observed threshold depolarizations.

7. The variation of the rheobase/brief-pulse threshold ratio conformed rather closely to that expected from the membrane time constant. However, this was to some extent fortuitous, the effect of subthreshold rectification being in part cancelled by a time-dependent change in voltage threshold.

8. It is proposed that the variation of excitability both across the motoneurone pool and within functional subgroups is governed chiefly by the variation in specific membrane resistivity and other membrane properties that co-vary with resistivity. Cell size itself, and factors co-varying with it, appear to play a more limited role.

INTRODUCTION

Evidence has been presented previously showing the existence of a systematic variation in intrinsic, largely size-unrelated, cellular properties determining input resistance among cat spinal motoneurones (Gustafsson & Pinter, 1984). It may be expected that this variation will function importantly in deciding the range of motoneurone excitability in response to synaptic currents. Nevertheless, existing evidence indicates that the range of motoneurone input resistance does not account for the larger range in excitability as measured by rheobase current (Fleshman, Munson, Sypert & Friedman, 1981). It is also known that certain types of motoneurones innervating fast-twitch muscle exhibit a rise in current threshold during applied current ramp stimulation (Burke & Nelson, 1971). Such results suggest differences in active cellular properties determining threshold conditions for spike initiation which may be related to the functional aspect of particular motoneurones (Fleshman *et al.* 1981), such as seems to be the case with passive membrane properties (Gustafsson & Pinter, 1984). One may imagine at least two ways in which such differences could arise. Since spike generation in motoneurones is initiated by a spike in the initial segment, it is evident that any systematic differences in threshold properties there will affect the over-all range of motoneurone excitability (Pinter, Curtis & Hosko, 1983). Conductance processes located in the soma-dendritic region which may be activated subthreshold to the initial segment spike also need to be considered since they could affect the absolute amount of current needed to evoke motoneurone firing but not necessarily the initial segment voltage threshold.

The present paper describes experimental results concerning the factors underlying variation of rheobase current threshold among cat motoneurones. In order to obtain threshold currents unaffected by differences in specific membrane resistivity and time-dependent changes in threshold conditions, thresholds for very brief current injections at the soma have also been determined and have been compared with rheobase currents obtained from the same cells. The results support the notion that intrinsic differences in threshold conditions unrelated to cell size exist for both types of activation among cat motoneurones and that the differences are probably related to motoneurone function.

METHODS

Data presented in this paper were obtained during experiments described previously (Gustafsson & Pinter, 1984), and that paper can be consulted for details concerning preparation and general experimental conditions and procedures regarding the measurements of motoneurone passive properties. Only procedures directly related to measurement of current threshold will be presented here.

Following impalement and a period of membrane potential stabilization, current thresholds for 0·5 ms depolarizing pulses were obtained. Spike amplitude at the time of threshold measurement was determined after increasing the current strength in order to minimize the initial segment–soma-dendritic spike delay. Threshold for long (50–60 ms) depolarizing pulses was determined next whereafter the short pulse measurement, as well as the spike amplitude measurement, was repeated. To obtain repeatable estimates of these thresholds, stable resting potentials were found to be absolutely necessary. Changes in either resting or spike potentials were reflected in changes of the current thresholds, most clearly in the short pulse threshold probably due to its numerically greater value and its smaller sensitivity to concomitant changes in input resistance and time-dependent conductance processes. Rheobase data presented here represent values that were bracketed by essentially identical short pulse threshold values obtained at the highest resting potentials observed in particular motoneurones.

The ultimate aim of an investigation such as this is to establish the current and voltage thresholds present in motoneurones at membrane potentials unaffected by synaptic activity and changes induced by micro-electrode impalement. While the first factor might be minimized by using heavily anaesthetized animals, the second factor is, as shown in the preceding paper (Gustafsson & Pinter, 1984), likely to have lowered the membrane potentials from pre-impalement levels. Since threshold values in an individual cell varied with changes in resting potential (see Results), the values given here are likely to be underestimates. On the other hand, the more direct aim of the present study was to compare threshold properties of motoneurones of different types, and it would seem sufficient to establish that a factor such as micro-electrode-induced damage has not introduced any selective bias to a particular motoneurone type. The state of the cells at the time of measurement of threshold values was assessed from the spike amplitude, the requirement being values exceeding 80 mV, or for cells with high resistance and long after-hyperpolarization (a.h.p.) durations, generally above 85 mV due to their larger action potential overshoots in comparison with the other types of motoneurones (Kuno, 1959; Burke & Nelson, 1971; Pinter *et al.* 1983).

In a majority of the motoneurones, membrane potential was followed on a Grass polygraph. The membrane potential at the time of threshold measurements was assessed from the absolute membrane potential measured following withdrawal of the micro-electrode from the cell, under the assumption that the extracellular reference potential measured had remained constant between the threshold measurements and the pipette withdrawal. These membrane potentials averaged −73·6 mV (−67 to −83 mV, $n = 91$) and the corresponding spike amplitude was 91·4 mV which was close to the average spike amplitude of 90·7 mV for the whole population of neurones ($n = 153$). The overshoot amplitude of 17·8 mV (91·4–73·6) at the time of threshold measurements was also close to that of 18·3 mV (89·5–71·2) obtained when using spike amplitudes and membrane potentials measured just prior to pipette withdrawal ($n = 116$, spike amplitude > 80 mV). These results suggest that the membrane potentials at the time of threshold measurements should be reasonably correct and that the value of 73·6 mV approximates that of the whole population of cells. This value should be compared to probable pre-impalement resting potentials of −80 mV or more (Brock, Coombs & Eccles, 1952; see also Jack, 1979; Gustafsson & Pinter, 1984).

In order to assess the degree of variability introduced into the threshold measurements by factors such as micro-electrode-induced injury, threshold values and other parameters have been compared with membrane potential and spike height. Of these two measures, the membrane potential would seem the most directly relevant, but is presently not available for the whole population of cells and its estimation may also be affected by problems related to variations in tip potentials. The spike height values are less affected by these technical problems and are available for the whole population of motoneurones. It is, on the other hand, not certain to which extent spike height can be used as an accurate measure of membrane potential. A linear regression between membrane potential and spike height, using values obtained just prior to pipette withdrawal, gave a significant correlation ($r = 0·68$) with a slope of 0·73 mV/mV (spike height/membrane potential) ($n = 116$,

spike height > 80 mV), but the plot showed a considerable scatter of spike height amplitude for a given membrane potential. As will be described in the Results section the motoneurones were subdivided into three groups, roughly corresponding to motoneurones projecting to types f.f. (III), f.r. (II) and s. (I) muscle units, respectively (Burke, 1981). When examined separately for these three groups of motoneurones, the overshoot averages were 16·3 mV (III), 18·4 mV (II) and 20·6 mV (I). This variation in the overshoot between different types of motoneurones (see above) may thus be partly responsible for the scatter in the over-all membrane potential–spike height relation. The membrane potential–spike height relations also differed between these groups, the slopes being 1·14 (I, $r = 0{\cdot}81$; $n = 38$), 0·99 (II, $r = 0{\cdot}81$; $n = 37$) and 0·54 (III, $r = 0{\cdot}58$; $n = 41$). The scatter of spike heights for a given membrane potential was however still considerable. It is thus clear that spike height should be used with some care as a measure of membrane potential, especially when used for the whole population of motoneurones. One may on the other hand not exclude that errors in the membrane potential measurements underlie part of the scatter.

Correlation coefficients given in the Results section were calculated according to a least-squares method (linear). Mean values are presented ±S.D., and unless otherwise stated correlation coefficients were significant at $P < 0{\cdot}001$.

RESULTS

Relation between rheobase current and estimated cell size

The current magnitude necessary to depolarize from resting potential to the critical firing level using long (50–60 ms) current pulses injected through the recording micro-electrode was examined in 153 motoneurones with stable spike amplitudes exceeding 80 mV. This rheobase current varied in magnitude from around 2 to 32 nA, as illustrated in Fig. 1 *A*. In Fig. 1 *B* and *C* rheobase current values are plotted against spike height (*B*) and membrane potential (*C*) and it may be seen that this variation in current was present at almost any given spike height or membrane potential, suggesting that the width of the distribution was minimally related to resting potential differences among the motoneurones. When plotted against membrane potential, rheobase current showed a significant correlation with resting potential, as expected from examinations of individual cells (see Methods). The correlation was, however, weak ($r = 0{\cdot}35$; $n = 91$), suggesting that about 90 % of the rheobase current variation observed among the motoneurones was independent of membrane potential variations.

In principle, one might expect this variation to be related to size differences among the motoneurones, those with smaller size having a correspondingly higher input resistance and smaller rheobase current. As shown previously (Gustafsson & Pinter, 1984), one may obtain an estimate of the cell surface area by calculating the total capacitance of individual motoneurone equivalent cylinders, and in Fig. 2 *A* this parameter has been plotted against the rheobase current. It can be observed that the current values tend to be small for the very smallest cells, and higher for the very largest ones, the over-all correlation being significant ($r = 0{\cdot}47$; $n = 95$). However, the range of rheobase currents greatly exceeds that of the estimated sizes, and over most of the size range there is little systematic relation between rheobase current and cell capacitance, the whole range of rheobase current values present at almost any given size. The same kind of relation can also be seen when plotting rheobase current against another tentative index of cell size, the conduction velocity (Fig. 2 *B*) (Cullheim, 1978; Kernell & Zwaagstra, 1981) or when using data only from motoneurones projecting to one group of muscles (Fig. 2 *C* and *D*). Since there was no

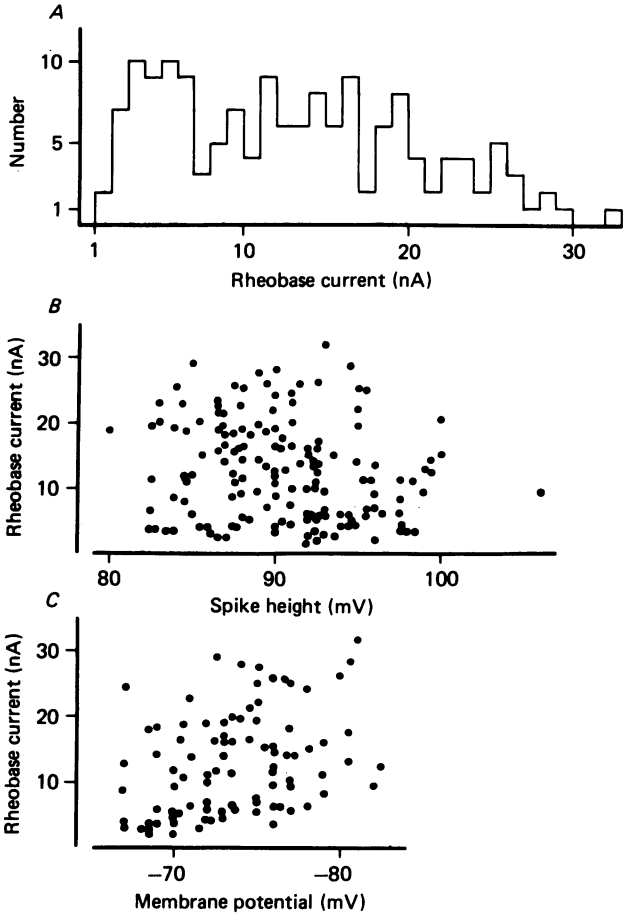

Fig. 1. Rheobase currents in motoneurones. *A*, distribution of the values for rheobase current. *B* and *C*, rheobase currents plotted against spike height (*B*) and membrane potential (*C*).

correlation between estimated cell size and membrane potential or spike height (not illustrated), these results suggest that motoneurone size itself is of limited importance in determining the over-all variation of rheobase current.

Relation between rheobase current and input conductance

As mentioned above, motoneurone input resistance (or input conductance) should be an important determinant of threshold for long duration excitatory currents such as rheobase. One may suppose that if threshold depolarizations (the difference between resting potential and firing level) were identical over the motoneurone pool, and if the membrane behaved linearly within this voltage range, then rheobase current and input conductance should be linearly related, with the slope providing the average value of threshold depolarization. Experimentally obtained values of input conductance and rheobase current are plotted in Fig. 3*A*. It may first be

observed that there exists a clear positive correlation between rheobase current and input conductance ($r = 0{\cdot}90$; $n = 153$). Moreover, the slope of the regression line (19·7 nA/μS) suggests that threshold depolarizations for rheobase are considerably higher than indicated in previous reports (cf. Pinter *et al.* 1983). In general agreement with the results by Fleshman *et al.* (1981), the range of rheobase current exceeds that

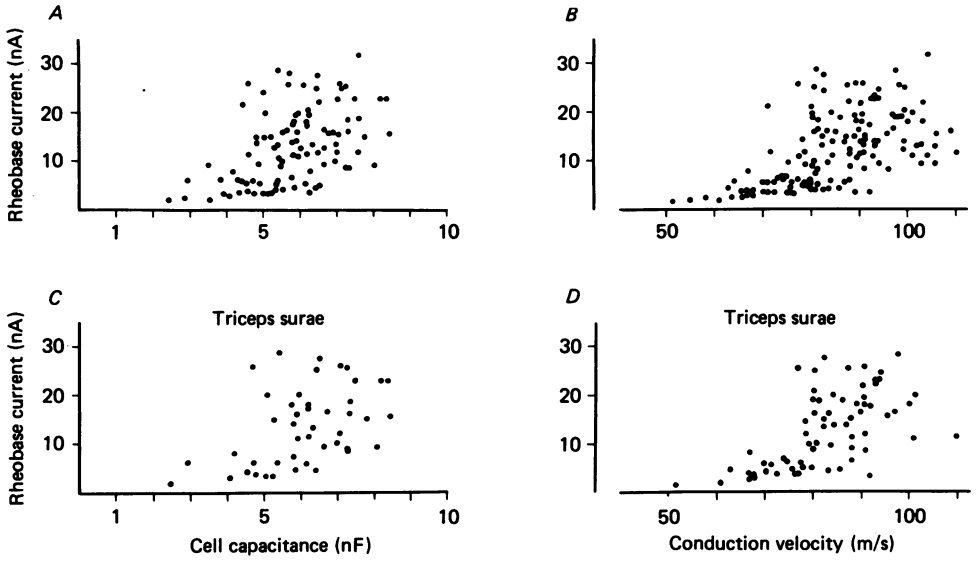

Fig. 2. Relations between rheobase current and cell 'size'. *A* and *C*, rheobase current plotted against total cell capacitance, for all cells (*A*) and for triceps surae motoneurones only (*C*). *B* and *D*, rheobase current plotted against axonal conduction velocity for all neurones (*B*) and for triceps surae motoneurones only (*D*).

of input conductance; in the present material the difference was almost 2-fold. Comparison of the spread of data points with the least-squares regression line provides some information concerning how this range discrepancy may arise. It may be observed that the data points from motoneurones possessing the lowest rheobase currents tend to fall below the line and that this tendency diminishes as both rheobase and input conductance increase. This tendency was sufficient to cause the Y intercept to deviate significantly from zero ($P < 0{\cdot}001$). It is thus apparent that rheobase and input conductance are not linearly related in the strictest sense. The data suggest that either threshold depolarizations are systematically lower among motoneurones possessing low rheobase current or that the motoneurone membrane does not behave linearly between the resting and threshold potential level, these alternatives being of course not mutually exclusive. There is no indication that these results are the consequence of using values from motoneurones projecting to different muscles. As shown in Fig. 3*C*, values taken from triceps surae motoneurones on the one hand (filled circles) and motoneurones to hamstring + instrinsic foot muscles on the other (open circles) are practically superimposable, and the correlations within these groups are statistically indistinguishable.

As shown by Gustafsson & Pinter (1984), the over-all range of motoneurone input resistance is approximated by the range of membrane time constant, and the two parameters are well correlated. A large proportion of the over-all variation of input conductance of motoneurones is thus likely to be explained by variation in specific membrane resistivity rather than size. Correspondingly, a good relation was observed

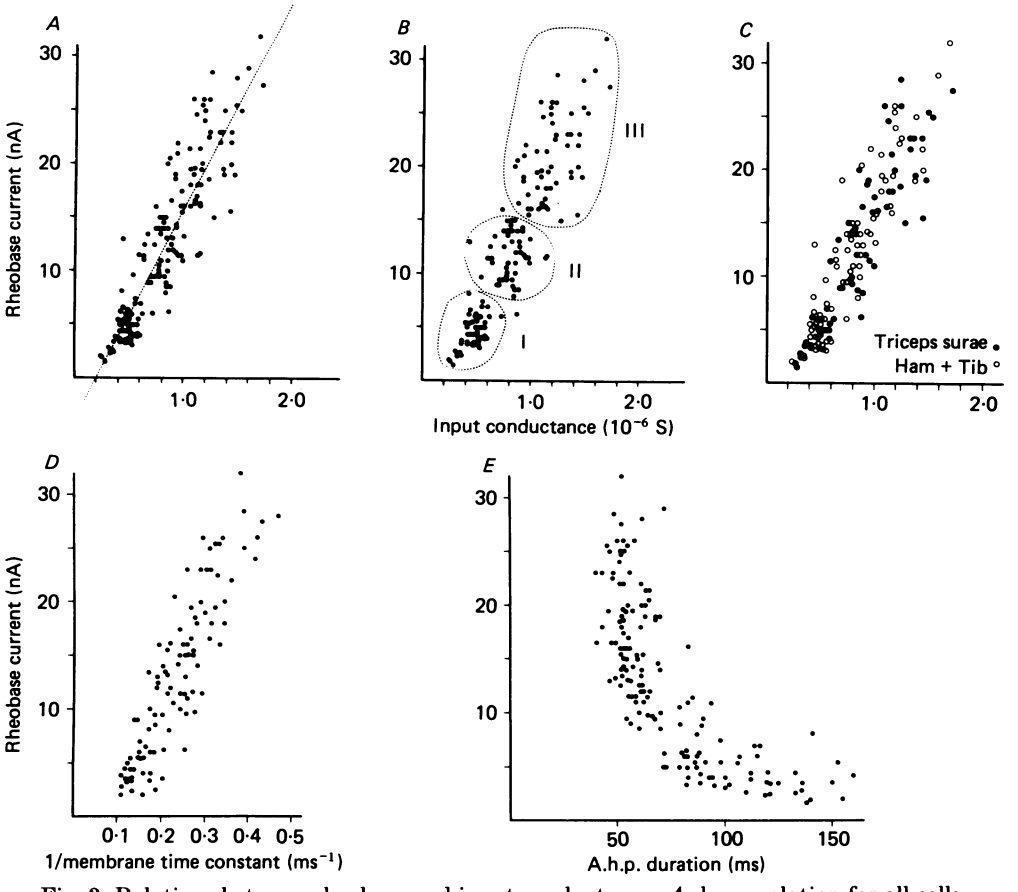

Fig. 3. Relations between rheobase and input conductance. *A* shows relation for all cells. The dotted line represents the least-squares regression line. *B* illustrates same plot with motoneurone subgroups indicated and *C* shows the relation for triceps surae, hamstring and intrinsic foot muscle motoneurones with symbols as indicated. *D*, relation between rheobase and the inverse of membrane time constant. *E*, plot of rheobase against a.h.p. duration.

when rheobase current was plotted against the inverse of the time constant, a parameter that can be considered an estimate of specific membrane conductivity (Fig. 3*D*, $r = 0{\cdot}88$; $n = 86$). It may be observed that the over-all relation in Fig. 3*D* is similar to that between rheobase and input conductance (Fig. 3*A*).

The rheobase current magnitude also varied in a systematic manner with the a.h.p. duration, as illustrated in Fig. 3*E*. The mean values of rheobase current for motoneurones with a.h.p. durations < 55 ms ($19{\cdot}2 \pm 5{\cdot}2$ nA, $n = 43$) and > 80 ms

(5·1 ± 2·4 nA, $n = 52$) indicate an average 4-fold variation of rheobase current over the a.h.p. range in comparison with a corresponding 2·3-fold range of input conductance and a 1·30-fold range of cell capacitance (or estimated surface area).

Motoneurone classification

It may be so that motoneurone properties do not vary according to a functional continuum but are related to the type of muscle unit innervated by the motoneurone. There exist essentially three types of muscle units: fast-fatiguable (f.f.), fast-fatigue-resistant (f.r.) and slow (s.) (Burke, 1981). Identification of unit type was precluded by the use of paralysing agents, but studies of type-identified motoneurones have suggested that these three types may be classified on the basis of rheobase current magnitude (Zengel, Munson, Sypert & Reid, 1982). Close inspection of Fig. 3*A* suggests the presence of three clusters of data points, and these have been circumscribed in Fig. 3*B* and designated as groups I, II and III, respectively. The mean rheobase currents for these groups, 4·5 ± 1·5 nA (I, $n = 51$), 11·7 ± 2·0 nA (II, $n = 44$) and 21·0 ± 4·0 nA (III, $n = 56$), are quite similar to mean values from type-identified motoneurones of cat medial gastrocnemius muscle (5·1 nA (s.), 12·8 nA (f.r.) and 19·7 nA (f.f.); Fleshman *et al.* 1981). These similarities in average values suggest a functional correspondence between the individual groups and types that is useful for an operational subdivision of motoneurones. It should be noted that this ad hoc classification imposes a somewhat lower variance of rheobase current within each group relative to experimentally determined rheobase current variances for type-identified motoneurones (see Fig. 4, Fleshman *et al.* 1981). There remains however an overlap of input conductance between groups which resembles that of type-identified neurones. In particular, the fractional increase in input resistance from group III to group I cells (1 : 1·4 : 2·8) was similar to that for f.f., f.r. and s. motoneurones in the material of Fleshman *et al.* (1 : 1·5 : 2·8). Alternatively, motoneurones with a.h.p. durations > 80 ms (slow) and < 55 ms (fast) may be compared. These groups possess average properties quite similar to groups I and III, respectively, but do not possess the artificially low variance of rheobase current imposed by the latter classification. The rheobase mean and variance for these groups (see previous section) are virtually identical to those observed for types s. and f.f. triceps surae motoneurones (Fleshman *et al.* 1981).

Estimation of threshold depolarization by the spike-height method

As mentioned above, one possibility for explaining the rheobase current-input conductance range discrepancy would be that the threshold depolarization systematically increases with rheobase current and input conductance. To explore this further, the threshold depolarization was evaluated by measuring the voltage drop caused by the rheobase current using a spike height method illustrated in Fig. 4*A*. This method was preferred over the direct measurement of the voltage drop because of difficulties in maintaining the bridge balance during injection of large rheobase currents. In cells where the compensation of the electrode resistance was judged to be reasonably adequate (as in Fig. 4*A*), the two measures were compared and, as shown in Fig. 4*B*, agreed rather well. The values obtained with the spike-height method are shown plotted against input conductance (G_{in}) in Fig. 4*C* and against

a.h.p. duration in Fig. 4*D*, and in each case significant correlations existed (*vs.* G_{in}, $r = 0{\cdot}45$; $n = 128$; *vs.* a.h.p., $r = -0{\cdot}59$; $n = 128$). The threshold depolarization was also correlated with the membrane time constant ($r = 0{\cdot}52$; $n = 89$) but only weakly with the cell capacitance ($r = 0{\cdot}22$; $n = 89$; $P = 0{\cdot}05$). A significant correlation was also obtained between rheobase current and threshold depolarization ($r = 0{\cdot}67$; $n = 128$).

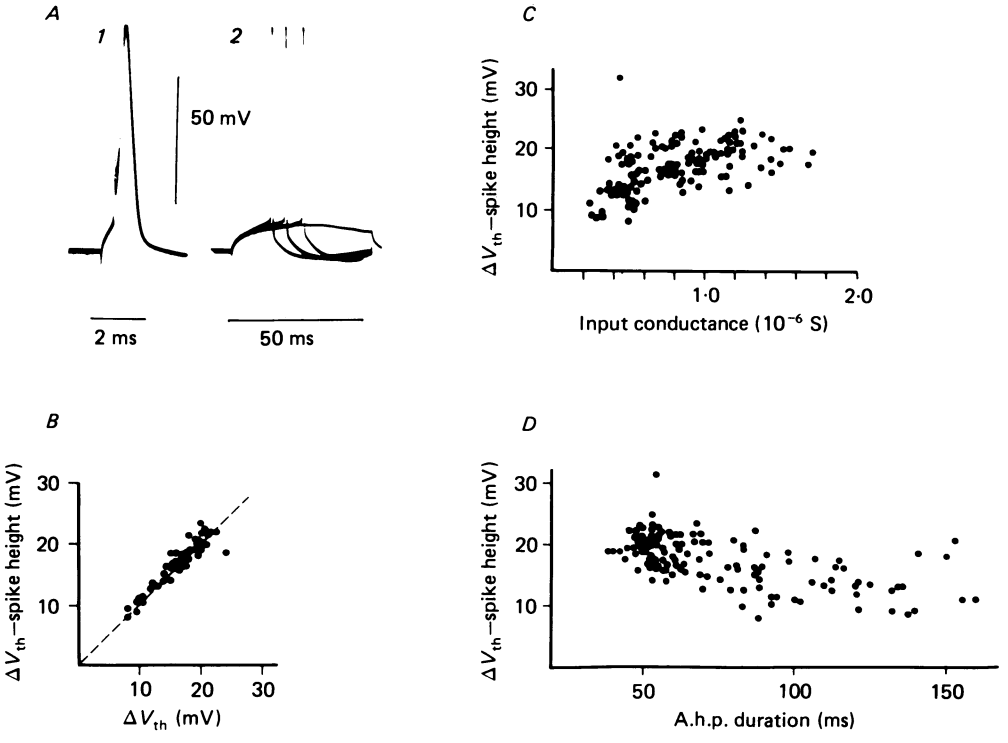

Fig. 4. Estimation of threshold depolarization. *A1* and *2* illustrates spike-height method for estimating threshold depolarization. The voltage measured between the spike peak and spike onset at rheobase current intensity (*A2*) is subtracted from the total spike amplitude obtained using a brief (500 μs) suprathreshold current pulse (*A1*). *B* illustrates good agreement between threshold depolarization (V_{th}) estimated by the spike-height method and that obtained by direct measurement in individual motoneurones where bridge balance was judged to be adequate (as in *A2*). *C* and *D* show, respectively, threshold depolarization (spike-height method) plotted against input conductance (*C*) and after-hyperpolarization duration (*D*).

When examined in the three motoneurone groups, the threshold depolarization for group I was significantly lower ($P < 0{\cdot}01$) than for the other groups, the averages being $14{\cdot}4 \pm 3{\cdot}7$ mV (I, $n = 46$), $18{\cdot}5 \pm 3{\cdot}5$ mV (II, $n = 38$) and $20{\cdot}1 \pm 2{\cdot}4$ mV (III, $n = 44$). In contrast to the over-all case, no significant correlations existed within groups I and III between threshold depolarization and either input conductance or a.h.p. duration (for group II see below). Even though group I motoneurones tended to have somewhat lower membrane potentials, it is not likely that the observed differences in threshold depolarization are directly related to resting potential

differences between the motoneurone groups; the mean resting potential for group I (for cells where this measurement was available) was −72·2 mV ($n = 32$) which is only slightly lower than those for the other two groups of cells (−74·8 mV, II, $n = 27$; −74·8 mV, III, $n = 28$). Since the average spike heights for these same cells were 91·9 mV (I), 93·1 mV (II) and 89·5 mV (III), and thus much the same as the average spike heights when including all typed cells (91·7 mV (I), 91·9 mV (II) and 89·2 mV (III)), these average resting potentials are likely to be representative for the whole population of cells within each group. It should also be considered that the threshold depolarization values differ less than 1 mV per millivolt difference in resting potential between the motoneurones (see below).

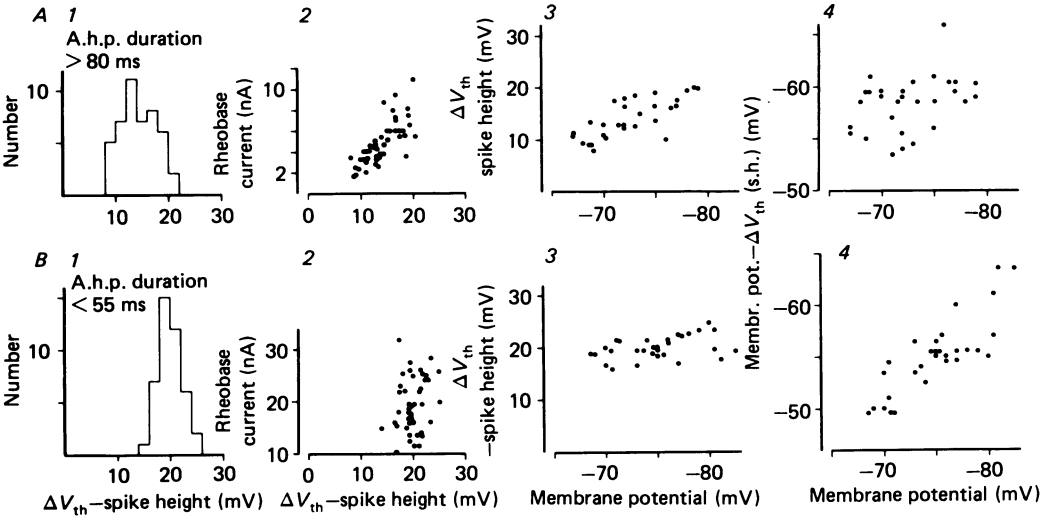

Fig. 5. Relations between threshold depolarization (V_{th}) and resting potential. *A1* and *2* show distribution of threshold depolarization (spike-height (s.h.) method) and plot of threshold depolarization and rheobase for motoneurones with a.h.p. duration > 80 ms. *A3* and *4* show resting potential plotted against threshold depolarization (*A3*) and absolute voltage threshold (*A4*) for the same group. *B1–4* are the same plots, but for motoneurones with a.h.p. duration < 55 ms.

The results thus suggest that differences in threshold depolarization do exist in the present material such that those motoneurones with low rheobase current also possess lower threshold depolarization, this effect not being directly explained by lower resting potentials. However, further analysis suggested that the situation might be more complex. When examining the relation between rheobase and threshold depolarization within groups, the correlation was quite strong for group I ($r = 0{\cdot}79$; $n = 46$), less so for group II ($r = 0{\cdot}60$; $n = 37$) and non-significant for group III ($r = 0{\cdot}28$, $n = 44$; $P > 0{\cdot}05$). The main features of this variable dependence of rheobase on threshold depolarization can be conveniently illustrated by comparing relations observed between motoneurones with a.h.p. duration < 55 ms (fast motoneurones) and > 80 ms (slow motoneurones) (Fig. 5). As with group I, the slow group showed a significant correlation between rheobase and threshold depolarization

($r = 0{\cdot}75$; $n = 46$, Fig. 5*A2*) whereas within the fast group, no significant trend existed ($r = 0{\cdot}32$; $n = 43$; $P > 0{\cdot}05$, Fig. 5*B2*). Resting potentials measured when rheobase was obtained are shown plotted against threshold depolarization for the slow (Fig. 5*A3*) and fast (Fig. 5*B3*) groups. It may be observed that for the fast group, threshold depolarization tended to remain around 20 mV independent of resting potential level ($r = 0{\cdot}37$; $n = 31$; $P > 0{\cdot}05$), while threshold depolarization for the slow group showed a significant trend to decrease over the resting potential range ($r = 0{\cdot}73$; $n = 29$). These results indicate that the absolute voltage threshold varied systematically for the fast group over the resting potential range, and a significant correlation was obtained ($r = 0{\cdot}83$; $n = 30$; Fig. 5*B4*). In contrast, this trend was of marginal significance for the slow group ($r = 0{\cdot}37$; $n = 29$; $P = 0{\cdot}05$) (Fig. 5*A4*). Nearly identical relations were observed for group III and I respectively, with group II exhibiting relations intermediate to these latter groups. The above analysis thus suggests that a considerable portion of the threshold difference may have arisen not because of lower resting potentials of slow motoneurones *per se* but because of a differential tendency of the absolute voltage threshold to 'adjust itself' to different resting potential levels (probably due to different degrees of soma leak), the adjustment being greatest among fast cells and least among slow cells. It is noteworthy that the regression equation relating threshold depolarization and resting potential in the slow group ($r = 0{\cdot}73$) and that relating abolute threshold and resting potential in the fast group ($r = 0{\cdot}81$) predict that no significant difference in either threshold depolarization or absolute voltage threshold would exist at a resting potential of -80 mV.

In contrast to the case with the resting potential, no differences existed between the fast and slow groups when threshold depolarizations were compared against spike height; both groups showed weak, but significant ($P < 0{\cdot}01$) correlations with similar slopes (slow, $r = 0{\cdot}42$; $n = 46$; slope = 0·3 mV/mV; fast, $r = 0{\cdot}38$; $n = 43$; slope = 0·22 mV/mV). Thus, to the extent that spike height reflects the resting potential, the variation of threshold depolarization with resting potential described above may be over-estimated for the slow group and underestimated for the fast group. This would indicate that the absolute threshold adjustment may be the same among motoneurones and that the threshold depolarization differences described above would exist also at -80 mV resting potential. However, the larger dependence of rheobase current on threshold depolarization shown above for the slow group clearly gives an independent indication that variation in some third factor, likely to be resting potential, had a larger effect on this group. Moreover, as discussed in the Methods section, the spike height may not be an absolutely accurate measure of membrane potential.

Subthreshold rectification

If the motoneurone membrane behaved linearly throughout the voltage interval from resting to threshold level, then the product of rheobase current and input resistance would predict the threshold depolarization. Comparison of this rheobase 'voltage' with the threshold depolarization measured by the spike height method can thus provide a means by which the presence of subthreshold rectification may be assessed. Fig. 6*A* illustrates this comparison for all motoneurones in which both

measurements were obtained, and shows that threshold depolarizations were generally larger than the corresponding rheobase 'voltages'. This lack of equivalence between the two parameters suggests that a subthreshold rectification process is operative among these cells, the net effect being apparently inward, Fig. 6*B* and *C* illustrates that the discrepancy (expressed as the ratio threshold depolarization/rheobase

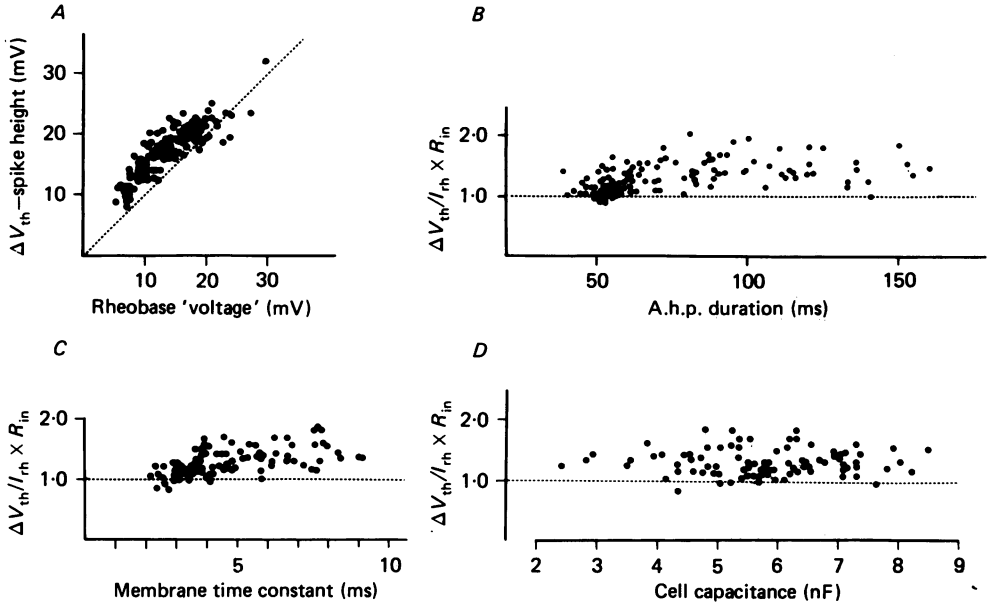

Fig. 6. Relation between threshold depolarization and rheobase 'voltage'. *A*, the threshold depolarization (spike-height method) plotted against rheobase 'voltage' i.e. the product of input resistance (R_{in}) and rheobase current (I_{rh}). The dashed line indicates equality between the values. *B*, *C* and *D*, show, respectively, the ratio between the estimated threshold depolarization and rheobase 'voltage' plotted against a.h.p. duration (*B*), membrane time constant (*C*) and total cell capacitance (*D*).

'voltage') tended to increase among motoneurones with longer a.h.p. duration or membrane time constant. Accordingly, group I motoneurones showed the greatest discrepancy, the rheobase 'voltage' being on the average 4·7 mV lower than the estimated threshold depolarization. The average difference for group II was 3·4 mV and that for group III was 2·1 mV. It may be noted that this difference between the groups corresponds rather well to that between the input resistances, suggesting that the magnitude of the current underlying the rectification may be rather similar among the motoneurone groups. Fig. 6*D* shows that the threshold depolarization/rheobase 'voltage' ratio bore no relation to estimated cell size.

The input resistance used for the 'rheobase voltage' is that measured with small hyperpolarizing current pulses applied from resting level. As shown first by Nelson & Frank (1967), the apparent input resistance of motoneurones tends to be larger in the depolarizing than in the hyperpolarizing direction. As illustrated in Fig. 7*A*, an increase in the voltage response often occurred rather close to the firing level. In

this example, the 3 nA pulses gave only a slightly larger voltage response in the depolarizing direction, a much larger discrepancy occurring with the 3·5 nA pulses and with the rheobase current intensity of 3·9 nA. Examination of current–voltage curves in some neurones ($n = 19$) showed that the voltage deflexion given by rheobase current intensity was 10–50 % greater than expected on the basis of the input

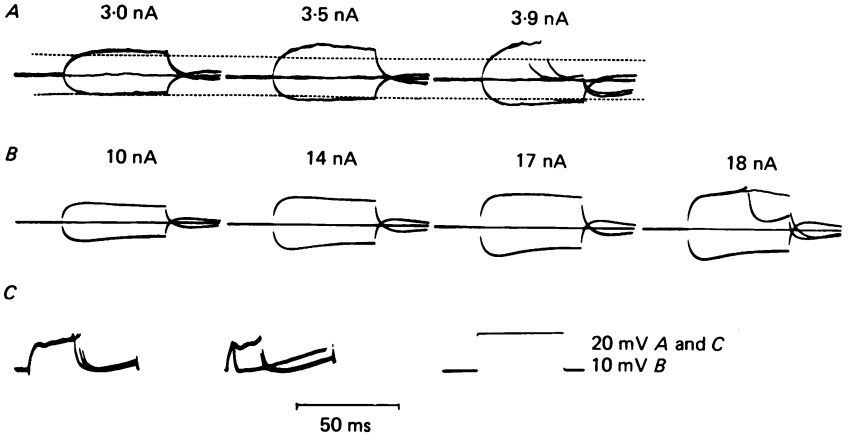

Fig. 7. Voltage responses to subthreshold membrane polarization. *A*, voltage deflexions caused by de- and hyperpolarizing current pulses of various magnitudes, as indicated. Note the larger increase in the depolarizing than in the hyperpolarizing direction with increasing current strength. *B*, same as in *A* but from a different motoneurone. Note here the change in the configuration of the depolarizing deflexion when approaching threshold level. *C*, from another motoneurone showing a notch on the rising phase of the depolarization.

resistance measured at resting level, suggesting that the discrepancy between the estimated threshold depolarization and rheobase 'voltage' may be accounted for by this rectification. We have, however, no independent evidence that this rectification should be relatively more developed in cells with long than with short a.h.p. duration as suggested by the threshold depolarization/rheobase 'voltage' ratio.

It was noted that the voltage deflexions produced by near-threshold current intensities were not only larger but often had a different shape with respect to those produced by lower current intensities. As illustrated from a rather typical cell in Fig. 7*B*, current pulses given at an intensity much less than rheobase produce de- and hyperpolarizing voltage deflexions that appear rather symmetrical. With increasing current strength, the depolarizing voltage deflexion changes in shape, the slow decay due to the sag process disappears and is replaced by a flattening of the trajectory and an upward deviation resulting in spike initiation. In a few neurones, the voltage resulting from the rheobase current even continued to rise throughout the pulse duration (50–60 ms), even though the deflexion in the hyperpolarizing direction had settled to a steady value. These changes in pulse shape with increasing current strength were not related to changes in electrode properties since they appeared when threshold was approached irrespective of current strength.

In a few other neurones, the response to depolarizing currents had a different

character, the depolarization developing a notch on the rising phase, followed by a slow depolarization to threshold level (Fig. 7*C*). Following a slight increase in current magnitude, the spike took off from the notch, often from an apparently lower threshold level (see below). Sometimes the spikes were generated at the notch even at the minimal intensities for spike generation. This type of voltage trajectory is suggestive of that which could be generated by the activation of an A-current (Galvan, 1982), possibly present in spinal motoneurones (Schwindt & Crill, 1980). However, the relative magnitude of the notch did not increase with steady membrane hyperpolarization, but rather with membrane depolarization, a behaviour not expected for an A-current. Moreover, this trajectory shape was occasionally also observed following deterioration of cells that previously showed trajectories as in Fig. 7*A* and *B*. It is thus likely that the notch represents some local response of the membrane, appearing in somewhat damaged cells. In view of this, the cells showing this kind of behaviour were excluded from the present analysis.

Factors determining rheobase current within groups of motoneurones

As described above, the over-all variation of rheobase current is determined by several factors among which input conductance plays a large role. Previously, Fleshman *et al.* (1981) reported that rheobase current among motoneurones of identical type was uncorrelated with input resistance. Such an observation suggests that the over-all correlation between rheobase current and input conductance is simply the result of pooling data from different motoneurone types, each type having properties different from the over-all relation. However, examination of the two extreme groups (I and III) of motoneurones in the present material revealed significant correlations between rheobase current and input conductance ($r = 0{\cdot}57$; $n = 51$ (I): $r = 0{\cdot}55$; $n = 56$ (III), see Fig. 3). To analyse further the factors underlying rheobase current variation within functionally restricted motoneurone groups, correlations between rheobase and various cell parameters were calculated for motoneurones with a.h.p. durations greater than 80 ms (slow) and less than 55 ms (fast). As shown in Table 1, rheobase current was well correlated with input conductance for each group; the regression lines passed close to the origin and had slopes similar to the rheobase 'voltage' for each group. It may also be seen that rheobase was significantly correlated with estimated threshold depolarization for the slow but not for the fast group (cf. above). In order to diminish the scatter introduced by variation in threshold depolarization, rheobase current values were normalized to the average threshold depolarization for each group, assuming direct proportionality. Since no significant correlations existed between threshold depolarization and any of the listed parameters for either group, this normalization had little effect on any of the regression equations (except, of course, those relating rheobase and threshold depolarization). For the slow group, however, the normalization produced a clear increase in the proportion of rheobase variance (r^2) explained by co-variation with input conductance and the inverse of membrane time constant. It is thus evident that for both groups, rheobase current variations are significantly associated with variance in specific membrane conductivity; the intercepts for each regression line are close to and do not differ significantly from zero. It is noteworthy, however, that the proportion of rheobase variance explained by co-variation with conductivity is

somewhat higher in the fast group (0·61) than in the slow group (0·43). When correlations were calculated between normalized rheobase and the present index of cell size (capacitance) the reverse emerged: rheobase was more strongly correlated with capacitance in the slow group (0·30) than for fast motoneurones (0·13), where the correlation was not significant ($P > 0·05$). It needs to be emphasized, however,

TABLE 1. Correlations and regression line parameters between rheobase current (Y) and other parameters (X) for slow (a.h.p. duration > 80 ms) and fast (a.h.p. duration < 55 ms) motoneurone groups. Correlation coefficients (r) are presented as the square, and the column headings designate values obtained from the raw material and after normalizing (norm.) individual rheobase values to the average threshold depolarization for each motoneurone group (see text). Sample size (n), regression slope and Y-intercept columns are similarly organized. Abbreviations and units: I_{rh}, rheobase current (nA); G_{in}, input conductance (μS); $1/\tau_m$, inverse of time constant (ms^{-1}); V_{th}, threshold depolarization (mV); Cap, total cell capacitance (nF)

		Slow motoneurones (a.h.p. duration > 80 ms)							
		r^2		n		Slope		Intercept	
Y	X	Raw	Norm.	Raw	Norm.	Raw	Norm.	Raw	Norm.
I_{rh}	G_{in}	0·55	0·71	52	46	12·8	9·4	−1·4	0·5
	$1/\tau_m$	0·19	0·43	27	26	29·1	31·5	0·3	0·2
	V_{th}	0·56	0·11	46	46	0·5	0·5	−1·6	3·1
	Cap.	0·29	0·30	27	26	1·0	1·2	−0·3	1·2
		Fast motoneurones (a.h.p. duration < 55 ms)							
		r^2		n		Slope		Intercept	
Y	X	Raw	Norm.	Raw	Norm.	Raw	Norm.	Raw	Norm.
I_{rh}	G_{in}	0·56	0·71	48	43	15·3	17·0	2·3	0·5
	$1/\tau_m$	0·62	0·61	38	36	70·0	68·4	−1·4	−0·6
	V_{th}	0·10	0·02	43	43	0·7	−0·3	4·9	24·3
	Cap.	0·06	0·13	38	36	1·3	2·0	11·2	7·2

that even though the correlation coefficient and regression slope for the normalized rheobase–capacitance relation for the fast group did not differ significantly from zero, the scatter was such that the Y intercept could not be distinguished from zero ($P > 0·05$).

In contrast to the cases of groups I and III, no significant rheobase–input conductance correlation existed within the raw material of group II ($r = 0·06$; $n = 44$; $P > 0·05$), similar to the case with type-identified m.g. (medial gastrocnemius) motoneurones (Fleshman *et al.* 1981). This appears to have been caused by a significant, negative correlation between threshold depolarization and input conductance ($r = -0·49$; $n = 38$); such a relation did not exist in any of the other groups. After confining the threshold depolarization dependence to only input conductance by normalizing each rheobase value to the average threshold depolarization (18 mV; rheobase *vs.* threshold depolarization, $r = 0·60$; $n = 38$), the rheobase–input conductance regression slope increased 10-fold and a significant correlation emerged ($r = 0·69$; $n = 38$). The negative relation between threshold depolarization and input conductance was probably the consequence of a similarly negative correlation between resting potential and input conductance ($r = -0·60$; $n = 27$) in conjunction

with a correlation between threshold depolarization and resting potential ($r = 0{\cdot}51$; $n = 25$). Since no other group showed a significant correlation between resting potential and input conductance, its existence within group II most likely signifies the presence of a sampling problem specific for this group. This seems further indicated by the fact that this correlation is negative; the exact opposite would be expected from theoretical considerations concerning the effects of micro-electrode impalement (Jack, 1979).

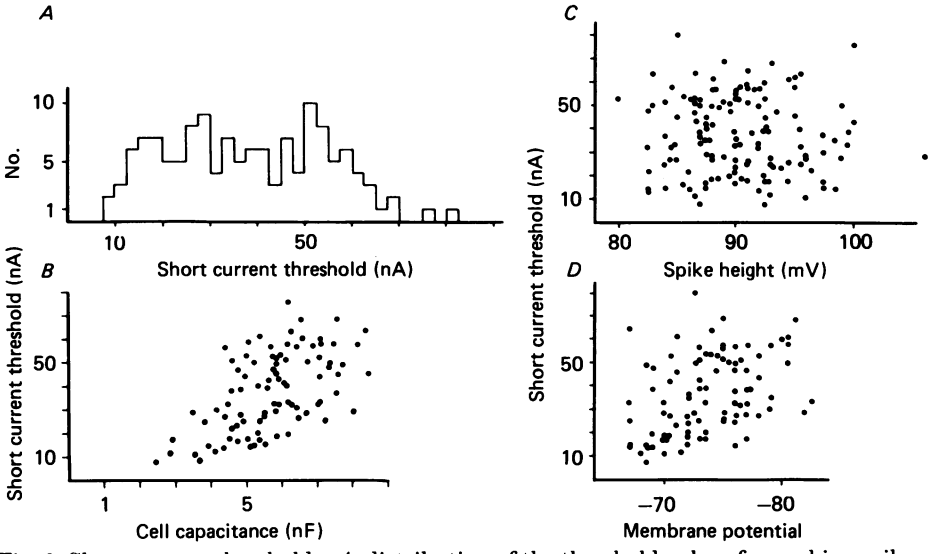

Fig. 8. Short current thresholds. *A*, distribution of the threshold values for evoking spikes with a 500 μs constant current pulse. *B*, relation between the threshold values and cell size, as estimated from total cell capacitance. *C* and *D*, short current thresholds plotted against spike height (*C*) and membrane potential (*D*).

Short current thresholds

According to strength–duration curve expectations, the amount of current required to initiate an action potential using a very brief pulse width should be primarily determined by the neuronal surface area, its electrotonic structure and the threshold depolarization. Such a threshold pulse should be largely independent of specific membrane resistivity and any subthreshold conductance processes that require more time to activate. A comparison of thresholds obtained using brief and long current pulses should thus provide useful information concerning the relative importance of various factors controlling cell excitability. Current thresholds using brief (500 μs) pulses were examined and compared with those using long pulses. Calculations performed on a compartmental model (Rall, 1964) showed that pulses of this duration were sufficiently brief to allow the current magnitude for a given model neurone to be virtually unaffected by changes in specific membrane resistivity in the experimental range (neuronal time constants 2·5–9·0 ms) even in the presence of soma leaks of appreciable magnitude (relative leaks up to 30%, expressed as a fraction of input conductance). As can be observed in Fig. 8*A*, the short current thresholds varied

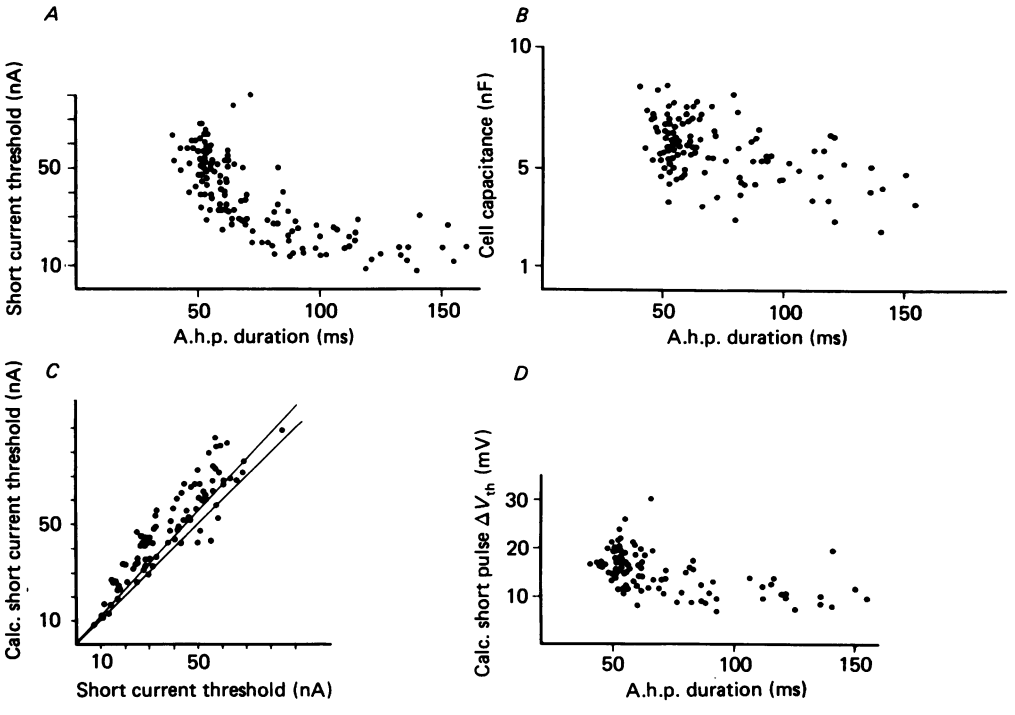

Fig. 9. Short current thresholds and a.h.p. duration. *A*, threshold values using 500 μs constant current pulses are plotted against a.h.p. duration. *B*, cell size, as estimated from total cell capacitance, plotted against a.h.p. duration. *C*, relation between calculated and experimental short current thresholds. The calculated thresholds were obtained as described in the text. The straight lines indicate the expected equality between the values given motoneurones with no soma leak (lower line) and with a 30 % soma leak (upper line). As indicated above, the voltage thresholds used in the calculations were those obtained by the spike-height method for rheobase current intensities. *D*, relation between estimated short pulse threshold depolarization and a.h.p. duration. This threshold depolarization was calculated as described in the text. The computations in the compartmental model were performed by numerical solution on a digital computer with time increments of 0·1 μs.

between 10 and 70 nA, a range about half that for the long pulses (2–32 nA). As illustrated in Fig. 8*C* and *D*, this range was present at almost any given spike height or membrane potential, and thus not likely to be directly related to resting membrane potential differences between the neurones.

The current thresholds showed an over-all tendency to increase with the estimated cell surface (Fig. 8*B*, $r = 0{\cdot}53$, $n = 85$). However, the scatter was considerable, with a 3–5-fold variation in current present for almost any given estimated size (except the very lowest ones). On the other hand, the short current thresholds were clearly related to the a.h.p. duration (Fig. 9*A*) with an almost 3-fold average variation over the a.h.p. range ($21{\cdot}4 \pm 8{\cdot}3$ nA, a.h.p.s > 80 ms, $n = 46$ to $52{\cdot}2 \pm 9{\cdot}9$ nA, a.h.p.s < 55 ms. $n = 42$). As illustrated in Fig. 9*B*, this variation was much greater than the estimated variation in size with a.h.p. duration, the size only increasing by around 50 % over the a.h.p. range. As mentioned above, short pulse current threshold is not directly

affected by the value of specific membrane resistivity. Even so, the short pulse current was strongly correlated ($r = 0{\cdot}74$; $n = 86$) with the inverse value of the membrane time constant, the relation arising indirectly via other factors that co-vary with resistivity.

To explore further the factors of importance in determining the range of current thresholds shown in Fig. 8*A*, 500 μs current pulses were injected into compartment one of compartmental neurone models. For each neurone, a 50-compartment cylinder was assembled using the measured values of input resistance, membrane time constant and electrotonic length. In this way, the surface area and geometry of each cell could be taken into account. Calculations were then performed to determine the current necessary to depolarize the first compartment to the rheobase threshold depolarization given by the spike-height method for each cell. In the presence of soma leak introduced by micro-electrode impalement, the actual neurone will not possess spatially uniform membrane resistivity. This will modify both the actual amount of injected current (5–10 % for a 30 % relative leak) as well as the compartmental parameters calculated assuming a non-leak situation. Calculations indicate that the net effect will cause the actual current threshold to be about 10 % greater than that calculated on the basis of uniform properties. The possible error introduced by the sag-process (Ito & Oshima, 1965) was investigated using the sag model described by Gustafsson & Pinter (1984). This indicated that there would be a 2–3 % difference between the actual and calculated currents (sag ratio = 0·70), a difference which can be neglected. Assuming a relative leak between zero and 30 %, the experimental and calculated values would not in theory be expected to be exactly equal, but to fall within the two lines in Fig. 9*C*. As shown by this Figure, there is a good proportionality between the measured and calculated values, implying that the range of experimental current threshold is well accounted for by taking into consideration the cell size, cell geometry and variations in threshold depolarization for long pulses. The calculated values are, however, somewhat larger than the experimental ones.

An alternative way of presenting the same type of calculation is to compute the depolarization resulting in the compartmental model by injecting the experimentally determined short current magnitude necessary to reach threshold. In this situation, the presence of leak will produce an error which was calculated to be around 1·5 mV underestimation of the depolarization with a 30 % relative soma leak (1·2–1·8 mV with time constant 2·5–10 ms). The computed values may be interpreted as an estimation of the threshold depolarization for the short current pulses and are plotted against the a.h.p. duration in Fig. 9*D*. The over-all relation is similar to that observed between a.h.p. duration and threshold depolarization (spike-height method). As expected from the above (Fig. 9*C*), the values for a given a.h.p. duration are somewhat lower than those estimated by spike height, being 16·6 mV ($n = 34$) and 11·1 mV ($n = 23$) for a.h.p. durations of < 55 ms and > 80 ms, respectively, compared to 20·2 and 14·4 mV obtained by the spike-height method. Similarly, when examined for each of the three groups of motoneurones, the estimated thresholds varied from 11·3 mV (I, $n = 24$) to 14·5 mV (II, $n = 22$) and to 17·2 mV (III, $n = 37$), thus showing much the same variation as seen with the long current pulses, but with each value about 3 mV lower.

The over-all pattern of correlations between the computed threshold depolarization

and resting potential was also generally similar to that observed with rheobase threshold depolarization (Fig. 5), although the group with a.h.p. duration < 55 ms showed a somewhat stronger relation ($r = 0{\cdot}47$; $n = 27$, $P < 0{\cdot}01$; a.h.p. duration > 80 ms, $r = 0{\cdot}79$; $n = 20$).

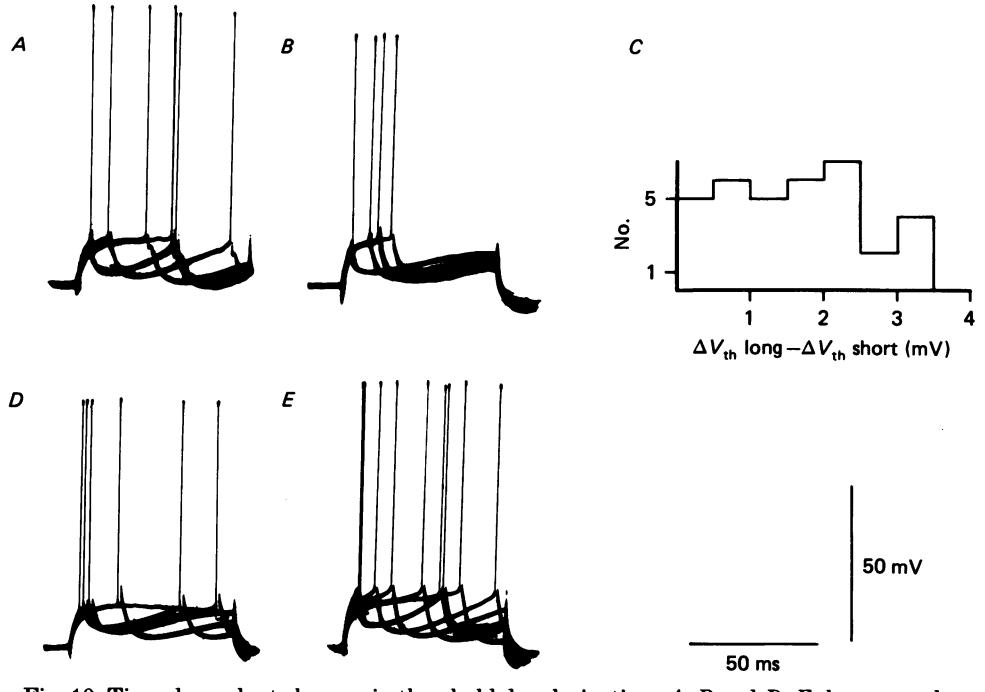

Fig. 10. Time-dependent changes in threshold depolarization. *A–B* and *D–E* show records from four different motoneurones. The threshold depolarization was estimated at different times from current onset, using the spike-height method. In each cell, the current intensity was increased from rheobase to produce spikes at various latencies. *C*, distribution of the difference between the threshold depolarization estimated with rheobase intensity and that observed with spike latencies of 2–4 ms for individual cells. Spikes have been retouched for clarity.

As mentioned above, part of the difference between calculated short pulse threshold and that given by the spike-height method may be due to the presence of soma leak. On the other hand, the voltage threshold may vary with time, increasing with increasing latency of the spike (see Grantyn, Grantyn & Schierwagen, 1983). To explore this alternative, the threshold depolarization was estimated in some neurones from spikes occurring late during a long pulse and early (2–4 ms) after current onset through the application of supra-rheobase intensities. For the neurones illustrated in Fig. 10, the peaks of the spikes (generated as first spikes) reach the same potential level independent of spike latency. Similarly, for the cell in Fig. 10*A*, the spikes seem to arise at much the same level, perhaps somewhat lower for the spike with the shortest latency. On the other hand, for the cell in Fig. 10*B*, the spike initiation level is clearly increasing with latency. The difference between threshold levels estimated at latencies of 2–4 ms and at rheobase current intensities, was measured in thirty-five

neurones, and varied between 0 and 3·5 mV, with a distribution as shown in Fig. 10*C*. The discrepancy of about 3 mV between the estimated threshold depolarizations for the long and short pulses can thus be explained by a change in voltage threshold with time, combined with the effect of a soma leak.

As can also be observed in Fig. 10*A*, *D* and *E*, the threshold depolarization, as estimated by the spike-height method, remains much the same for the second spikes following first spikes evoked at a short latency. In twenty neurones where second spikes appeared with similar interspike intervals as shown above, the threshold depolarization remained generally within a millivolt or less of that of the first spike with a similar latency from current onset. Only occasionally were the estimated threshold differences greater, then having measured values of 1·5–2·5 mV. We did thus not observe a 40–100 % increase from the first to a second spike, as reported by Schwindt & Crill (1983).

Relation between short and long current pulse thresholds

If specific electrical properties were identical over the motoneurone pool, the ratio between the long and short current pulses would be constant, the value depending primarily upon the specific membrane resistivity and capacitance, possible voltage threshold shifts and any contributions from subthreshold activated conductance processes. The specific electrical properties are, however, not uniform, and as illustrated in Fig. 11*A* the long/short current pulse ratio changes appreciably with a.h.p. duration, being high in cells with short a.h.p. duration (0·37, a.h.p. durations < 55 ms) and small in cells with long durations (0·25, a.h.p. durations > 80 ms). Similarly, there was a systematic decrease in this ratio from type III cells (0·39) to type II (0·33) and type I (0·24) cells. This result shows that the excitability variation over the motoneurone pool increases with increased duration of the stimulating current.

A likely explanation for the variation of this ratio with a.h.p. duration (or motoneurone type) is that the specific resistivity is higher in cells with longer a.h.p. duration as suggested by their longer time constants (Gustafsson & Pinter, 1984; cf. Kernell & Zwaagstra, 1981; Burke, Dum, Fleshman, Glenn, Lev-Tov, O'Donovan & Pinter, 1982). As shown in Fig. 11*B*, the current pulse ratio was also clearly related to the variation of the membrane time constant. To investigate how closely the variation of the current pulse ratio conformed to that expected from the variation in passive properties, size and geometry, long pulse (50 ms) and short pulse (500 μs) current thresholds for a given threshold depolarization were calculated using compartmental models of each neurone (see above). These computations produced values that closely followed the lower line in Fig. 11*B*. The same calculations were also performed in the presence of a relative leak of 30 % and gave the upper line in the graph. It may be observed that the experimental values tend to follow the theoretical curves rather well but show a tendency to be lower except at the very shortest values of time constant. Any increase in threshold depolarization occurring between the short and long threshold pulses would tend to increase the ratio while the subthreshold rectification present for the long pulse would tend to decrease the ratio. The graph in Fig. 11*B* thus seems to indicate that these opposing factors cancel each other rather well, perhaps with some predominance of the rectification effect.

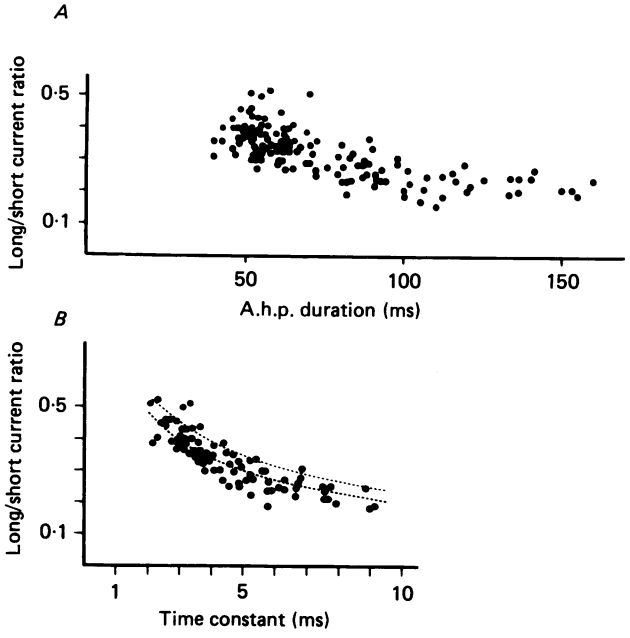

Fig. 11. Relation between short and long current thresholds. *A*, the ratio between the long (50 ms) and short (500 μs) pulse current thresholds plotted against a.h.p. duration. *B*, the same ratio plotted against the membrane time constant. The dashed lines indicate the theoretical ratios in equivalent cylinder models of the motoneurones, given no activation of subthreshold conductance processes and with equal threshold depolarizations for both pulses. The lower line shows the theoretical relation in the absence of soma leak, the upper line in the presence of a 30% soma leak.

Excitability variations among motoneurone groups with inputs of different spatial location

The differences in rheobase and short pulse current thresholds observed between the various motoneurone groups are of course only relevant for a somatically located input, the likely position of the micro-electrode. To explore differences which might exist at various other input locations, computations were performed on compartmental neurone models which incorporate the known differences in passive membrane properties between fast and slow motoneurones. Two models were used, equipped with parameters derived from the average input resistance, time constant and electrotonic lengths of group I (slow) and group III (fast) motoneurones (Gustafsson & Pinter, 1984). In each, currents were injected into various compartments, and the resulting voltage change in the first compartment was calculated. The short current pulse had a time course and shape resembling the synaptic current underlying single-fibre e.p.s.p.s (Iansek & Redman, 1973) and the long pulse had a duration of 50 ms. Using brief currents of identical intensity injected into compartment 1, the computed e.p.s.p. peaks were reached after about 0·5 ms, the amplitude being 73% larger in the slow than in the fast model neurone. This difference is about twice that

expected from the 35% difference in surface area between the two models and is largely related to geometrical differences between the models; the equivalent cylinder representation of the slow motoneurone possesses a smaller diameter with respect to its length relative to the fast motoneurone (Gustafsson & Pinter, 1984). This implies that for a similar threshold depolarization, about 30% more current (applied at the soma) would be needed to reach threshold in the fast motoneurone model after the 35% difference in surface area is taken into account. With an input located further out in the dendritic tree, however, the effect of geometry would be expected to decrease; the computations showed that the difference in short pulse threshold between the models due to this effect would be reduced (from 30%) to 10% for input located halfway out on the dendritic tree. As discussed by Pinter *et al.* (1983), the spatial distribution of Ia synapses can be approximated by locating the current input in compartments 2, 3 and 4 of an 8-compartment model in which compartment 1 represents the soma region. Calculations performed for the brief current input equally distributed to these compartments showed the differences in current threshold to be about 15%, the lower value being for the slow model. This value is of interest since it suggests that passive factors (excluding size) would in themselves produce Ia e.p.s.p.s 15% larger in the slow motoneurone for an identical density of synaptic input over the motoneurone pool.

For a long current pulse injected into compartment 1, the threshold was 2·6 times greater in the fast model, a difference directly related to the measured variation in input resistance between the two motoneurone groups. In contrast to the case with brief current input, the difference did not decrease when the input was moved into the dendritic region; when the input was halfway out the ratio was even slightly greater (2·8). For an input resembling the group Ia spatial distribution, the ratio was 2·7. These calculations suggest that the rheobase current variation between the motoneurone groups for somatic current input will also be present for distributed dendritic inputs, even to a slightly larger degree. It should be noted, however, that these calculations do not take into account any possible differential spatial variation of the sag process or of subthreshold rectification.

DISCUSSION

The purpose of the present study was to examine the relative contributions made by various post-synaptic factors in determining variations in intrinsic excitability over the motoneurone pool. To accomplish this, thresholds for very brief currents and for longer duration (rheobasic) currents have been determined and compared with other membrane properties among functionally distinct groups of motoneurones. The motoneurones were not identified from the mechanical properties of their muscle units but were instead grouped according to their a.h.p. duration or rheobase current magnitude. These classifications produced groups of motoneurones, of which the two extreme ones can be considered as fast and slow groups, respectively, with properties quite similar although perhaps not identical to the type f.f. and s. groups. Using this classification, the results demonstrate that for an average estimated size difference of less than 50%, the fast motoneurones needed on average about 2·5 times more current when activated by brief pulses, and about 4 times more rheobase current than

the slow cells. This shows that variations in threshold currents are to a large extent determined by factors other than motoneurone size. In fact, taken at face value, the present results suggest that the post-synaptic contribution to the variation of motoneurone functional threshold is only to a limited extent related to motoneurone size or size-related factors but is instead related to intrinsic membrane properties which vary little with respect to size. There are, however, several aspects of this work which need to be considered before a more detailed discussion of this point is undertaken.

A first point to consider is that these results have been obtained from motoneurones in which the electrode impalement itself may have produced changes in neuronal properties determining excitability. As discussed by Gustafsson & Pinter (1984) (see also Jack, 1979), resting potentials of undamaged motoneurones may, under the present conditions, be about −80 mV or more, which would mean that the present sample of motoneurones is depolarized on average by at least 7 mV. Such an impalement-induced depolarization would have two direct effects on the recorded threshold currents: an enhancing effect on the rheobase currents due to the increase in input conductance caused by the soma leak, and a depressing effect due to an expected decrease in threshold depolarization. However, the effects of leak-induced input conductance changes are likely to be rather small, being on average 10–15% (Gustafsson & Pinter, 1984). With respect to the expected decrease in threshold depolarization, Pinter *et al.* (1983) showed that there appears to be an adjustment of the absolute threshold voltage in response to impalement-induced resting potential variations. In the over-all resting potential range of −50 to −80 mV, there was an approximate 7 mV threshold change per difference of 10 mV in resting potential and types f. and s. motoneurones showed much the same behaviour. According to this result, the present threshold depolarizations should only be decreased by about 2 mV on average from the pre-impalement level, causing a decrease in rheobase current corresponding to the increase produced by the leak. It would then seem that the present estimates of threshold currents should be similar to those present in non-impaled neurones.

In the present study, with a resting potential range of −67 to −80 mV, fast motoneurones also displayed a significant correlation between resting potential and absolute voltage threshold with a slope quite similar to the over-all slope reported by Pinter *et al.* (1983). However, for slow motoneurones this tendency was not significant, while the correlation between threshold depolarization and resting potential was significant. It thus appears that within the present resting potential range, fast and slow motoneurones were differentially affected by the resting potential variations, in such a way that the absolute threshold level adjusted itself to a larger extent in fast than in slow motoneurones. This result would suggest that the estimated differences in rheobase current and threshold depolarization between fast and slow motoneurones observed in these experiments may be over-estimated. In fact, as mentioned in the Results, the relevant regression equations predict that both threshold depolarization and absolute voltage threshold would be nearly identical for these groups at a resting potential of −80 mV. There is of course a degree of uncertainty associated with such predictions, since the average resting potentials of both groups were clearly less than −80 mV and there were, in particular for the slow

group, few motoneurones in this high resting potential range. Moreover, when using spike height as a measure of membrane potential, this differential sensitivity did not emerge. However, spike height may not be an accurate measure of membrane potential (see Methods), and the better correlation between rheobase current and threshold depolarization in the slow than in the fast group provides independent evidence of a differential threshold adjustment in this resting potential range. It thus seems likely that differences in the way slow and fast motoneurones respond to depolarization induced by impalement may have produced a substantial portion of the difference in threshold depolarization observed here between the two groups. If so, the rheobase and short-pulse current differences between non-injured fast and slow motoneurones would be somewhat less than observed in the present experiments, the extrapolated average values for rheobase current at a resting potential of -80 mV being 6·7 and 22 nA for group I and III cells, respectively, compared to the 4·4 and 20·8 nA observed in this work. The average rheobase current variation between the fast and the slow groups of cells should then be about 3-fold rather than 4-fold (see above). Similarly, the over-all range in rheobase current would change from a 16-fold variation (2–32 nA) to an 11-fold variation (3–33 nA).

An important consideration in this context is whether such a differential threshold adjustment could also operate under physiological conditions. If so, a steady synaptic depolarization might have little influence on the excitability of fast motoneurones but would lower the threshold depolarization of the slow ones. The present results would suggest the existence of an intrinsic variation in excitability that may not exist at a true resting level but would appear during synaptic depolarization, perhaps being analogous to the differential degree of accommodation described by Burke & Nelson (1971). In three fast motoneurones examined at different potential levels following 'spontaneous' resting potential shifts, voltage threshold adjustments were observed but the changes were only around 3 mV per change of 10 mV in resting potential even minutes after the change. This variation was much the same as that observed for similarly 'spontaneously' changing slow motoneurones ($n = 8$). This observation may indicate that the larger threshold adjustment observed for the fast motoneurones in the present experiments and over-all by Pinter *et al.* (1983), must operate on an even slower time scale, or be partly associated with conditions established at the time of impalement not directly related to the membrane potential itself. Clearly, more experimental data must be provided to decide this issue.

The existence of voltage threshold adjustments, be they 3 or 7 mV per change of 10 mV in resting potential, may have further consequences for the interpretation of experimental results. If we assume that the threshold adjustment is associated with a slow inactivation of the voltage-sensitive sodium channels responsible for the spike in the initial segment, or a shift in their activation curve, it does not seem obvious whether other voltage-dependent conductances operating in the sub- or near-threshold region would be similarly affected. Under such conditions, the particular balance of ionic currents observed at threshold in neurones with low resting potentials (< -70 mV) may not accurately portray that present had the neurones been investigated at their proper resting level. The state of the sodium channels, for example, with respect to inactivation characteristics may also not be the same when threshold level is shifted. The lack of apparent voltage threshold shift from the first

to the second spike observed in the present work, compared to the large shifts observed by Schwindt & Crill (1983) may possibly be explained on such a basis.

A second consideration is the manner in which the present threshold depolarizations have been estimated and how these estimates compare with previously obtained values. Two different approaches were used here; the threshold depolarization was calculated via a compartmental model as the voltage displacement produced by the short current threshold pulse, or measured as the voltage drop given by the rheobase current using a spike-height method. The possible error introduced by a soma leak and the sag process for the short current threshold was calculated and found to be small. The estimations of rheobase current threshold depolarization via the spike-height method were also well correlated with those measured directly from seemingly well balanced voltage responses. The estimated threshold depolarizations obtained by the two techniques differed by a few millivolts, a discrepancy that can be related partly to a soma leak and partly to a real shift in voltage threshold with time. Such a shift was also observed when estimating threshold depolarization at the beginning and at the end of a long pulse using the spike-height method, and has also been described for other central neurones (Grantyn *et al.* 1983).

The threshold depolarizations generally varied between 10 and 25 mV for the rheobase pulse, with the over-all distribution peak between 15 and 20 mV, the values being about 3 mV lower for the short pulse estimations. These threshold values are significantly higher than in previous reports where the average values were about 10 mV. For example, in the study by Pinter *et al.* (1983), in which spikes were initiated by monosynaptic e.p.s.p.s, threshold depolarizations measured from the e.p.s.p.s were about 9 mV on average, and no differences between type f. and s. motoneurones were observed. Part of the discrepancy between the present results and those of Pinter *et al.* (1983) may be related to resting potential differences between the two sample populations. When selecting type s. motoneurones from the results of Pinter *et al.* (1983) with resting potential in the same range as those for the present sample (-65 to -80 mV), the average threshold depolarization was about 13 mV, which is similar to the present estimates for slow motoneurones. For their type f. motoneurones, the average threshold depolarization for the same range of resting potential was about 12 mV, which is considerably lower than that for the present sample of fast motoneurones. It seems possible that most of this difference can be explained as being the result of a sampling problem in the work reported by Pinter *et al.* (1983). For instance, if threshold depolarization of type f. cells is as high as indicated by the present results, then it would seem difficult to cause orthodromic firing of those motoneurones that have high resting potentials and small amplitude e.p.s.p.s even if temporal facilitation was used. It thus seems likely that there is an under-representation of type f.f. motoneurones in the upper resting potential range of the results of Pinter *et al.* (1983).

With regard to the over-all relationship between rheobase and input conductance, the present results generally agree with those of Fleshman *et al.* (1981) in that a strong correlation existed between the two parameters and the over-all range of rheobase exceeded that of input conductance. Our results indicate that at least for the present sample this range discrepancy arises because of differences in threshold depolarization and subthreshold rectification among the sample motoneurones, and this could in

principle account for the over-all results of Fleshman *et al.* (1981). However, beyond this point of general agreement, there exist several discrepancies between the two sets of results. For example, the average rheobase 'voltages' (the product of rheobase and input resistance) of types f.f. and s. motoneurones reported by Fleshman *et al.* (11·8 and 8·7 mV, respectively) are considerably lower than for the present groups III and I and for the fast and slow groups classified according to a.h.p. duration (see Results). Since the average rheobase values for the various groups agree quite well with the corresponding types, these differences indicate that the input resistance estimates of Fleshman *et al.* (1981) are considerably lower. This may be related to differences in technique (see Gustafsson & Pinter, 1984 for present method) or perhaps to differences in criteria for cell acceptance. A more serious difference is that Fleshman *et al.* (1981) reported that no significant correlations existed between rheobase and input resistance within motor unit type, whereas the present results contained significant correlations within the raw data groups obtained from fast and slow motoneurones. It does not seem likely that the differences between the present results and those of Fleshman *et al.* (1981) are the consequence of the various procedures used to classify motoneurones. As described above, there is a good agreement of average rheobase values between corresponding types and the present groups, and the fractional increases of both rheobase and input resistance were also similar. One possibility for explaining the negative findings of Fleshman *et al.* (1981) emerged from consideration of the present group II (see Results) where an apparent sampling problem specific for this group served to minimize the rheobase–input conductance correlation in the raw material.

It should also be clear that if rheobase current was uncorrelated with input resistance within type, the 3-fold variation in input resistance within type observed by Fleshman *et al.* (1981) would require a similar variation in some other factor determining rheobase current. This would imply, for example, that threshold depolarization should be positively correlated with input resistance within type, being three times smaller in those with the lowest input resistance (or largest size according to Fleshman *et al.* (1981). Comparing this presumed large variation in threshold depolarization within type to the much smaller difference in rheobase 'voltage' between types evident in the material of Fleshman *et al.* (see above), a presynaptic input with equal density and efficacy over the motoneurone pool would then give rise to the paradoxical result that the largest motoneurones within each type would, almost independently of type, be recruited first and the smallest ones last. Any preferential recruitment of the presumed small type s. motoneurones would have to rely upon a greater density or larger efficacy of the presynaptic input. A random recruitment within type would only occur if the synaptic input within type were correlated with input resistance, which however does not seem to be the case (Fleshman, Munson & Sypert, 1981).

The present results suggested that part of the rheobase current variation was related to differences in subthreshold rectification among the motoneurones. This rectification was not measured directly but appeared as a discrepancy between the estimated threshold depolarization and the product of rheobase current and input resistance (measured close to resting level). This difference was practically always positive, indicating that the apparent cell input resistance increased when threshold

was approached. Such an increase of input resistance with membrane depolarization (anomalous rectification) is a well known feature of motoneurones (Nelson & Frank, 1967), and measurements of voltage drops produced by current pulses also suggested that the anomalous rectification in principle could account for the observed discrepancy between threshold depolarization and rheobase 'voltage'. The existence of a rectification in this direction was further suggested from the observed relations between the long and short current thresholds. Since the threshold depolarization tended to increase with time, an absence of subthreshold rectification would have led to long/short current pulse ratios higher than expected from the membrane properties measured at resting level. The ratios tended instead to be lower than expected, a finding which can be explained by a rectification in the observed direction.

The subthreshold rectification estimated in the present work differed among the motoneurones, being both relatively and absolutely larger in slow than in fast motoneurones. It was thus positively correlated with a.h.p. duration and membrane time constant, but showed no correlation with total cell capacitance. This result should probably not be taken to indicate that the inward current, likely underlying anomalous rectification in this potential range (Liebl & Lux, 1975; see also Hotson, Prince & Schwartzkroin, 1979), should be of greater magnitude in slow than in fast cells. Instead, the variation in rectification corresponded closely to the variation in input resistance between the different groups of motoneurones, suggesting no systematic variation in the underlying current. This result is consistent with the observation that the magnitude of the I_i inward current, possibly underlying the rectification in this potential range, appears to be similar over the motoneurone pool (Schwindt & Crill, 1980). It should be noted that the systematic variation in excitability introduced by this factor is only secondary to the systematic variation in passive membrane properties.

The present study also showed a good agreement between the experimental long/short current pulse ratios and those expected on the basis of passive membrane properties measured at resting level. This result may seem somewhat surprising in view of the apparent complexity of nerve cell membranes with a number of time and voltage dependent conductance processes operating also in the sub- and near-threshold membrane potential regions. However, if one examines motoneurones with brief time constants (< 4 ms) for which the agreement looks the best, one may see that the good fit is to some extent fortuitous. As indicated by the discrepancy between threshold depolarization and rheobase 'voltage', such cells showed some subthreshold rectification, giving a decrease in the expected threshold level for the long pulse corresponding to about 2 mV on average. On the other hand, as indicated by threshold depolarization measurements at short and long intervals using the spike-height method, there was a threshold shift of about the same value, increasing the threshold level for the long pulse. Thus, taking into account these two factors whose effects can be evaluated independently of the current threshold measurements themselves, the good agreement can in fact be expected.

To the extent that a third factor influences the current thresholds differentially, there must be an additional fourth factor balancing its effect. For example, voltage-clamp experiments have alluded to the presence of a fast transient outward current in motoneurones (Schwindt & Crill, 1980). Such a current might activate fast

enough to affect the short current threshold, but would inactivate and have little effect on the long pulse current. As mentioned in the Results section, inspection of the trajectories resulting from rheobase current pulses gave no clear indication of the presence of such a current. However, one cannot exclude that with this type of current-clamp activation the action of this current is obscured by the other currents present, and/or that it is more effectively triggered in isolation by the short pulse. The good agreement between the experimental and theoretical ratios suggests, however, that if this current is present in motoneurones and does significantly affect the short pulse current threshold, it must be well balanced by another as yet undetermined factor.

A 7-fold variation of short-pulse current threshold and an over-all variation in rheobase current about twice that value was observed. From the analysis, the variation in short-pulse current thresholds was explained by taking into account variations in size, geometry and threshold depolarization, the additional increase in range for rheobase currents given by variations in resistivity and subthreshold rectification. As should be clear from the discussion above (see also Gustafsson & Pinter, 1984), each of these determining factors has been obtained in indirect ways and each is also subject to problems of interpretation, for example with respect to the resting potential dependence of threshold depolarization. Any conclusion as to the exact role of any of these factors in setting current thresholds must clearly remain provisional. Nevertheless, if cell capacitance is taken as a size estimate (see Gustafsson & Pinter, 1984), it is clear that its 3-fold variation can only directly account for a minor part of the rheobase current range. Any dependence of rheobase current on size could only arise to the extent that the other factors determining current threshold are size-related. However, as shown here, none of these factors show much correlation with cell capacitance, and the over-all correlation between rheobase current and capacitance is also rather low ($r = 0{\cdot}47$). This result implies that only about 25 % of the over-all variation in rheobase current can be explained on the basis of size or size-related factors.

As discussed previously (Gustafsson & Pinter, 1984), the key factor deciding the functional variation of input resistance over the motoneurone pool seems to be specific membrane resistivity rather than size. The present results also showed a very strong correlation ($r = 0{\cdot}88$) between rheobase current and the inverse value of the time constant, suggesting that about 80 % of the over-all 16-fold variation in rheobase current is explained by variation of membrane resistivity. This strong co-variation is partly related to the direct effect of resistivity itself (about 4-fold over-all range), but is to a large extent also related to the correlation shown to exist between the time constant and other factors setting current threshold such as geometry, threshold depolarization and subthreshold rectification. It was also noted that the short current thresholds, despite being independent of resistivity itself, were better correlated with the inverse value of the time constant than with cell capacitance. It thus seems that specific membrane resistivity may be viewed as the key factor underlying the over-all variation in rheobase current. The higher threshold currents observed for fast motoneurones are then more a consequence of their lower specific membrane resistivity in association with more expansive dendritic trees, smaller subthreshold rectification and higher threshold depolarizations, than of larger surface areas.

As discussed by numerous authors (Henneman, Somjen & Carpenter, 1965; Henneman & Olson, 1965; Milner-Brown, Stein & Yemm, 1973; Fleshman *et al.* 1981; Harrison, 1983), the most effective manner of recruitment seems to be one in which muscle units of successively greater tension are recruited in an orderly manner. This would insure that at any operating force, the force added by recruitment of extra units would be proportional to that force level. Hence, fine gradation of force output is preserved independently of the operating level. To achieve this type of behaviour, it would seem reasonable to structure the motoneurone pool in such a way that the over-all range of muscle unit tension is somehow matched by or correlated with the range of motoneurone excitability. It has been suggested that the key organizational factor underlying this structure is size (Henneman *et al.* 1965; Henneman & Olson, 1965; Henneman & Mendell, 1981); the smallest motoneurones innervate the lowest tension producing units, and all other factors deciding excitability, such as synaptic input, or basic cellular features, such as voltage threshold, would be organized in relation to cell size to ensure that smaller motoneurones are recruited first. However, it is difficult to reconcile all of the assumptions of this view with recent experimental findings. In particular, both direct horseradish peroxidase (Ulfhake & Kellerth, 1982; Burke *et al.* 1982) and indirect electrophysiological estimates of cell size (Gustafsson & Pinter, 1984) indicate that motoneurones of similar size can innervate muscle units of widely different mechanical characteristics, and the average size differences between motoneurones innervating slow and fast muscle units seem to be much less than previously believed. The alternative view that size is only of relatively limited importance in deciding the range of functional excitability is strongly indicated by the present data, at least with respect to its importance as a key post-synaptic factor.

When considering how the motoneuronal pool should be structured to achieve the functional goal mentioned above, it may seem reasonable that the range of motoneurone excitability should be set by intrinsic factors largely independent of size itself, these factors being correlated with muscle unit tension. With an equal density of synaptic innervation over the motoneurone pool, such an organization would allow for the standard scheme of recruitment mentioned above. Any other use of the motoneurone pool outside this recruitment pattern could then be achieved by a specific use of the synaptic input. As shown in the present study, there exists a large variation in excitability over the motoneurone pool, and specific membrane resistivity seems to be the intrinsic factor best correlated with it. Although information about the relation between resistivity and muscle unit tension is still lacking, the fact that resistivity (or time constant) is well correlated with a.h.p. duration and input resistance and increases systematically from the present presumed s., to f.r., and to f.f. groups (Gustafsson & Pinter, 1984), suggests that resistivity should also be well correlated over-all with muscle unit tension. Consequently, one may suggest that the motoneurone pool is so structured such that the variation in functional excitability is set by resistivity and resistivity-related intrinsic factors, possibly in conjunction with a resistivity-related variation in synaptic input.

While this type of structure may operate over-all, one may ask to what extent this may also be true within a motor unit type. For example, it might be argued that the strong over-all relation between rheobase and time constant is not indicative of

a functional continuum but is rather the result of pooling data from distinctly different motoneurone subgroups in which the observed relations are not present. However, this does not seem to be directly the case, since significant correlations between rheobase and the inverse of time constant were present also within the present motoneurone subgroups, and it seems likely that the same should be true within motor unit types as well. The results thus suggest that the factors which organize the over-all range of motoneurone intrinsic excitability are operative, at least to a considerable extent, also within functional subgroups.

We wish to thank Mrs R. Larsson for expert technical assistance. This work was supported by the Swedish Medical Research Council (Project 05180) and by Magnus Bergvalls Stiftelse.

REFERENCES

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *Journal of Physiology* **117**, 431–460.

BURKE, R. E. (1981). Motor units: anatomy, physiology and functional organization. In *Handbook of Physiology*, sect. 1, *The Nervous System*. vol. 2, *Motor Control*, part 2. ed. BROOKS, V. B., pp. 345–422. Bethesda, MD: American Physiological Society.

BURKE, R. E. & NELSON, P. G. (1971). Accommodation to current ramps in motoneurons of fast and slow twitch motor units. *International Journal of Neuroscience* **1**, 347–356.

BURKE, R. E., DUM, R. P., FLESHMAN, J. W., GLENN, L. L., LEV-TOV, A., O'DONOVAN, M. J. & PINTER, M. J. (1982). An HRP study of the relation between cell size and motor unit type in cat ankle extensor motoneurones. *Journal of Comparative Neurology* **209**, 17–28.

CULLHEIM, S. (1978). Relations between cell body size, axon diameter and axon conduction velocity of cat sciatic alpha-motoneurons stained with horseradish peroxidase. *Neuroscience Letters* **8**, 17–20.

FLESHMAN, J. W., MUNSON, J. B. & SYPERT, G. W. (1981). Homonymous projection of individual group Ia-fibers to physiologically characterized medial gastrocnemius motoneurons of the cat. *Journal of Neurophysiology* **46**, 1339–1348.

FLESHMAN, J. W., MUNSON, J. B., SYPERT, G. W. & FRIEDMAN, W. A. (1981). Rheobase, input resistance and motor-unit type in medial gastrocnemius motoneurons in the cat. *Journal of Neurophysiology* **46**, 1326–1338.

GALVAN, M. (1982). A transient outward current in rat sympathetic neurones. *Neuroscience Letters* **31**, 295–300.

GRANTYN, R., GRANTYN, A. & SCHIERWAGEN, A. (1983). Passive membrane properties, after-potentials and repetitive firing of superior colliculus neurons studied in the anaesthetized cat. *Experimental Brain Research* **50**, 377–391.

GUSTAFSSON, B. & PINTER, M. J. (1984). Relations among passive electrical properties of lumbar α-motoneurones of the cat. *Journal of Physiology* **356**, 401–431.

HARRISON, P. J. (1983). The relationship between the distribution of motor unit mechanical properties and the forces due to recruitment and to rate coding for the generation of muscle force. *Brain Research* **264**, 311–315.

HENNEMAN, E. & MENDELL, L. M. (1981). Functional organization of motoneuron pool and its inputs. In *Handbook of Physiology, The Nervous System, Motor Control*, sect. 1, vol. II, part 1, chap. 11, pp. 423–507. Bethesda, MD: American Physiological Society.

HENNEMAN, E. & OLSON, C. B. (1965). Principles in the design of muscles. *Journal of Neurophysiology* **28**, 581–598.

HENNEMAN, E., SOMJEN, G. & CARPENTER, D. O. (1965). Functional significance of cell size in spinal motoneurons. *Journal of Neurophysiology* **28**, 560–580.

HOTSON, J. R., PRINCE, D. A. & SCHWARTZKROIN, P. A. (1979). Anomalous inward rectification in hippocampal neurons. *Journal of Neurophysiology* **42**, 889–895.

IANSEK, R. & REDMAN, S. J. (1973). The amplitude, time course and charge of unitary excitatory post-synaptic potentials evoked in spinal motoneurone dendrites. *Journal of Physiology* **234**, 665–688.

ITO, M. & OSHIMA, T. (1965). Electrical behaviour of the motoneurone membrane during intracellularly applied current steps. *Journal of Physiology* **180**, 607–637.

JACK, J. J. B. (1979). An introduction to linear cable theory. In *The Neurosciences; Fourth Study Program*, ed. SCHMITT, F. O. & WORDEN, F. G., pp. 423–437. Cambridge, MA: MIT Press.

KERNELL, D. & ZWAAGSTRA, B. (1981). Input conductance, axonal conduction velocity and cell size among hindlimb motoneurones of the cat. *Brain Research* **204**, 311–326.

KUNO, M. (1959). Excitability following antidromic activation in spinal motoneurones supplying red muscles. *Journal of Physiology* **149**, 374–393.

LIEBL, L. & LUX, H. D. (1975). The action of Co, Ba and Verapamil on anomalous rectification in cat spinal motoneurons. *Pflügers Archiv*, **355**, suppl., R80.

MILNER-BROWN, H. S., STEIN, R. B. & YEMM, R. (1973). The orderly recruitment of human motor units during voluntary isometric contractions. *Journal of Physiology* **230**, 359–370.

NELSON, P. G. & FRANK, K. (1967). Anomalous rectification in cat spinal motoneurons and effect of polarizing currents on excitatory postsynaptic potentials. *Journal of Neurophysiology* **30**, 1097–1113.

PINTER, M. J., CURTIS, R. L. & HOSKO, M. J. (1983). Voltage threshold and excitability among variously sized cat hindlimb motoneurons. *Journal of Neurophysiology* **50**, 644–657.

RALL, W. (1964). Theoretical significance of dendritic trees for neuronal input–output relations. In *Neural Theory and Modeling*, ed. REISS, R. F., pp. 73–93. Stanford: Stanford University Press.

SCHWINDT, P. C. & CRILL, W. E. (1980). Properties of a persistent inward current in normal and TEA-injected motoneurons. *Journal of Neurophysiology* **43**, 1700–1724.

SCHWINDT, P. C. & CRILL, W. E. (1983). Factors influencing motoneuron rhythmic firing: results from a voltage clamp study. *Journal of Neurophysiology* **48**, 875–890.

ULFHAKE, B. & KELLERTH, J-O. (1982). Does α-motoneurone size correlate with motor unit type in cat triceps surae? *Brain Research* **251**, 201–209.

ZENGEL, J. E., MUNSON, J. B., SYPERT, G. W. & REID, S. A. (1982). Can motor unit type be predicted on the basis of the motoneuron membrane properties? *Neuroscience Abstracts* **12**, 958.