

DIFFERENTIATION OF MOTONEURONES AND SKELETAL MUSCLES IN KITTENS

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SUMMARY

1. Isometric contractions of the medial gastrocnemius (fast twitch) and soleus (slow twitch) muscles were recorded in kittens ranging in age from 3 to 112 days, as well as in adult cats.

2. It was confirmed that the speed of contraction of the gastrocnemius muscle becomes progressively faster during the first few weeks after birth, whereas contraction times of the soleus muscle show little changes or a slight prolongation during the period of post-natal development.

3. The properties of gastrocnemius (fast alpha) and soleus (slow alpha) motoneurones were examined with intracellular electrodes in kittens at three different stages; 16–20, 61–71 and 100–112 days in age.

4. The axonal conduction velocities of both gastrocnemius and soleus motoneurones increased monotonically throughout the period of development and showed no correlation with post-natal changes in contraction times of the innervated muscles.

5. The duration of after-hyperpolarization in soleus motoneurones became progressively longer with age, while that in gastrocnemius motoneurones remained virtually unchanged during development.

6. The relation between the duration of after-hyperpolarization and the axonal conduction velocity in kitten motoneurones was similar to that observed in axotomized motoneurones of adult cats.

7. It is suggested that fast and slow alpha motoneurones show post-natal differentiation in terms of the duration of after-hyperpolarization and that axotomy leads to 'dedifferentiation' of the motoneurone properties.

8. Post-natal changes in the contractile properties of skeletal muscles were independent of the changes in the duration of after-hyperpolarization of the innervating motoneurones. However, it remains uncertain whether muscle differentiation is independent of the discharge pattern of the innervating motoneurones.

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INTRODUCTION

All skeletal muscles in new-born kittens show uniformly slow contraction (Denny-Brown, 1929; Koschtjanz & Rjabinowskaja, 1935). During the first few weeks after birth, the speed of contraction of many muscles (e.g. gastrocnemius) becomes progressively faster while other muscles (e.g. soleus) maintain their slow contraction times (Buller, Eccles & Eccles, 1960*a*; Buller & Lewis, 1965*a*; also, cf. Close, 1964). Thus, differentiation into the fast and slow twitch muscles is complete in early post-natal life. However, the speed of muscle contraction can be transformed, at least in part, if the nerves to the two types of muscles are sectioned and cross-united before, or even after, muscle differentiation is established (Buller, Eccles & Eccles, 1960*b*; Eccles, Eccles & Kozak, 1962; Buller & Lewis, 1965*b*; also, cf. Close, 1969). These observations suggest two features for the differentiation processes during development: (1) motoneurone differentiation may precede muscle differentiation because the muscle properties depend on the motoneurone properties; (2) the properties of the motoneurons innervating fast twitch muscles (e.g. gastrocnemius) may change during development because it is these muscles that show post-natal changes.

In adult cats, the motoneurons innervating fast twitch muscles usually have a faster axonal conduction velocity (fast alpha motoneurons) and show a shorter after-hyperpolarization following each action potential than motoneurons subserving the slow twitch (e.g. soleus) muscles (Eccles, Eccles & Lundberg, 1958; Kuno, 1959; Burke, 1967; Kuno, Miyata & Muñoz-Martinez, 1974). It is generally agreed that the duration of after-hyperpolarization limits the maximum frequency at which the motoneurone can discharge (Kernell, 1965; Gustafsson, 1974). It has been suggested that the difference in discharge frequency between fast (30–60/sec) and slow (10–20/sec) alpha motoneurons (Granit, Henatsch & Steg, 1956; Granit, Phillips, Skoglund & Steg, 1957) may be one of the factors responsible for the different contractile properties of the innervated muscles (Buller *et al.* 1960*b*). In fact, when the fast twitch muscles are subject to daily stimulation at a rate of 10/sec, contraction times of the muscles are significantly prolonged (Eccles *et al.* 1962; Salmons & Vrbová, 1969). Similarly, the speed of contraction of the denervated slow twitch muscle in rats increases after daily stimulation at 100/sec (Lomo, Westgaard & Dahl, 1974). Furthermore, in individual motor units, the duration of after-hyperpolarization in motoneurons has a positive correlation with contraction times of the innervated muscle fibres (Devanandan, Eccles & Westerman, 1965; Burke, 1967; R. E. Burke, personal communication). A question may then arise as to whether differentiation of

skeletal muscles may be associated with post-natal changes in discharge frequency and in the duration of after-hyperpolarization of the innervating motoneurons. It is conceivable that all motoneurons in new-born kittens may uniformly have a long after-hyperpolarization and that the post-natal speeding of contraction of fast twitch muscles may result from progressive decrease in the duration of after-hyperpolarization of the motoneurons.

The present study was undertaken to examine the above possibility. The questions to be tested are: (1) do motoneurons show post-natal differentiation with respect to the duration of after-hyperpolarization? (2) if so, does differentiation occur only in fast alpha motoneurons? (3) does motoneurons differentiation precede muscle differentiation?

METHODS

The experiments were performed on kittens, ranging from 3 to 112 days in age and from 110 to 1050 g in weight. The animal was anaesthetized by i.p. injection of sodium pentobarbitone (25–40 mg/kg), and the brain was anaemically destroyed by bilateral occlusion of the common carotid and vertebral arteries. The spinal cord was transected at the first cervical level, and the brain rostral to the section was pithed. The animal was then maintained on artificial respiration. The rectal temperature was kept between 35 and 38° C by external heat.

The lumbosacral spinal cord was exposed by laminectomy. The dorsal roots were sectioned on the left side from the sixth lumbar through the first sacral segments. In the left hind leg, the nerves to the medial gastrocnemius (fast twitch), lateral gastrocnemius (fast twitch) and soleus (slow twitch) muscles were dissected. Each of these muscle nerves was cut distally and prepared for electrical stimulation. Intracellular recording from spinal motoneurons was identified by the generation of antidromic action potentials in response to stimuli applied to the central ends of the cut muscle nerves. The glass micro-electrodes used for intracellular recording were filled with 2 M potassium acetate. Their resistance was between 10 and 30 M Ω . To minimize disturbance from respiratory movements, the exposed spinal cord was covered with 2% agar dissolved in Hartmann solution. The properties of spinal motoneurons were measured in terms of the axonal conduction velocity and the duration of after-hyperpolarization by the methods described in a previous report (Kuno *et al.* 1974).

At the beginning of every experiment, isometric twitch characteristics of the medial gastrocnemius and soleus muscles were observed in a warm paraffin oil at 37–38° C. The distal tendons of these muscles were cut, and the distal portion of the muscles was freed of surrounding tissues. The cut tendon was attached to a force displacement transducer (Grass FT 0.03 or FT 10) operated into a carrier amplifier (Tektronix 3C66). The initial tension was adjusted at the optimum level at which the twitch tension was maximum (Buller *et al.* 1960*a*). Muscle contractions were also recorded in seven adult cats (2.0–3.9 kg in weight) for which an initial tension of 100 g was used (Burke, 1967). Contraction times measured at this level (100 g) of initial tension might have been slightly longer than those observed at the 'optimum' initial tension (see Results).

In four kittens, the electromyogram (e.m.g.) was recorded from the medial gastrocnemius and soleus muscles with pairs of enamel coated copper wires (40 gauge).

A pair of the recording wires was inserted into each muscle under anaesthesia with methoxyflurane (Penthrane; Abbott Laboratories). The recording wires and the connectors (Winchester plugs) were securely anchored to connective tissues with fine silk thread (Fischbach & Robbins, 1969; Nelson, 1969). The animal recovered from anaesthesia within 1–2 hr after the operation. The e.m.g. was then recorded on an analogue magnetic tape for 30–60 min through long flexible cables while the animal was unrestrained and walking on the experimental table.

RESULTS

Muscle differentiation. Fig. 1 illustrates isometric contractions of the medial gastrocnemius (single arrows) and soleus (double arrows) muscles evoked by nerve stimulation in 20-day-old (*A*) and 112-day-old (*B*) kittens. The contraction times in the fast twitch muscle (gastrocnemius) were appreciably shortened with age, whereas those in the slow twitch

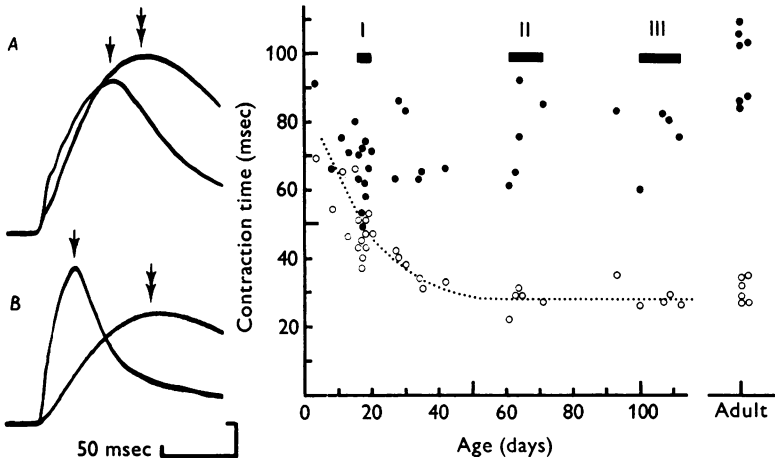


Fig. 1. Isometric contractions of medial gastrocnemius (single arrows) and soleus (double arrows) muscles recorded in 20-day-old (*A*) and 112-day-old (*B*) kittens. Vertical calibration: 4 g for soleus and 20 g for gastrocnemius in *A*; and 40 g for soleus and 100 g for gastrocnemius in *B*. Graph on the right, relations between contraction time and age for medial gastrocnemius (open circles) and soleus (filled circles) muscles. Upper horizontal bars indicate three post-natal stages (I, II, III) selected for measurements of motoneurone properties.

muscle (soleus) showed little changes or a slight prolongation during development. The relationship between contraction times and age is shown on the right side in Fig. 1 for the fast (open circles) and slow twitch (filled circles) muscles. The post-natal changes of contraction time in the fast twitch muscle (dotted curve) were essentially the same as those reported previously (Buller *et al.* 1960*a*; Buller & Lewis, 1965*a*). Contraction

times of the soleus muscle (filled circles) varied considerably from animal to animal at any developmental stage, so that the time course of the post-natal changes was difficult to delineate precisely (Fig. 1). However, it is clear that the difference in contraction times between the two types of muscles is more prominent at late stages of development (after 60 days) than at early stages (before 20 days) and that differentiation into the fast and slow twitch types is achieved largely by a progressive speeding of contraction of the fast twitch muscle (Fig. 1).

The question posed in the present study was whether post-natal differentiation of the two types of muscles is associated with changes in the properties of the motoneurons innervating these muscles. To examine this question, intracellular recording from spinal motoneurons was made in kittens at three different stages indicated by horizontal bars (I, II, III) in Fig. 1. Stage I included kittens between 16 and 20 days of age. The kittens at Stage II and Stage III were aged between 61 and 71 days and between 100 and 112 days, respectively. It should be noted that muscle differentiation is still incomplete at Stage I, whereas Stage II and Stage III are virtually the same with respect to the degree of muscle differentiation.

Contraction times of the soleus muscle were longer in adult cats than those in old kittens (100–112 days). This tendency can be noticed also in previous reports (Fig. 3 in Buller *et al.* 1960*a*; Fig. 2 in Buller & Lewis, 1965*a*). However, our results for contraction times of the soleus muscle in adult cats (97 msec; Fig. 1, Table 1) were appreciably longer than the previous values (70–85 msec; Buller *et al.* 1960*a*; Buller & Lewis, 1965*a*). On the other hand, contraction times of the gastrocnemius muscle of adult cats in our experiments (31 msec; Fig. 1, Table 1) were comparable to those reported by Buller *et al.* (1960*a*). In adult cats, contraction times tend to be longer at initial tensions greater than about 50 g (Burke, 1967; also, cf. Methods). However, this tendency has been found to be less marked in the soleus than in the gastrocnemius muscle (Burke, 1967). No adequate explanation was given to long contraction times of the soleus muscle observed in adult cats.

Motoneurone differentiation. Fig. 2 illustrates antidromic action potentials recorded from medial gastrocnemius and soleus motoneurons in 16-day-old (*A*) and 112-day-old (*C*) kittens. As depicted by changes in latencies of the action potentials (Fig. 2*A, C*), the axonal conduction velocity increased with age in both gastrocnemius and soleus motoneurons. After-hyperpolarization following an action potential evoked in these motoneurons by intracellular stimulation is shown in Fig. 2*B, D*. The duration of after-hyperpolarization in gastrocnemius motoneurons shows little difference between the two kittens (Fig. 2*B, D*), whereas that in soleus motoneurons appears to increase with age.

Fig. 3 shows the mean values for the duration of after-hyperpolarization (middle) and axonal conduction velocity (bottom) of gastrocnemius (open circles) and soleus (filled circles) motoneurons at different post-natal stages (I, II, III). The open circles in Fig. 3 included the results

obtained from both medial and lateral gastrocnemius motoneurons since their electrophysiological properties were indistinguishable at any developmental stage examined. The axonal conduction velocity of gastrocnemius and soleus motoneurons (Fig. 3, bottom; also, cf. Table 1) increased monotonically throughout the period of development (Ridge, 1967; Bagust, Lewis & Westerman, 1974; also, cf. Nyström, 1968). There was no obvious correlation between post-natal changes in contraction times of the fast and slow twitch muscles (Fig. 3, top) and changes in axonal conduction velocity of their motoneurons (Fig. 3, bottom).

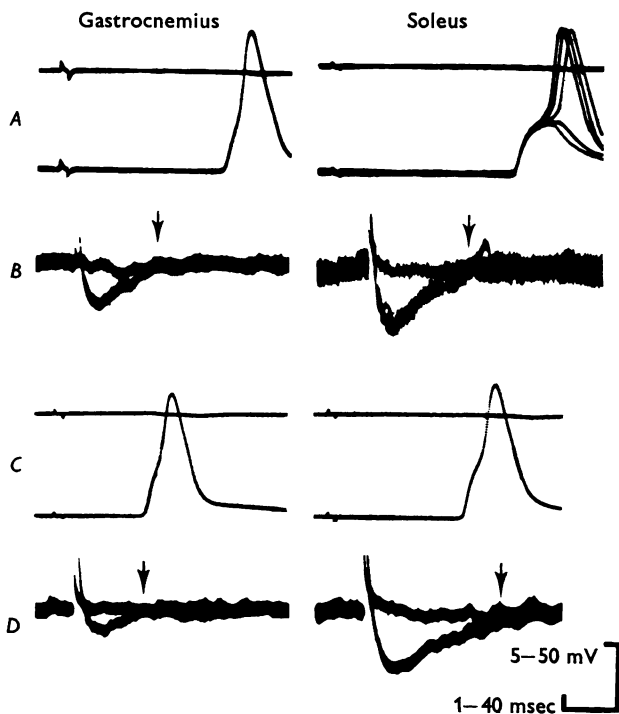


Fig. 2. Resting and antidromic action potentials recorded from medial gastrocnemius and soleus motoneurons (*A, C*). *B, D*, responses evoked in the same motoneurons by intracellular stimulation. Arrows indicate termination of after-hyperpolarization. *A, B*, from a 16-day-old kitten. *C, D*, from a 112-day-old kitten. 50 mV, 1 msec calibration for *A, C*. 5 mV, 40 msec calibration for *B, D*.

The difference in the duration of after-hyperpolarization between gastrocnemius and soleus motoneurons became progressively more prominent with age (Fig. 3, middle; also, cf. Table 1). Thus, spinal motoneurons apparently show post-natal differentiation with respect to the

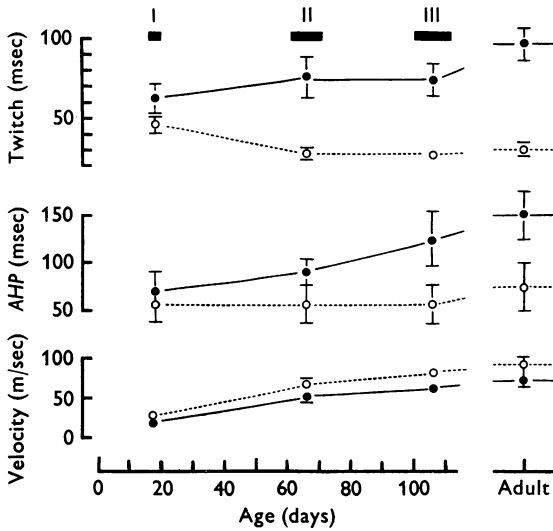


Fig. 3. Time courses of the changes in twitch times of medial gastrocnemius (open circles) and soleus (filled circles) muscles (top), the duration of after-hyperpolarization (middle, *AHP*) and the axonal conduction velocity (bottom) of medial and lateral gastrocnemius (open circles) and soleus (filled circles) motoneurons. Each point represents the mean with s.d. of observation (vertical bars). Upper horizontal bars indicate three post-natal stages (I, II, III) selected for measurements.

duration of after-hyperpolarization. However, the post-natal increase in the duration of after-hyperpolarization was observed only in soleus motoneurons, whereas this parameter in gastrocnemius motoneurons remained unchanged during development (Fig. 3, middle). This is in contrast with the behaviour of the muscles in which post-natal changes in the contractile properties are more dominant in the gastrocnemius than in the soleus muscle (Fig. 1). Also, soleus motoneurons showed a further increase in the duration of after-hyperpolarization (Fig. 3, middle, filled circles), while contraction times of the soleus muscle remained relatively constant at Stages II and III (Fig. 3, top, filled circles). From the results illustrated in Fig. 3, it seems clear that post-natal changes in the speed of muscle contraction are not correlated with those in the duration of after-hyperpolarization of the innervating motoneurons.

Fig. 4A shows the relation between the duration of after-hyperpolarization and the axonal conduction velocity for gastrocnemius and soleus motoneurons recorded at different developmental stages. The properties of ten soleus (filled circles) and twenty-nine gastrocnemius motoneurons (open circles) examined at Stage I showed a considerable overlap as illustrated by continuous and dashed line boundaries (left outermost in

Fig. 4A). The properties of ten soleus and thirty-five gastrocnemius motoneurons observed at Stage II are similarly shown by continuous and dashed line boundaries, respectively (Fig. 4A). The results from kittens at Stage III (eleven soleus and twenty gastrocnemius motoneurons) are presented only by the mean values (large filled and open circles), for

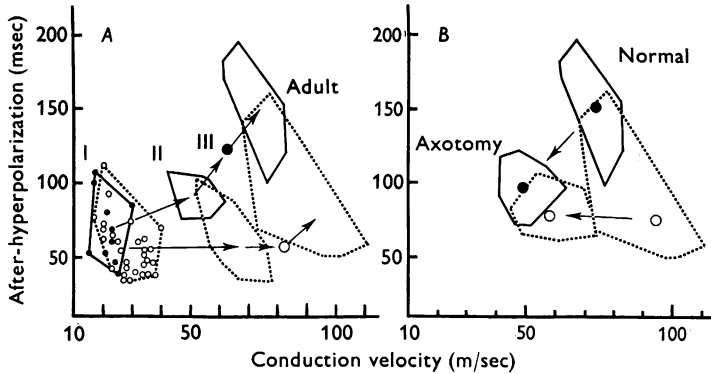


Fig. 4. Relations between the duration of after-hyperpolarization and the axonal conduction velocity of motoneurons. *A*, changes in the properties of gastrocnemius (open circles and dashed line boundaries) and soleus (filled circles and continuous line boundaries) motoneurons at different stages (I, II, III) in kittens up to normal adults. Small open and filled circles represent the results from individual motoneurons (Stage I). Large open and filled circles (Stage III) represent the mean values. Arrows are connected between the mean values at adjoining stages. *B*, similar to *A*, but the results from adult cats under normal conditions (right) and 110–119 days after section of the gastrocnemius and soleus muscle nerves (left). The results from normal and axotomized adult cats were reproduced from those in Kuno *et al.* (1974).

simplicity, without their boundaries (Fig. 4A, III). Post-natal differentiation of the two groups of motoneurons thus appears to follow the direction indicated by arrows connected between the mean values at adjoining stages (Fig. 4A). These changes in the motoneurone properties may be compared to the changes produced by chronic section of the motor axons (axotomy) in adult cats. Fig. 4B shows the shift of the properties of soleus (continuous line boundaries) and gastrocnemius motoneurons (dashed line boundaries) about 110 days after axotomy in adult cats (Kuno *et al.* 1974). The motoneurone properties in kittens (Fig. 4A) were strikingly similar to those of axotomized motoneurons in adult cats (Fig. 4B, left). Thus, the motoneurone properties seem to be 'dedifferentiated' by axotomy in the sense that the differences in properties between soleus and gastrocnemius motoneurons become less prominent and that their properties mimic the motoneurone properties during the period of differentiation.

Discharge frequency of motoneurons. A lack of correlation between the speed of muscle contraction and the duration of after-hyperpolarization of motoneurons during development (Fig. 3) suggests that muscle differentiation may be independent of the discharge pattern of the innervating motoneurons. However, it might be argued that regulation of discharge frequency of motoneurons by after-hyperpolarization may be different between kittens and adult cats. To test this possibility, motoneurons

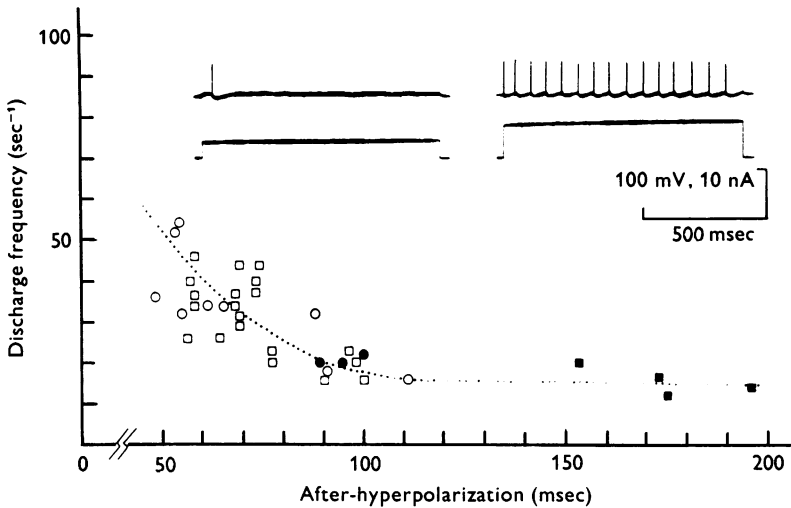


Fig. 5. Inset, responses of a gastrocnemius motoneurone (upper traces) recorded from a 112-day-old kitten to depolarizing current pulses (lower traces) applied through the intracellular electrode. Left, at rheobase. Right, at intensity twice the rheobase. Spikes retouched. Lower graph, the relation between the frequency of motoneurone discharge evoked by intracellular stimulation at an intensity twice the rheobase and the duration of after-hyperpolarization for gastrocnemius (open circles) and soleus (filled circles) motoneurons in kittens and gastrocnemius (open squares) and soleus (filled squares) motoneurons in adult cats. Five motoneurons were examined in four kittens, 20–30 days in age, and seven motoneurons in two kittens, 107 and 112 days in age.

were excited by applying depolarizing current pulses through the intracellular electrode. Rheobasic current was determined for each motoneurone by gradually increasing the stimulus intensity (Fig. 5, inset, left record), and multiple discharge was evoked in the motoneurone at a current intensity twice the rheobase (Fig. 5, inset, right record). The discharge frequency was estimated by the number of action potentials occurring during the first 500 msec of the depolarizing pulse. Fig. 5 shows the relation between the discharge frequency and the duration of after-hyperpolarization for gastrocnemius (open circles) and soleus motoneurons (filled

circles) recorded in kittens, ranging in age from 20 to 112 days. Distribution of these points was approximately the same as that for gastrocnemius (open squares) and soleus motoneurons (filled squares) observed in adult cats (Fig. 5). Therefore, it is concluded that the role of after-hyperpolarization in controlling discharge frequency of motoneurons is essentially the same in both kittens and adult cats.

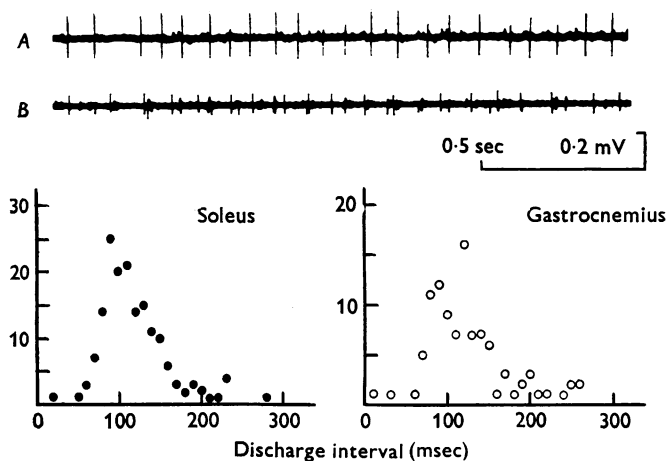


Fig. 6. *A*, e.m.g. recorded from the soleus muscle in a 19-day-old kitten. *B*, e.m.g. recorded from the medial gastrocnemius muscle in a 20-day-old kitten. Lower graphs show interval distributions of single motor unit discharges analysed from the records illustrated in *A* and *B*.

The duration of after-hyperpolarization may limit the maximum discharge frequency of motoneurons, but the general pattern of discharge also depends on the amount of depolarization resulting from excitatory synaptic bombardment to which the motoneurons are subject. Attempts were made to record discharges of individual motor units from skeletal muscles. Fig. 6 shows some examples of e.m.g. recorded from the soleus (*A*) and medial gastrocnemius (*B*) muscles in kittens at Stage I. Discharges of single motor units could occasionally be identified unambiguously, but in most cases the e.m.g. showed activity of several motor units with similar wave forms (Fig. 6*A, B*). Activity of particular motor units was selected by scanning details of configuration of every discharge expanded at a fast sweep in a large-screen oscilloscope connected to a digital computer (Bessou & Perl, 1969; also, cf. Fischbach & Robbins, 1969). After sorting, activity produced by a given motor unit was analysed by plots of interval distribution of discharge (Fig. 6, lower graphs) and by instantaneous frequencies as a function of time. In total, five medial gastrocnemius and eleven soleus motor units were examined in two kittens at Stage I and two

kittens at Stage II. The modal frequency of motor unit discharge varied from 5 to 20/sec in different units for both soleus and gastrocnemius muscles. The mean discharge frequency of motor units in the gastrocnemius muscle of rats and kittens has been reported to increase with age (Bursian & Sviderskaya, 1971). However, in our experiments, there was no indication that the modal frequency of motor unit discharge is different between the two post-natal stages examined. The modal discharge frequency observed in these experiments was appreciably lower than the mean frequency (30–60/sec) recorded from gastrocnemius motoneurons in adult cats (Granit *et al.* 1956, 1957). However, it should be noted that the impulse-sorting procedure could be applied only to those motor units which showed sustained discharge for several seconds. Therefore, the samplings of motor units would have been biased in favour of 'tonic' motoneurons (Granit *et al.* 1957; Burke, 1968). In fact, the average modal frequencies of five gastrocnemius (17/sec) and five soleus (12/sec) motor units measured by the same procedure in one adult cat were similar to those observed in kittens. Occasional bursting activity was noticed in the gastrocnemius muscle of kittens. However, because of a mixture of simultaneous activity arising from a number of different motor units, quantitative analyses of their frequencies were difficult. Thus, it remained uncertain whether the discharge frequency of 'phasic' gastrocnemius motoneurons may change during development.

DISCUSSION

Table 1 summarizes the present results. The contractile properties of gastrocnemius and soleus muscles as well as their motoneurone properties were significantly different ($2P < 0.05$ by two tail *t* tests) at every stage examined. The only exception was probably the difference in the duration of after-hyperpolarization between gastrocnemius (56 msec) and soleus (69 msec) motoneurons at Stage I, in which the level of significance was doubtful ($0.05 < 2P < 0.10$). The axonal conduction velocities of gastrocnemius and soleus motoneurons increased progressively during development, the former being consistently faster by about 30% than the latter at every stage (Ridge, 1967; cf. Nyström, 1968; Bagust *et al.* 1974). Obviously, these changes had no relationship with post-natal shortening or prolongation in contraction times of their muscles (Table 1). Similarly, post-natal speeding of contraction of the gastrocnemius muscle was not associated with any change in the duration of after-hyperpolarization of the innervating motoneurons (Table 1). Also, soleus motoneurons continued to increase in the duration of after-hyperpolarization throughout the period of development, independent of the changes in contraction

times of the soleus muscle (Table 1). It seems reasonable to suggest that there is no causal relationship between the duration of after-hyperpolarization of motoneurons and contraction times of the innervated muscles at early developmental stages (Stage I to Stage III).

TABLE 1. Properties of muscles and motoneurons in kittens at different stages and in adult cats

	Muscle twitch time (msec)	Conduction velocity (m/sec)	AHP duration (msec)
	Gastrocnemius		
Stage I	46 ± 5 (9)	29 ± 6 (30)	56 ± 18 (29)
Stage II	28 ± 4 (5)*	68 ± 7 (38)*	57 ± 18 (35)
Stage III	27 ± 1 (4)	82 ± 7 (21)*	57 ± 20 (20)
Adult	31 ± 4 (6)	94 ± 9 (56)*	75 ± 25 (53)*
	Soleus		
Stage I	63 ± 9 (9)	22 ± 5 (11)	69 ± 24 (10)
Stage II	76 ± 13 (5)*	52 ± 6 (13)*	91 ± 12 (10)*
Stage III	74 ± 10 (4)	63 ± 5 (13)*	122 ± 32 (11)*
Adult	97 ± 10 (7)*	74 ± 8 (21)*	150 ± 25 (17)*

* Significant ($2P < 0.05$ by two tail t tests) difference from the corresponding value at the preceding stage.

Stage I: 16–20 days old. Stage II: 61–71 days old. Stage III: 100–112 days old. All values give the mean ± s.d. of observation. Numbers of muscles or motoneurons examined in parentheses. AHP, after-hyperpolarization. Adult values, except muscle properties, from Kuno *et al.* (1974).

A question then arises as to whether post-natal differentiation of the fast and slow twitch muscles is independent of the discharge pattern of the innervating motoneurons. A relatively short duration of after-hyperpolarization in kitten motoneurons (Table 1; but, cf. Eccles, Shealy & Willis, 1963) agrees with the short subnormality period of monosynaptic reflexes following antidromic activation of motoneurons in kittens (Wilson, 1962). Also, there seems little doubt that the duration of after-hyperpolarization is a post-synaptic determinant for the frequency at which the motoneurons can discharge in kittens as well as in adult cats (Fig. 5). However, it is uncertain whether the duration of after-hyperpolarization may indeed reflect the discharge frequency of motoneurons under physiological conditions. For example, phrenic motoneurons of the cat have generally a short after-hyperpolarization (Gill & Kuno, 1963*a*), but their discharge frequency in quiet breathing is relatively low (5–30/sec; Pitts, 1946) probably because of irregularly spaced synaptic bombardment superimposed on the underlying synaptic depolarization (Gill & Kuno, 1963*b*). It is possible that kitten motoneurons may show similar be-

haviour, particularly if one takes into account marked depression of excitatory synaptic potentials observed in kitten motoneurons by repetitive afferent stimulation (Eccles & Willis, 1965; also, cf. Wilson, 1962). Unfortunately, attempts to determine the discharge pattern of individual motor units in kittens (Fig. 6) failed to provide unequivocal answers. Thus, there remain two alternative possibilities, depending on the relation between the duration of after-hyperpolarization and the motoneurone discharge frequency: (1) if motoneurone discharges in kittens are largely determined by the duration of after-hyperpolarization, post-natal muscle differentiation must be independent of the motoneurone discharge pattern but (2) if the duration of after-hyperpolarization in kittens does not represent the frequency of motoneurone discharge, a possibility still exists that muscle differentiation may depend on the discharge pattern of the innervating motoneurons.

From the present results, it seems clear that post-natal changes in the duration of after-hyperpolarization in soleus motoneurons are still proceeding even over 100 days after birth (Fig. 3; Table 1). Therefore, as far as this parameter is concerned, motoneurone differentiation is preceded by differentiation of the contractile properties of the muscles. Thus, differentiation of the motoneurone properties may be entirely independent of the muscle properties. Alternatively, motoneurons may acquire their 'normal' properties only after development of the skeletal muscles. If the latter were the case, a progressive change in the motoneurone properties may be attributed to the influence from the growing skeletal muscles.

A similar increase in the duration of after-hyperpolarization was also present in gastrocnemius motoneurons during the period from Stage III to adult cats (Fig. 3; Table 1). However, this parameter in gastrocnemius motoneurons remained constant at early developmental stages (Stage I to Stage III), while the contractile properties of the muscle were changing (Fig. 3; Table 1). In this respect, the differentiation processes seem to be different between gastrocnemius and soleus motoneurons. When the spinal cord is isolated from descending and afferent impulses in kittens a few days old, the development of the contractile properties of the gastrocnemius muscle still follows a normal time course, whereas the soleus muscle fails to maintain the slow contraction times (Buller *et al.* 1960*a*). It is possible that 'inductive' functions exerted from motoneurons to muscles and/or in the converse direction during development may be different between the gastrocnemius and soleus systems.

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