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DISPARITY OF MOTONEURONE AND MUSCLE DIFFERENTIATION FOLLOWING SPINAL TRANSECTION IN THE KITTEN

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SUMMARY

1. The spinal cord of kittens, 3–5 days of age, was transected at the lower thoracic level. Isometric contractions of the medial gastrocnemius and soleus muscles as well as intracellular potentials of their motoneurones were recorded after varying post-operative periods of up to 110 days. Similar observations were made 52–59 days after cord transection in adult cats.

2. In cord-transected kittens, contraction time of the gastrocnemius muscle showed normal development, whereas the soleus muscle failed to maintain slow contraction. In adult cats, cord transection increased the speed of contraction in the soleus muscle without significant changes in contraction times of the gastrocnemius muscle.

3. Soleus motoneurones showed a normal post-natal increase in the duration of afterhyperpolarization (a.h.p.) up to a certain stage (61–71 days in age) following cord transection. However, the subsequent increase in the duration of a.h.p. of soleus motoneurones observed in normal kittens was lacking in cord-transected kittens. It is suggested that soleus motoneurones show two stages of differentiation in terms of the duration of a.h.p.

4. In adult cats, cord transection caused a decrease in the duration of a.h.p. of soleus motoneurones approximately to the value observed at the end of the first stage of differentiation in kittens.

5. The duration of a.h.p. of gastrocnemius motoneurones remained virtually unchanged following cord transection in both kittens and adult cats.

6. The positive correlation between the duration of a.h.p. of soleus motoneurones and contraction time of the innervated muscle fibres normally observed in kittens and adult cats was absent following cord transection.

7. It was assumed that alterations in contraction time of the muscle following cord transection are due to virtual elimination of motoneurone discharge and that the duration of a.h.p. reflects the discharge pattern of motoneurones under normal conditions. Based on these assumptions, a possible process for normal post-natal differentiation of motoneurone and muscle is proposed.

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INTRODUCTION

In the preceding paper (Czéh, Gallego, Kudo & Kuno, 1978), it has been suggested that alterations in motoneurone properties induced by transection of the spinal cord result from a decrease in muscle activity and that normal motoneurone properties in an adult are thus maintained, at least in part, by an influence from the innervated muscle. The question to be examined in the present study is whether an influence from the muscle may also be involved in the process of motoneurone differentiation during post-natal development in kittens.

In new-born kittens, both the gastrocnemius and soleus muscles show uniformly slow contractions (Denny-Brown, 1929). During the first few weeks after birth, the speed of contraction of the gastrocnemius muscle becomes progressively faster while the soleus muscle maintains its slow contraction (Buller, Eccles & Eccles, 1960*a*; Buller & Lewis, 1965*a*; Mann & Salafsky, 1970; Huizar, Kuno & Miyata, 1975; Hammarberg & Kellerth, 1975*a*). During this period of development, the duration of after-hyperpolarization (a.h.p.) following each action potential in soleus motoneurones is gradually prolonged (Huizar *et al.* 1975; also, cf. Hammarberg & Kellerth, 1975*b*). The duration of a.h.p. is generally assumed to limit the maximum frequency at which the motoneurone can discharge (Eccles, Eccles & Lundberg, 1958; Kuno, 1959; Kernell, 1965). Also, the duration of a.h.p. appears to be the only electrophysiological parameter of motoneurones which is 'coupled' to the contractile properties of the muscle in adult cats (Huizar, Kudo, Kuno & Miyata, 1977). It is thus conceivable that the duration of a.h.p. of motoneurones is linked to the pattern of motoneurone discharge which in turn may be the primary determinant of the contractile properties of the muscle (Eccles, Eccles & Kozak, 1962; Salmons & Vrbová, 1969; Lømo, Westgaard & Dahl, 1974; Buller & Pope, 1977).

When the spinal cord is transected at the upper lumbar level in new-born kittens, the soleus muscle is no longer capable of maintaining slow contraction (Buller *et al.* 1960*a*; also, cf. Hoh & Dunlop, 1975). The question may then arise as to whether the failure of differentiation of the soleus muscle under such conditions is associated with a failure of differentiation of the motoneurones in terms of the duration of a.h.p. The present results suggest that soleus motoneurones may show two stages of post-natal differentiation and that the initial stage is independent of the condition of the muscle, whereas the second stage may require an influence from the muscle.

METHODS

The spinal cord was transected between the tenth and twelfth thoracic segments in kittens, 3–5 days of age, under methoxyflurane (Penthrane; Abbott Laboratories) anaesthesia with aseptic precautions. In early experiments, the urinary bladder of operated animals was manually emptied every day for a post-operative period of 10–20 days. However, this procedure was later found to be unnecessary since spontaneous micturition was restored within one day after cord transection in both male and female kittens.

After varying post-operative periods, the animal was deeply anaesthetized by I.P. injection of sodium pentobarbitone. In some experiments, the brain was ischaemically impaired by bilateral occlusion of the common carotid and vertebral arteries. After cessation of respiration and dilatation of the pupils, the animal was maintained on artificial respiration, and the spinal cord was transected at the first cervical level. In other experiments, the animal was maintained on natural respiration, employing a supplementary dose of sodium pentobarbitone

(8–10 mg/kg; I.P.) whenever flexion reflexes to noxious stimuli were recovered. The results obtained from these two preparations were essentially the same. Intracellular recordings from medial gastrocnemius and soleus motoneurones were performed in a manner described in a previous report (Huizar *et al.* 1975). In a few experiments the peripheral connexions of the soleus nerve with the muscle were left intact in order to measure isometric contractions of individual motor units following intracellular stimulation of the motoneurone (Devanandan, Eccles & Westerman, 1965; Burke, 1967; Kuno, Miyata & Muñoz-Martinez, 1974). Electrophysiological properties of motoneurones were examined only in those cells with an action potential in excess of 70 mV. In order to minimize the possible bias of sampling, the maximum number of cells recorded from one kitten was limited to five soleus and eight gastrocnemius motoneurones.

At the beginning of every experiment, isometric contractions of the medial gastrocnemius and soleus muscles evoked by nerve stimulation were recorded in a warm (37 °C) paraffin oil by the methods detailed previously (Kuno *et al.* 1974; Huizar *et al.* 1975). For measurements of contractions of the whole muscle or individual motor units (see above), the initial tension was adjusted to the level at which the maximum twitch tension was obtained (Buller *et al.* 1960*a*; Huizar *et al.* 1975). The rectal temperature of the animal was kept between 36 and 38 °C by external heat throughout the experiment. However, the pool temperature around the hind leg muscles ranged from 30 to 33 °C during the experiment. This might have caused an overestimate of the contraction time of motor units (Gordon & Phillips, 1953). Contraction time of individual motor units measured by intracellular stimulation of the motoneurone was longer by 14 %, on the average, than that of the whole muscle recorded at 37 °C.

For comparison with the results obtained from kittens, six adult female cats (2·5–3·0 kg in body wt.) were subjected to cord transection at the twelfth thoracic level under anaesthesia with sodium pentobarbitone. Electrophysiological properties of medial gastrocnemius and soleus motoneurones were examined 52–59 days after cord transection. The experimental procedures used for adult cats were basically the same as those employed for kittens, except that isometric contractions of the muscle were measured at an initial tension of 100 g (Burke, 1967; Kuno *et al.* 1974). Also, motoneurones with an action potential of less than 75 mV were excluded from the analysis in the case of adult cats. Some of the results obtained from adult cats were included in the preceding paper (Czéh *et al.* 1978).

RESULTS

Muscle differentiation following cord transection. In agreement with previous observations (Buller *et al.* 1960*a*), transection of the spinal cord of new-born kittens resulted in a striking increase in the speed of contraction of the soleus muscle. Fig. 1 illustrates isometric contractions of the medial gastrocnemius (single arrows) and soleus (double arrows) muscles in two different kittens (*A*, *B*) whose spinal cords were transected 5 days after birth. In a 21-day-old kitten (Fig. 1*A*), both the gastrocnemius and soleus muscles showed a relatively long contraction time, but in a 104-day-old kitten (Fig. 1*B*), contraction times of both the muscles became short. In fact, in this particular animal, the speed of contraction was slightly faster in the soleus muscle than in the gastrocnemius muscle (Fig. 1*B*). The relation between contraction times and age in cord-transected kittens is summarized on the right of Fig. 1 for the medial gastrocnemius (open circles) and soleus (filled circles) muscles. For comparison, post-natal changes in contraction time of the medial gastrocnemius muscle observed in normal kittens (Huizar *et al.* 1975) are shown by a continuous curve in the same figure. Contraction times of the soleus muscle varied considerably from animal to animal during normal development, but their values were enclosed in the range shown by a cross-hatched area in the Figure (see Huizar *et al.* 1975). In cord-transected kittens, the changes in contraction time of the medial gastrocnemius muscle (open circles) with age followed the time course observed in normal kittens (continuous curve). In contrast, the soleus muscle showed a significant increase in the speed of contraction within 35–55 days after cord transection (filled circles).

In adult cats, the mean contraction time of the soleus muscle measured 52–59 days after cord transection was 63 msec which was significantly ($2P < 0{\cdot}001$ by two-tailed *t* tests) shorter than the normal, adult value (97 msec Huizar *et al.* 1975), as shown by an arrow on the right of Fig. 1. Under such conditions, the medial gastrocnemius muscle showed no significant changes in contraction time (Fig. 1, open circle and horizontal bar in adult; also, see Table 1). Evidently, it was only the soleus muscle that failed to maintain normal contractions following cord transection in both kittens and adult cats.

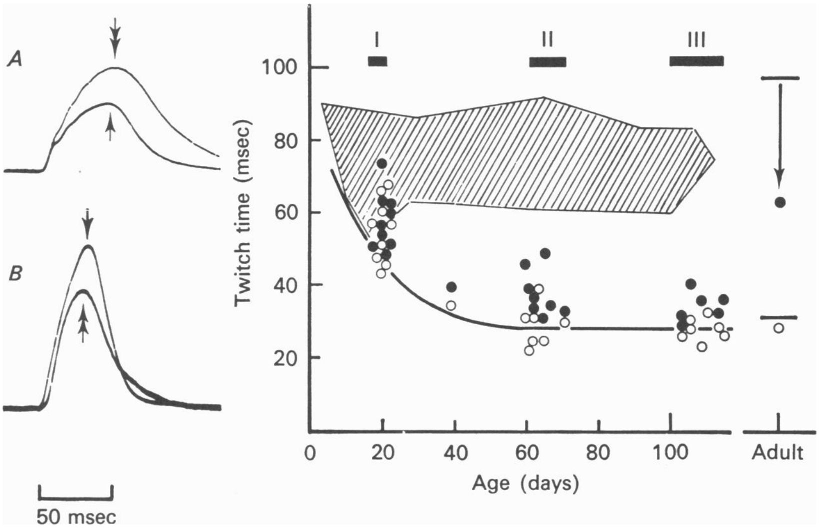

Fig. 1. Isometric contractions of medial gastrocnemius (single arrows) and soleus (double arrows) muscles recorded in 21-day-old (*A*) and 104-day-old (*B*) kittens whose spinal cords were transected 5 days after birth. Twitch tensions: 85 g (*A*) and 864 g (*B*) in gastrocnemius; 26 g (*A*) and 102 g (*B*) in soleus. Graph on the right, relations between contraction time and age for medial gastrocnemius (open circles) and soleus (filled circles) muscles after cord transection at 3–5 days of age. Normal developmental changes in contraction time for gastrocnemius (continuous curve) and soleus (cross-hatched area) muscles are reproduced from the results by Huizar *et al.* (1975). Adult: horizontal bars, control values; open (gastrocnemius) and filled (soleus) circles, 52–59 days after cord transection in adult cats. Upper horizontal bars indicate three post-natal stages (I, II, III) selected for measurements of motoneurone properties.

In contrast to the present results and those observed by Buller *et al.* (1960*a*) and Hoh & Dunlop (1975), Davis & Montgomery (1977) have recently reported that both the soleus and flexor digitorum longus muscles show a significant *decrease* in the speed of contraction within 3 weeks after isolation of the lumbosacral spinal cord (Tower's operation; Tower, 1937) in adult cats. The results obtained by Davis & Montgomery (1977) have also suggested that the decrease in the speed of muscle contraction might have occurred for only short periods after cord isolation. In our experiments, the mean contraction time of the soleus muscle showed no significant changes 1 week (96 msec; $n = 6$) or 2 weeks (86 msec; $n = 7$) after transection of the spinal cord, compared with the normal, adult value (97 msec). We have no explanation for the discrepancy between our results and those by Davis & Montgomery (1977).

Motoneurone differentiation following cord transection. In order to test whether the failure of muscle differentiation following cord transection is associated with a failure of motoneurone differentiation, intracellular recordings from spinal motoneurones were performed in cord-transected animals. Motoneurone properties were examined at three post-natal stages indicated by horizontal bars in Fig. 1 (stage I, 16–21 days in age; stage II, 61–71 days in age; stage III, 100–115 days in age) as well as in adult cats 52–59 days after cord transection. Fig. 2*a* shows antidromic action potentials of soleus motoneurones recorded from a 19-day-old kitten (*A*) and a 64-day-old kitten (*B*) whose spinal cords were transected 5 days after birth. The axonal conduction velocity was 27 m/sec in the former and 53 m/sec in the latter. The a.h.p. following action potentials evoked by intracellular stimulation is shown in Fig. 2*b*. As indicated by arrows in Fig. 2*b*, the duration of a.h.p. in soleus motoneurones appears to increase with age in cord-transected kittens. The contraction time of the whole soleus muscle measured at the beginning of the experiment was 53 msec in

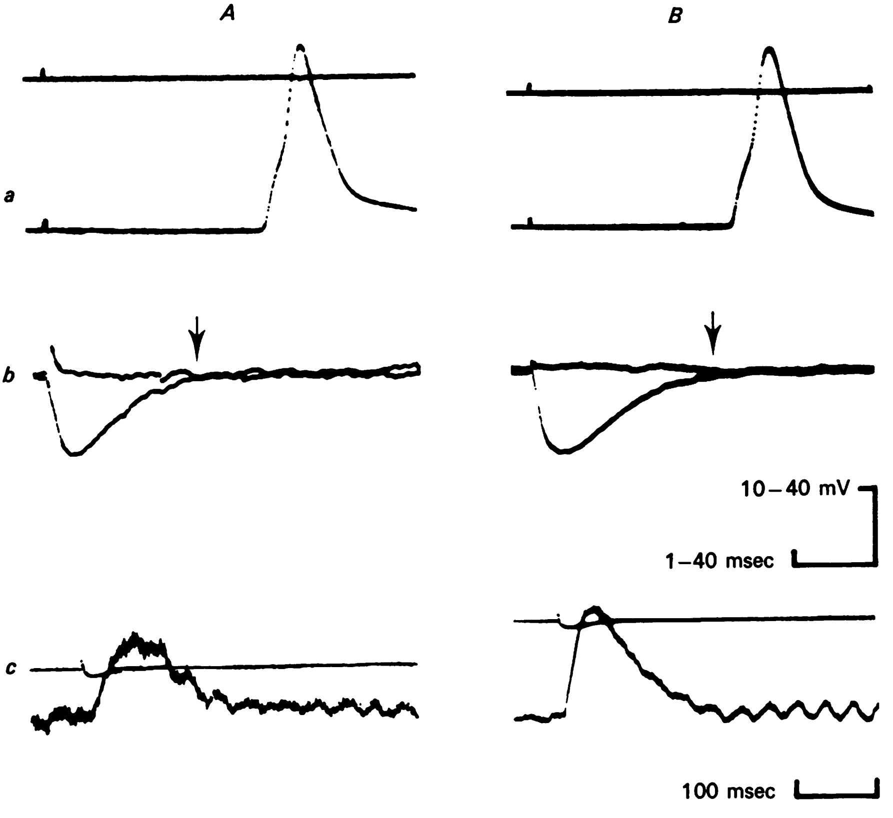

Fig. 2. Resting and antidromic action potentials (*a*) and afterhyperpolarization (a.h.p.) (*b*) recorded from soleus motoneurones in 19-day-old (*A*) and 64-day-old (*B*) kittens whose spinal cords were transected 5 days after birth. Arrows in *b* indicate termination of a.h.p. 40 mV, 1 msec calibration for *a*; 10 mV, 40 msec calibration for *b*. *c*, isometric contractions of motor units produced by intracellular stimulation of the motoneurones. Twitch tensions: 0·3 g in *Ac*, 0·9 g in *Bc*. Contraction times: 64 msec in *Ac*, 37 msec in *Bc*.

the 19-day-old kitten and 31 msec in the 64-day-old kitten. The difference in contraction time between the two animals can also be noticed in isometric contractions of the motor units evoked by intracellular stimulation of the soleus motoneurones (Fig. 2*c*, lower traces). Thus, while the soleus muscle apparently fails to maintain the slow contraction following cord transection (Fig. 2*c*), the motoneurones seem to show post-natal differentiation with respect to the axonal conduction velocity as well as to the duration of a.h.p. (Fig. 2*b*), at least to a certain stage.

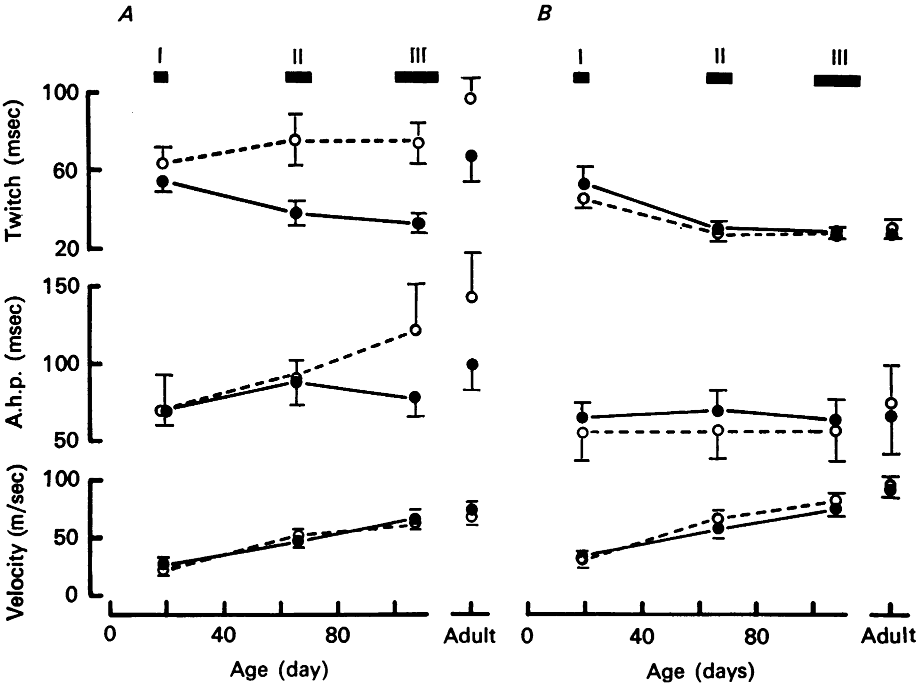

Fig. 3. Time courses of the changes in twitch time (top) of the whole soleus (*A*) and medial gastrocnemius (*B*) muscles, the duration of afterhyperpolarization (a.h.p. middle) and the axonal conduction velocity (bottom) of soleus (*A*) and medial gastrocnemius (*B*) motoneurones in normal (open circles) and cord transected (filled circles) kittens. Adult: open circles, normal adult cats; filled circles, 52–59 days after cord transection in adult cats. Each point represents the mean with S.D. (vertical bars). Upper horizontal bars indicate three post-natal stages (I, II, III) selected for measurements.

Fig. 3 shows the changes in mean contraction times (top) with age in normal (open circles) and cord-transected (filled circles) kittens for the soleus (*A*) and medial gastrocnemius (*B*) muscles. The soleus muscle in cord-transected kittens showed a significant decrease in contraction time at stage II (61–71 days) and stage III (100–115 days) compared with the values obtained from normal kittens of the corresponding ages (also, see Table 1). On the other hand, post-natal changes of contraction time in the medial gastrocnemius muscle were practically unaffected by cord transection (Fig. 3*B*, top). Consequently, the soleus and gastrocnemius muscles became indistinguishable in terms of contraction time at stage III in cord-transected kittens (Fig. 3*A* and *B*, top).

Post-natal changes in the duration of a.h.p. are illustrated in the middle of Fig. 3

for soleus (*A*) and gastrocnemius (*B*) motoneurones in normal (open circles) and cord-transected (filled circles) kittens. In contrast to the striking changes in contraction time of the soleus muscle (Fig. 3*A*, top), an increase in the duration of a.h.p. of soleus motoneurones from stage I to stage II was equally present in both normal and cord-transected kittens (Fig. 3*A*, middle). The difference in the values between stage I (69 msec) and stage II (88 msec) in cord-transected kittens was highly significant ($0{\cdot}005 > 2P > 0{\cdot}001$) as was the case for normal kittens (69 msec and 91 msec; also, see Table 1). Therefore, differentiation of soleus motoneurones at this stage in terms of the duration of a.h.p. appears to be independent of the condition of the innervated muscle. However, a further increase in the duration of a.h.p. from stage II to stage III observed in normal kittens (Fig. 3*A*, middle, open circles) was lacking in cord-transected kittens (Fig. 3*A*, middle, filled circles).

In adult cats, the average duration of a.h.p. of soleus motoneurones was decreased from 144 msec to 101 msec 52–59 days after cord transection (Fig. 3*A*, middle, adult). The latter value was not significantly ($0{\cdot}10 > 2P > 0{\cdot}05$) different from the average value observed at stage II in normal kittens (91 msec). As shown in Czéh *et al.* (1978), the changes of soleus motoneurones induced by cord transection in adult cats can be prevented when activity of the muscle is maintained by daily stimulation. Similarly, the failure of differentiation of soleus motoeurones from stage II to stage III in cord-transected kittens might be attributed to a lack of muscle activity. Thus, the present results may be explained adequately by assuming that soleus motoneurones show normally two phases of differentiation: the first phase is independent of the condition of the muscle, whereas the second phase fails to occur in cord-transected kittens or can be eliminated ('dedifferentiation') by cord transection in adult cats. The average duration of a.h.p. at stage III in cord-transected kittens (78 msec) was not significantly ($0{\cdot}10 > 2P > 0{\cdot}05$) different from that at stage II (88 msec). However, the significance of the difference ($0{\cdot}02 > 2P > 0{\cdot}01$) in the mean value between stage I (69 msec) and stage III (78 msec) was also marginal in cord-transected kittens (see Discussion for significance limit). Therefore, it is possible that an influence from the muscle may be required for the *maintenance* of the first phase of differentiation as well as for the *initiation* of the second phase of differentiation. While this possibility remains uncertain, there seems little doubt that normal soleus motoneurones have at least two phases of differentiation in terms of the duration of a.h.p. which can be distinguished by cord transection (Fig. 3*A*, middle).

Post-natal changes in the duration of a.h.p. of medial gastrocnemius motoneurones were generally similar in both normal and cord-transected kittens (Fig. 3*B*, middle). There was, however, a slight but significant difference in the mean value at stage II between normal and cord-transected kittens (Table 1). The explanation of this result is not clear.

The axonal conduction velocities of soleus (*A*) and gastrocnemius (*B*) motoneurones increased progressively during post-natal development in cord-transected kittens (Fig. 3*A* and *B*, bottom, filled circles). This behaviour qualitatively agrees with morphological observations that the post-natal growth of fibre diameter in the ventral root is not affected by cord transection in kittens (Hildebrand, 1976). However, the conduction velocities of gastrocnemius motoneurones at stage II and stage III in cord-transected kittens were slightly but significantly slower than the corresponding values (Table 1).

Interactions between motoneurone and muscle. The duration of a.h.p. in motoneurones begins to show a positive correlation with contraction time of the innervated muscle fibres at about six weeks of age in normal kittens (Hammarberg & Kellerth, 1975*b*). This correlation is maintained in adult cats (Devanandan *et al.* 1965; Burke, 1967; Hammarberg & Kellerth, 1975*b*; Huizar *et al.* 1977). Furthermore, a decrease in the duration of a.h.p. of 'intact' soleus motoneurones following partial denervation of the muscle is subsequently associated with a decrease in contraction time of the innervated muscle fibre (Huizar *et al.* 1977). It may be assumed that the correlation between the two parameters is mediated by the pattern of motoneurone discharge (see Introduction). Thus, if motoneurone discharge is significantly eliminated following cord transection, one might expect that the two parameters may be divorced in cord-transected animals. To test this possibility, contraction times of individual motor units were measured following intracellular stimulation of soleus motoneurones at stage III (100–115 days in age) in cord-transected kittens. Fig. 4*A* illustrates the relation between the duration of a.h.p. of soleus motoneurones and contraction time of the motor units. It is clear that no correlation exists between the two parameters in cord-transected kittens (Fig. 4*A*, continuous line; $r < 0{\cdot}05$). Fig. 4*B* shows similar results obtained 52–59 days after cord transection in adult cats. The relation between the two parameters (Fig. 4*B*, continuous line; $r = 0{\cdot}34$) showed a tendency similar to that observed in normal, adult cats (dashed line; $r = 0{\cdot}72$; see Huizar *et al.* 1977), but its correlation was statistically insignificant ($0{\cdot}10 > 2P > 0{\cdot}05$). These results are consistent with the assumption that in cord-transected animals, motoneurone discharge is virtually eliminated or at least significantly reduced.

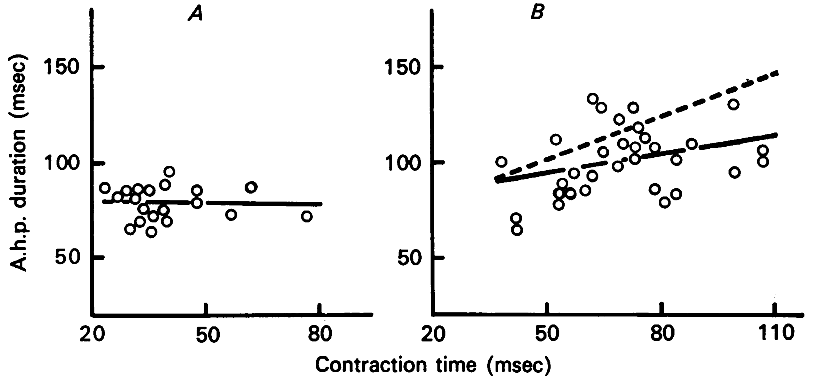

Fig. 4. Relations between the duration of after-hyperpolarization (a.h.p.) of soleus motoneurones and the contraction time of the motor units in cord transected kittens, 100–115 days of age (*A*) and in adult cats 52–59 days after cord transection (*B*). The correlation coefficients of the regression lines (continuous lines) are less than 0·05 in *A* and 0·34 in *B*. Dashed line in *B*, regression line (correlation coefficient = 0·72) observed for normal adult cats (Huizar *et al.* 1977).

TABLE 1. Properties of motoneurone and muscle in normal and cord transected animals

		Stage I (12–21 days)	Stage II (61–71 days)	Stage III (100–115 days)	Adult
Gastrocnemius					
Twitch time: (msec)	Normal	46 ± 5 (9)	28 ± 4 (5)	27 ± 1 (4)	31 ± 4 (6)
	Cord section	53 ± 9 (7)	30 ± 6 (7)	28 ± 3 (7)	28 ± 3 (7)
A.h.p. duration: (msec)	Normal	56 ± 18 (29)	57 ± 18 (35)	57 ± 20 (20)	75 ± 25 (53)
	Cord section	65 ± 10 (25)	70 ± 14 (26)*	64 ± 17 (53)	66 ± 25 (49)
Conduction velocity: (m/sec)	Normal	29 ± 6 (30)	68 ± 7 (38)	82 ± 7 (21)	94 ± 9 (56)
	Cord section	30 ± 4 (25)	56 ± 6 (26)*	75 ± 7 (53)*	95 ± 11 (49)
Soleus					
Twitch time: (msec)	Normal	63 ± 9 (9)	76 ± 13 (5)	74 ± 10 (4)	97 ± 10 (7)
	Cord section	54 ± 5 (7)	38 ± 6 (8)*	33 ± 5 (6)*	63 ± 13 (7)*
A.h.p. duration: (msec)	Normal	69 ± 24 (10)	91 ± 12 (10)	122 ± 32 (11)	144 ± 28 (93)
	Cord section	69 ± 9 (12)	88 ± 16 (11)	78 ± 12 (26)*	101 ± 18 (31)*
Conduction velocity: (m/sec)	Normal	22 ± 5 (11)	52 ± 6 (13)	63 ± 5 (13)	69 ± 8 (94)
	Cord section	27 ± 5 (12)	47 ± 5 (11)	68 ± 7 (26)	75 ± 6 (31)*

* Significant ($2P < 0{\cdot}01$ by two-tailed t tests) difference from the corresponding normal value. All values give the mean ± S.D. Numbers of sample in parentheses. Cord transected adult cats were examined 52–59 dyas after the operation. Normal values, from Huizar *et al.* (1975) for kittens and from Huizar *et al.* (1977) for adult cats. A.h.p., afterhyperpolarization.

DISCUSSION

The present results are summarized in Table 1. As pointed out by Buller & Lewis (1965*a*), the maturity of kittens varies considerably from one litter to another. It was difficult to assess how variation in level of maturity might affect the average values obtained from a limited number of normal and spinal preparations at each stage of development. Because of this uncertainty, the level of statistical significance was limited to $2P < 0{\cdot}01$ by a two-tailed t test, and attention was focused only on those parameters which showed clear differences between normal and cord-transected animals.

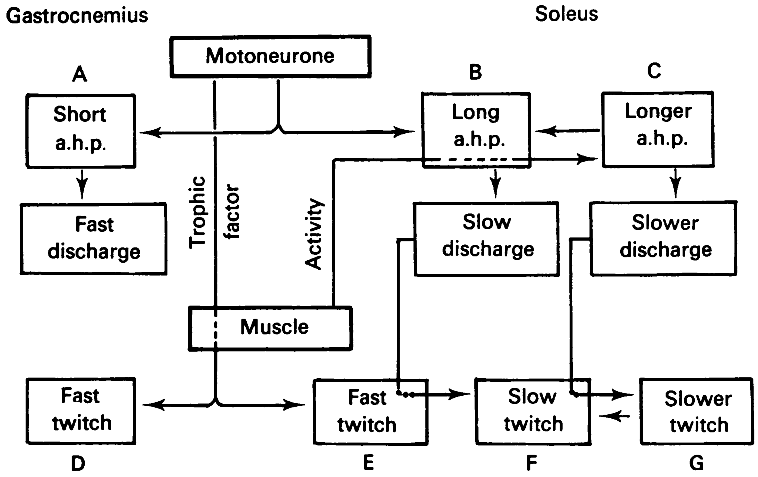

Fig. 5. A hypothetical diagram for normal post-natal differentiation of motoneurones and muscles. A.h.p., afterhyperpolarization. Explanation, see text.

From the data presented in Table 1 (also, see Fig. 3), it seems clear that chronic cord transection significantly increases the speed of contraction of the soleus muscle in both kittens and adult cats without affecting the contractile properties of the gastrocnemius muscle (also, see Buller *et al.* 1960*a*, *b*; Hoh & Dunlop, 1975; but cf. Davis & Montgomery, 1977). Thus, the average contraction times of the gastrocnemius (28 msec) and soleus (33 msec) muscles were not significantly ($0{\cdot}10 > 2P > 0{\cdot}05$) different at stage III in cord-transected kittens. If motoneurone discharge is assumed to be virtually eliminated by cord transection, as suggested from the above data (Fig. 4), the present results imply two possibilities: (1) normally discharging gastrocnemius motoneurones have the same effects on the muscle as do quiescent motoneurones; (2) the normally existing difference in contraction time between the gastrocnemius and soleus muscles is achieved by the normal discharge pattern of soleus motoneurones.

Fig. 5 illustrates a possible process for normal post-natal differentiation of motoneurone and muscle, which seems to be consistent with the present results as well as with the currently available information. This hypothetical process detailed below is based on two assumptions: (1) alterations in muscle properties observed following cord transection are due to virtual elimination of motoneurone discharge; (2) the duration of a.h.p. reflects the discharge frequency of motoneurones under normal conditions.

During development, motoneurones may differentiate into those with a short a.h.p. (A, e.g. gastrocnemius) and those with a long a.h.p. (B, e.g. soleus). This process appears to be independent of an influence from the muscle since motoneurone differentiation at this stage (stage II in Fig. 3) is equally present in both normal and cord-transected kittens. The a.h.p. in the latter (B) may be further prolonged (longer a.h.p.; C) by some signal associated with muscle activity. Thus, cord-transected kittens fail to differentiate from state B to state C. Similarly, in adult cats cord transection leads to 'dedifferentiation' from state C to state B, and this conversion in differentiation can be prevented when muscle activity is maintained by daily stimulation of the peripheral nerve (Czéh *et al.* 1978).

In new-born animals, all the skeletal muscles are uniformly slow in contraction (Denny-Brown, 1929). If motoneurone activity is virtually absent, both the gastrocnemius and soleus muscles appear to differentiate into a fast-contracting type (D and E), as seen at stage III in cord-transected kittens (Table 1). However, if muscle activity is eliminated by denervation, both the gastrocnemius and soleus muscles are known to *decrease* the speed of contraction in kittens (Lewis, 1973) as well as in adult cats (Eccles *et al.* 1962; Lewis, 1972; also, see Lømo *et al.* 1974). Thus, the lack of muscle activity produces different effects on the speed of muscle contraction, apparently depending upon the presence or absence of normal innervation. Therefore, the post-natal speeding of muscle contraction may be assumed to be induced by some trophic factors emanating from the motoneurones, which are equally present in both gastrocnemius and soleus motoneurones.

The duration of a.h.p. in soleus motoneurones starts to increase between 20 and 60 days of age in normal kittens (Fig. 3*A*, middle, open circles; see Huizar *et al.* 1975). Thus, the resultant slow discharge pattern of soleus motoneurones may counteract the post-natal speeding of the soleus muscle induced by trophic factors from the motoneurones, thereby leading from state E to state F. This is consistent with the observations that duration of a.h.p. has no correlation with contraction time of the innervated muscle fibres in kittens younger than 2–5 weeks in age (Hammarberg & Kellerth, 1975*b*).

When the duration of a.h.p. of soleus motoneurones is further prolonged (state C), the resultant slower discharge pattern of the motoneurones may further decrease the speed of contraction of the soleus muscle, reaching the adult value (state G). Transition between states F and G as well as between states B and C appears to be reversible in adult cats. Thus, partial denervation of the soleus muscle causes a decrease in the duration of a.h.p. (from C to B) of 'intact' soleus motoneurones, which is followed by a decrease in contraction time (from G to F) in the innervated muscle fibres (Huizar *et al.* 1977). Similarly, cord transection of adult cats causes a shift from state C to state B presumably by a decrease in muscle activity and results in a shift from state G to state F presumably by virtual elimination of motoneurone discharge.

The absence of changes in contraction time of the gastrocnemius muscle following cord transection is puzzling. For this behaviour, three possibilities may be considered. The first possibility may be that the gastrocnemius muscle is relatively insensitive to the pattern of motoneurone discharge. However, this possibility is unlikely since fast-contracting muscle decreases its speed of contraction after cross-innervation by the soleus nerve (Buller *et al.* 1960*b*; Eccles *et al.* 1962; Buller & Lewis, 1965*b*) or following daily stimulation of the nerve at low frequencies (Eccles *et al.* 1962; Salmons & Vrbová, 1969; Buller & Pope, 1977). The second possibility is that the contractile properties of the muscle may be influenced only by a slow discharge pattern of motoneurones (Buller *et al.* 1960*b*). This possibility seems incompatible with the observations that contraction time of the denervated soleus muscle of the rat becomes significantly faster by daily stimulation at 100/sec than at 10/sec (Lømo *et al.* 1974). Thus, the most likely possibility would be that the firing of fast-discharging gastrocnemius motoneurones is only transient ('phasic') or infrequent, so that the innervated muscle is practically unaffected by the 'instantaneous' discharge frequency of motoneurones under normal conditions. In fact, it has been shown that the maximum amplitude of excitatory synaptic potentials produced in fast gastrocnemius motoneurones is too small to be recruited during standing and walking movements (Burke, Rymer & Walsh, 1976). Furthermore, in fast gastrocnemius motor units, no correlation can be seen between the duration of a.h.p. and contraction time of muscle fibres (Hammarberg & Kellerth, 1975*b*; R. E. Burke, personal communication). The implication of the third possibility is that an increase in the speed of contraction of the soleus muscle following cross-innervation by fast-motor nerves could be entirely due to the deprivation of the slow soleus motoneurone discharge, but not to any additional influences from the newly innervating motoneurones.

The hypothetical diagram shown in Fig. 5 is, of course, by no means complete, and further tests will be required for each step illustrated in the diagram as well as for the two assumptions on which the proposed process is based. However, this overview picture may be helpful in posing some specific questions for further studies.

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