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EFFECTS OF AXOTOMY ON THE DISTRIBUTION OF PASSIVE ELECTRICAL PROPERTIES OF CAT 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

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SUMMARY

1. Previously obtained experimental results concerning the effect of axotomy on motoneurone passive electrical properties have been re-analysed. As shown earlier, axotomy causes an average increase of motoneurone input resistance, membrane time constant and after-hyperpolarization duration.

2. The present analysis suggests that the increased input resistance is related to a higher specific membrane resistivity, a decreased cell size and an altered dendritic geometry. The results also suggest that the change takes place only in neurones projecting to fast-twitch muscle units and produces in them passive electrical properties normally exhibited only by motoneurones projecting to slow-twitch units.

3. Based on the notion that axotomy causes a 'dedifferentiation' of motoneurone properties, the present results might be taken to indicate that undifferentiated motoneurones are slow in character.

4. A possible scheme in which a post-natal differentiation of motoneurone properties may lead to muscle differentiation is discussed.

INTRODUCTION

Lumbar α-motoneurones projecting to different types of muscle units show a systematic variation in cellular properties that is likely to be an adaptation to functional demand. For example, motoneurones projecting to fast-twitch muscle units are known to have larger sizes, lower input resistances and shorter post-spike hyperpolarizations (a.h.p.s) than those projecting to slow-twitch units (e.g. Eccles, Eccles & Lundberg, 1958; Kuno, 1959; Burke, 1967; Burke, Dum, Fleshman, Glenn, Lev-Tov, O'Donovan & Pinter, 1982; Ulfhake & Kellerth, 1982). Following interruption of the connexion between motoneurone and muscle through axotomy, some of these differences diminish, the a.h.p. durations and input resistances of motoneurones to the slow-twitch soleus and the mainly fast-twitch medial gastrocnemius muscles becoming more similar to each other (Kuno, Miyata & Munoz-Martinez, 1974). As discussed by Huizar, Kuno & Miyata (1975), these changes may be seen as a 'dedifferentiation' to a state present in new-born kittens where at least the a.h.p. durations are fairly similar among the motoneurones.

Motoneurones of different types probably also differ with respect to specific

membrane resistivity and dendritic geometry (Kernell & Zwaagstra, 1981; Burke *et al.* 1982; Ulfhake & Kellerth, 1982; Gustafsson & Pinter, 1984*a*), and the function of a motoneurone may be more tightly coupled to resistivity and geometry than to size (Gustafsson & Pinter, 1984*a*). One would then expect these parameters also to change with axotomy such that 'dedifferentiated' distributions of values are obtained. In the preceding paper (Gustafsson & Pinter, 1984*a*), the interrelations among motoneurone passive cell properties such as size, specific resistivity and geometry, and their relations with motoneurone type were described using electrophysiological measurements only. Such data have also been obtained in a previous report (Gustafsson, 1979) dealing with the electrical properties of axotomized motoneurones but were then not analysed with respect to these considerations. In the present paper the same data will be presented in a form providing a better description of the nature of the changes in motoneurone passive electrical properties after axotomy.

METHODS

The experimental results presented were obtained from fourteen cats taken from two previous experiments (Gustafsson, 1979; Gustafsson, Katz & Malmsten, 1982). In seven of the cats, motoneurones were axotomized, either by an intradural section of the L7 and S1 ventral roots 2–3 weeks before the experiments, or by section of the nerves to the medial gastrocnemius and soleus muscles close to the muscle 4 and 8 weeks before the experiments. Seven unoperated cats were used as controls. For further methodological details the papers cited above can be consulted. The present analysis of the previously obtained measurements is based on assumptions about the motoneuronal geometry, the effect of micro-electrode-induced soma leak and of membrane resistance non-linearities discussed in detail in the preceding paper (Gustafsson & Pinter, 1984*a*).

RESULTS

In Fig. 1 are illustrated the changes produced by axotomy on some motoneurone parameters; input resistance, membrane time constant, a.h.p. duration and total cell capacitance. As indicated by the arrows under each distribution, axotomy causes an average increase in input resistance (*A*), membrane time constant (*B*) and a.h.p. duration (*C*), and a decrease in cell capacitance (*D*). Assuming a constant specific membrane capacitance within a motoneurone and among motoneurones (normal and axotomized), these capacitance values give a measure of motoneurone surface area and thus suggest that axotomy decreases motoneurone surface areas. These results show that after axotomy, motoneurones on average become smaller, and obtain a higher specific membrane resistivity and a longer a.h.p. duration. Examination of the graphs in Fig. 1 does however suggest that these changes are not due to a general shift of the same distribution of values to a new level. It can be noted first that the values in the axotomized population, with few exceptions, are contained within the distribution of values of normal motoneurones, which suggests that no 'new' type of motoneurone is created. A second feature is that the normally skewed distributions of input resistance, time constant and a.h.p. duration are replaced by what appear to be narrower and more Gaussian distributions of values. The narrower distribution is especially evident with respect to a.h.p. duration ($P < 0{\cdot}001$, F-test), and is of border-line significance also for the capacitance (or size) estimates.

Among normal motoneurones certain relations have been shown to exist between these different electrical parameters (Gustafsson & Pinter, 1984*a*). For example, motoneurones with higher input resistances display longer membrane time constants likely caused by a higher membrane resistivity. As suggested by the graph in Fig. 2*A*, the increase in input resistance following axotomy seems to parallel a similar increase

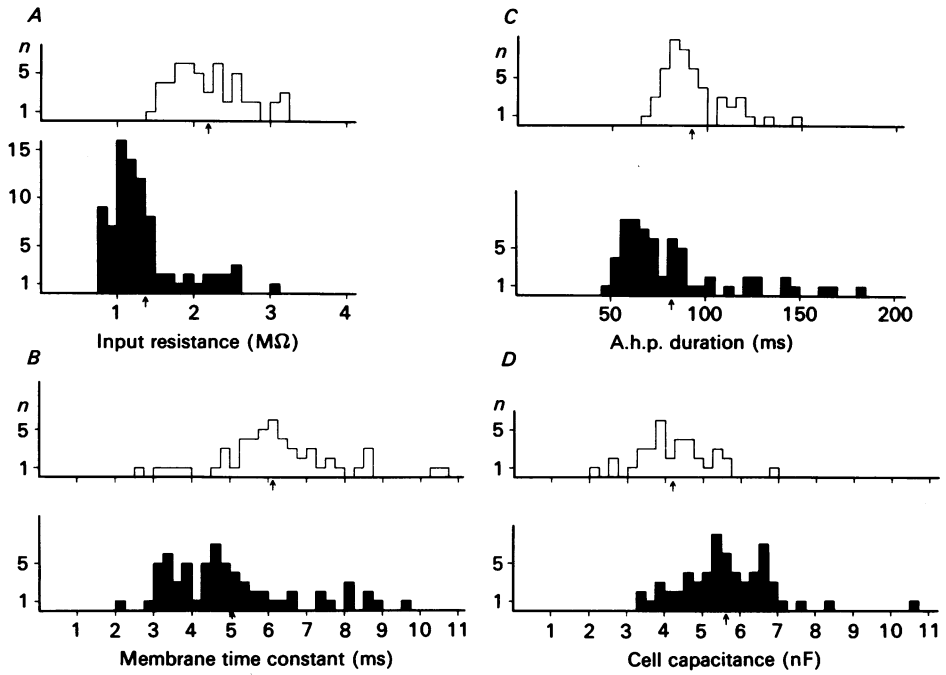

Fig. 1. Distribution of electrical properties among normal and axotomized motoneurones. *A–D*, distributions of input resistance (*A*), membrane time constant (*B*), a.h.p. duration (*C*) and total cell capacitance (*D*). The distributions of values for normal motoneurones are shown in the filled histograms and for axotomized in the open ones. The cell capacitance values were obtained from the input resistance, the time constant and electrotonic length values as described by Gustafsson & Pinter (1984*a*). The average value for each distribution is indicated by an arrow below the abscissa.

in time constant as present among the normal motoneurones (see also Fig. 3, Gustafsson & Pinter, 1984*a*).

As discussed in the preceding paper, with a constant dendritic geometry the electrotonic length of motoneurones should vary with membrane time constant such that a plot of electrotonic length against the inverse square root of the time constant should yield a straight line passing through the origin. In a normal population this was not found to be the case, a regression line intersecting the ordinate far above the origin. As shown in Fig. 2*B*, a regression for the total population of values (normal plus axotomized) shows a similar small variation of electrotonic length with time constant, the line intersecting the ordinate at an electrotonic-length (*L*) value of 1·0 compared to 0·9 in a normal population (Fig. 4, Gustafsson & Pinter, 1984*a*). Examination of the graph also shows that the values for normal and axotomized

motoneurones are well intermingled for a given time-constant value. This result then suggests that the increase in membrane resistivity following axotomy is associated with the same change in dendritic geometry as that observed between normal motoneurones of low and high resistivity.

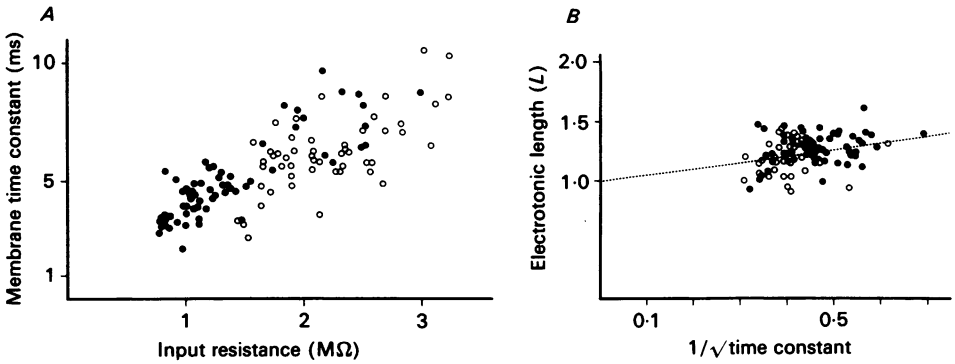

Fig. 2. Relations between input resistance, time constant and electrotonic length. Values from normal and axotomized motoneurones are shown with filled and open circles, respectively. *A*, membrane time constant plotted against input resistance. *B*, electrotonic length plotted against the inverse square root of the time constant. The dashed line is the result of a linear regression performed on all the values (normal plus axotomized).

Relation with a.h.p. duration

In a normal population of motoneurones, input resistance and membrane time constant increase over-all in a rather proportional manner with a.h.p. duration, and there is a decrease in cell size as estimated from total capacitance (Gustafsson & Pinter, 1984*a*). These relations can also be observed for the present control material in Fig. 3, although the rather few cells with long a.h.p. durations make these trends slightly less evident than shown previously. Nevertheless, it can be observed that the changes occurring in the a.h.p. duration with axotomy are largely reflected in similar changes in the other parameters. There are however some discrepancies most evident in the relation between input resistance and a.h.p. duration. First, within the normal population, a.h.p. durations around 70–100 ms can be associated with lower (around 1 MΩ) as well as higher (around 2 MΩ) input resistances. However, within this a.h.p. range the axotomized motoneurones show exclusively the higher input-resistance values. In Fig. 3*B* is plotted the input resistance–a.h.p. duration relation for normal motoneurones taken from Gustafsson & Pinter (1984*a*), but with presumed type F motoneurones (groups II + III) and presumed type S motoneurones (group I) indicated separately. As judged from this graph, the axotomized motoneurones within this a.h.p. duration range are all within the type S range of the normal distribution.

As shown previously (Kuno *et al.* 1974), axotomy decreased the a.h.p. duration of soleus motoneurones but had little effect on their input resistance. Similarly, the average input resistance of axotomized soleus motoneurones ($n = 6$) among the present data was exactly the same (2·55 MΩ) as the average resistance of soleus motoneurones ($n = 14$) in the study of Gustafsson & Pinter (1984*a*). This fact may then explain the somewhat higher input resistances at a.h.p. durations around 100 ms in

the axotomized material encountered only at longer a.h.p. durations in the normal population.

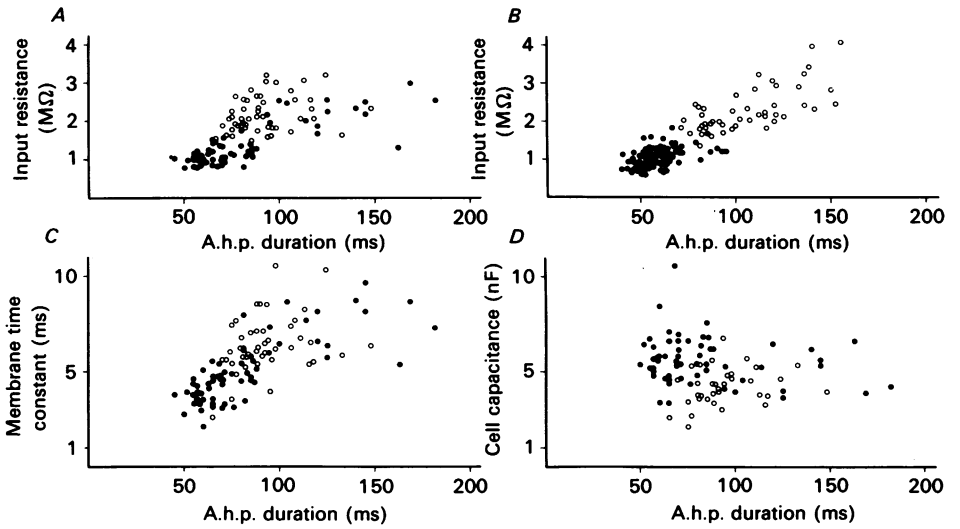

Fig. 3. Relations between passive cell properties and a.h.p. duration. *A*, input resistance plotted against a.h.p. duration. Values from normal and axotomized motoneurones are shown with filled and open circles, respectively. *B*, the same plot but performed with measurements taken from Gustafsson & Pinter (1984*a*). The filled circles are values taken from cells presumed to be projecting to type FF and FR muscle units (groups II and III), while the open ones correspond to presumed type S motoneurones (group I). *C* and *D*, values from normal and axotomized motoneurones are shown with filled and open circles, respectively. *C*, membrane time constant plotted against a.h.p. duration. *D*, total cell capacitance plotted against a.h.p. duration.

Relation between membrane resistivity and cell size

In the preceding paper, a simple model of the motoneurone pool was presented in which motoneurones were distributed with equal density with respect to surface area, specific membrane resistivity and electrotonic length, with the two latter parameters independent of surface area but themselves interrelated. When plotting membrane time constant against cell capacitance, motoneurones in such a pool would then distribute themselves with equal density within a rectangle such as that in Fig. 4*B*. The experimental distribution of values from normal and axotomized motoneurones is shown in Fig. 4*A*, and shows as previously found (Gustafsson & Pinter, 1984*a*) that membrane time constant varies little with the estimated size. In Fig. 4*B* these values have been enclosed by a rectangle containing most of the experimental values. It can be observed that the bulk of the normal distribution (filled circles) is situated in the lower right part of the rectangle, covering the upper two-thirds of the size range and the lower third of the time constant or resistivity range. On the other hand, the axotomized motoneurones are largely contained in the lower two-thirds of the size range and the upper two-thirds of the resistivity range together with a few of the

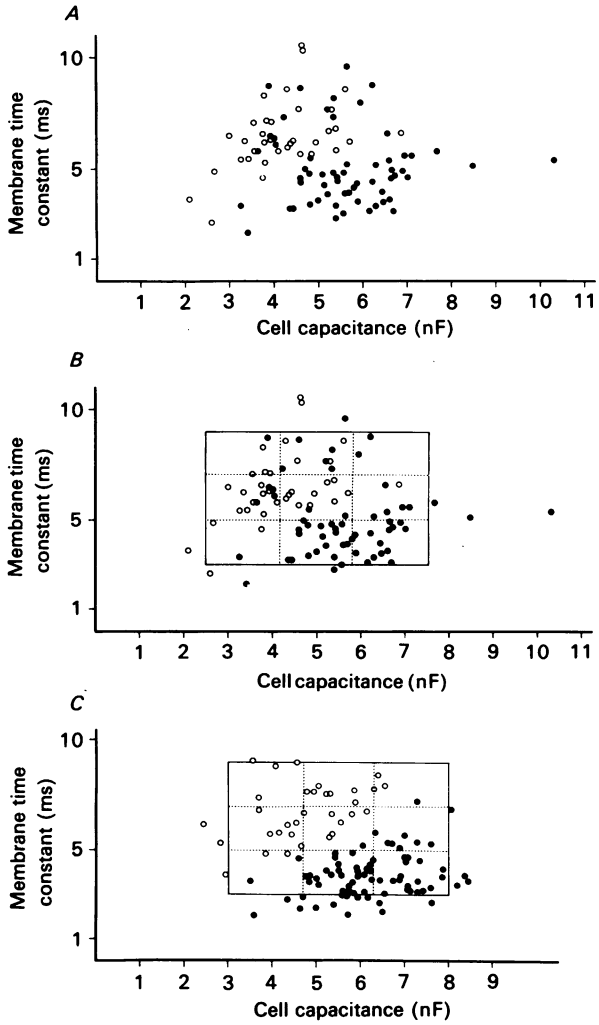

Fig. 4. Relation between membrane time constant and cell capacitance. *A*, time constant plotted against cell capacitance for normal (filled circles) and axotomized (open circles) motoneurones. *B*, the same plot as in *A* but with the values enclosed by a rectangle, representing a simple model of a motoneurone pool. *C*, membrane time constant plotted against cell capacitance for values taken from Gustafsson & Pinter (1984*a*). Presumed type FF and FR motoneurones (groups II and III) are shown with filled circles, and presumed type S motoneurones (group I) with open circles. The values are enclosed by a rectangle similar to that in *B*, but shifted 0·5 nF along the abscissa. This shift was done in order to compensate for the somewhat larger average capacitance values obtained in these later experiments.

control neurones. It can be noted that this difference between normal and axotomized motoneurones corresponds well to the shift within a similar rectangle (Fig. 4*C*) when moving from presumed type F motoneurones (II+III) to presumed type S motoneurones (I) in the normal population of motoneurones obtained by Gustafsson & Pinter (1984*a*).

DISCUSSION

In the present paper, previously obtained data from axotomized motoneurones have been re-examined to provide a better description of the nature of the transformation occurring in motoneurone passive electrical properties following axotomy. As was described earlier (Gustafsson, 1979), axotomy causes a considerable increase in average motoneurone input resistance. The present analysis suggests that this change arises from an increase in specific membrane resistivity (as judged by an increased membrane time constant), a decrease in motoneurone surface area as indicated by the decreased average total cell capacitance, and a change in dendritic geometry. These alterations occur, however, in a specific way. In a normal population, motoneurones projecting to presumed type F muscle units are quantitatively distinct from those projecting to presumed type S units in terms of these passive electrical membrane properties (Gustafsson & Pinter, 1984*a*; see also Kernell & Zwaagstra, 1981; Burke *et al.* 1982; Ulfhake & Kellerth, 1982). Following axotomy, these distinctions are absent, and what remains is a more homogeneous ensemble of motoneurones resembling the presumed type S motoneurones of adult cat.

As discussed by Gustafsson & Pinter (1984*a*), the type of analysis employed here is based solely on electrophysiological measurements and is thus subject to certain assumptions and possible errors. The over-all similarity with the results of Gustafsson & Pinter (1984*a*) suggests, however, that the present control material is not subject to any additional or exceptional errors. Moreover, assuming that axotomy does not produce any violation concerning the assumptions of the equivalent cylinder model, it seems reasonable to believe that at least the relative changes indicated by the electrophysiological measurements reflect actual alterations in cellular properties caused by axotomy.

The analysis thus suggests that when adult motoneurones are physically disconnected from muscle, they acquire passive electrical properties of type S motoneurones. This would imply that the properties of type S motoneurones themselves do not change following axotomy. As shown by Kuno *et al.* (1974) and by the present study, the input resistance of soleus motoneurones (type S) is little changed following axotomy and thus, presumably, neither are other properties determining input resistance. It may be further expected that the parameter distributions following axotomy should resemble distributions of values obtained from type S motoneurones. Owing to the limited sampling from type S motoneurones in control animals, no detailed comparison can be made in this regard. However, comparison between the histograms of Fig. 1 with those in Fig. 7 of Gustafsson & Pinter (1984*a*) reveals great similarities in the ranges and distributions of values from axotomized and type I presumed type S motoneurones (compare also Fig. 4*B* and *C* in the present paper). Taking averaged values for input resistance, time constant, cell capacitance and a.h.p. duration for axotomized and group I motoneurones respectively, these values are 2·19 and 2·33 MΩ, 6·21 and 6·76 ms, 4·2 and 4·7 nF, and 92 and 106 ms. These values can be related to the corresponding values for presumed type FF motoneurones (group III motoneurones) from the same study, 0·87 MΩ, 3·29 ms, 6·4 nF and 54 ms. As can be seen in Fig. 4, the total cell capacitance estimates were on the average somewhat lower in the present material than in Gustafsson & Pinter (1984*a*).

If allowance is made for this small discrepancy (which seems related to lower estimates of the electrotonic lengths), the agreement between estimated sizes of axotomized and group I motoneurones is even better than indicated above. As described earlier (Gustafsson, 1979), the average increase in membrane time constant was not associated with any detectable changes in average electrotonic length following axotomy. Had there been no changes in neuronal geometry following axotomy, a decrease in electrotonic length would be expected according to cable theory (cf. Jack, Miller, Porter & Redman, 1971; Gustafsson & Pinter, 1984*a*). The apparent absence of such an effect suggests that the transformation induced in a normal type F motoneurone by axotomy not only involves its specific resistivity and surface area but also produces a change in over-all dendritic geometry which parallels that observed when moving from type F to type S motoneurones in a normal population, that is the appearance of less expansive dendritic trees (cf. Gustafsson & Pinter, 1984*a*). Retraction of dendritic branches such as that seen after axotomy of brain-stem motoneurones (Sumner & Watson, 1971) may thus be a feature of this kind of transformation induced by axotomy.

In the distinction between types F and S motoneurones, the duration of the a.h.p. plays a significant role, the a.h.p. in F motoneurones being appreciably shorter than in S motoneurones (Eccles *et al.* 1958; Kuno, 1959; Burke, 1967). As described by Kuno *et al.* (1974), axotomy decreases the a.h.p. duration of soleus motoneurones, and produces some increase in the a.h.p. duration of those with short a.h.p.s. Shortening of a.h.p.s was not very apparent in the present material, presumably due to the small number of control motoneurones with long a.h.p. duration. On the other hand, an increase of a.h.p. duration was more apparent. Comparison of the histograms in Fig. 1*C* revealed the virtual absence of a.h.p. durations in the 50–70 ms range among axotomized motoneurones, a range in which a high proportion of values are found in the normal population. A similar upward shift in the distribution of values is indicated in Kuno *et al.* (1974) but is less evident. Considering the presence of both increases and decreases in a.h.p. duration, it may be imagined that axotomized motoneurones acquire an intermediate a.h.p. duration relative to the normal range of 40–200 ms (i.e. between that of type F and S motoneurones). It should be noted, however, that at least in our hands the range of a.h.p. durations obtained from axotomized motoneurones is mainly in the province of normal group I (presumed type S), and as shown above, the average group I a.h.p. duration is rather close to that of axotomized motoneurones. One may then believe that axotomized motoneurones are also slow with respect to the a.h.p. duration, albeit in the lower part of the normal type S range. Axotomized motoneurones are, however, not similar to normal type S motoneurones in all respects. For example, as shown previously (Heyer & Llinas, 1977; Gustafsson, 1979) the frequency–current curves of axotomized motoneurones are considerably steeper than normally observed and the current underlying the a.h.p. is less. Axotomized motoneurones also often show small spike-like partial responses to afferent stimulation and have more prominent hump-type delayed depolarizations than observed normally.

As discussed by Kuno *et al.* (1974), the changes in a.h.p. duration induced by axotomy may be seen as a 'dedifferentiation' of the motoneurone properties. Such a throw-back to more immature properties is also indicated by the appearance of

dendritically initiated partial spike responses to afferent stimulation (Kuno & Llinas, 1970), a property also suggested for kitten motoneurones (Kellerth, Mellström & Skoglund, 1971). One may then in an analogous manner propose that the suggested transformation of type F motoneurones into type S motoneurones with respect to passive electrical membrane properties represents a feature of this dedifferentiation. It should be noted that this is not a complete transformation into dedifferentiated kitten motoneurones since motoneurone sizes are still those of an adult cat. Nevertheless, the present results might be taken to indicate that undifferentiated motoneurones are slow in character and that post-natal development leads to the emergence of fast characteristics while the slow motoneurones do not change with respect to these passive electrical characteristics. If this is true, it implies an homology between motoneurone and muscle, both starting out as slow. Based on the effect of axotomy, one may speculate that the differentiation into fast and slow motoneurone characteristics may be related to the establishment of neuromuscular contact, the muscle connexion in an unspecified way enabling the onset of differentiation. It should be noted that motoneurones destined to be fast are then developed into a less excitable state by the reduction in input resistance, due to resistivity, size and geometry changes, and possibly also by other intrinsic differences making F motoneurones less excitable than S motoneurones (Burke & Nelson, 1971; Fleshman, Munson, Sypert & Friedman, 1981; Kernell & Monster, 1981; Gustaffson & Pinter, 1984*b*). As suggested by the results of Gallego, Huizar, Kudo & Kuno (1978), a quiescent motoneurone would lead to a speeding of its muscle unit from the slow contraction present at birth. One may then surmise that a post-natal differentiation into type F motoneurones, leading into a developing decrease in their excitability and a reduction in their motor activity, would result in the observed muscle unit speeding (cf. Gallego *et al.* 1978). On the other hand, the relatively more excitable type S motoneurones would be more active, which would, as discussed by Gallego *et al.* (1978), counteract the muscle unit speeding. It should be recognized that this scheme whereby motoneurone differentiation leads to muscle differentiation remains speculative since it is not known whether kitten motoneurones actually are all slow at birth, if differentiation of motoneurone excitability properties precedes muscle differentiation or if such a motoneurone differentiation leads to the presumed change in firing activity.

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