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POLYNEURONAL INNERVATION OF KITTEN SKELETAL MUSCLE

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SUMMARY

1. Isometric contractions of kitten soleus and flexor hallucis longus (FHL) muscles have been examined for evidence of polyneuronal innervation. The sum of tetanic tensions of two almost equal divisions of the ventral roots was greater than the tetanic tension elicited by stimulating both the divisions simultaneously. The difference was large in kittens aged about 3 days, was less at 2 weeks and was small or absent at 6 weeks.

2. A tetanus elicited from one division of the ventral root potentiated a twitch elicited from the other root division. The time course of this potentiation was similar to that of post-tetanic potentiation induced from the same root from which the twitch was elicited.

3. It is concluded that polyneuronal innervation exists in the kitten limb muscles.

4. The observed degree of tension excess was less if the divisions of the root were not equal. The observations were compatible with a model which assumed a random distribution of nerve axons to the muscle fibres.

INTRODUCTION

It has been shown that the mean isometric tetanic tension of motor units, measured relative to the tension of the whole muscles, are greater in kittens than in adult cats (Bagust, Lewis, Luck & Westerman, 1972). One explanation of this would be that muscle fibres are innervated by more than one motoneurone in the kitten, a condition that is rare in the adult cat (Brown & Matthews, 1960). Redfern (1970) examined end-plate potentials in the new-born rat diaphragm and showed that there was frequently more than one end-plate on a muscle fibre. The rigid interpretation of this is that there is multiple innervation of muscle fibres but not

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necessarily polyneuronal innervation since the end-plates could be derived from branches of one axon. Moreover, in the newborn rat the innervation of skeletal muscle is less mature than the cat (Zelená & Hník, 1963) and so mechanical evidence for polyneuronal innervation has been looked for by the methods used by Brown & Matthews (1960) in the adult cat.

METHODS

Kittens of known age were anaesthetized with sodium pentobarbitone 20–40 mg/kg injected I.P.; additional doses were administered intravenously as required. Flexor hallucis longus (FHL) and soleus muscles were prepared, other muscles in the limb were denervated and the spinal roots were exposed by techniques similar to those described for the adult by Lewis, Luck & Knott (1972). In older kittens the leg was sealed into a Perspex bath by cutting the skin around the upper thigh and tying the skin edges around a flange on a hole which was cut at an angle through one side of the bath (cf. Close, 1964). Two clamps, which held the upper and lower ends of the tibia and fibula, were supported on rods which passed through water-tight seals in the floor of the bath. The limb was rigidly fixed and could be immersed in liquid paraffin in the bath. The paraffin here and in a back pool and the animal's body were maintained at $37{\cdot}5 \pm 0{\cdot}2°$ C by high gain temperature servos using thermistor probes. In the youngest kittens the lower leg was held by transfixing the bones with stout needles held in pin chucks and a pool was formed from agar (5% solution in saline) poured into a polystyrene former placed around the leg. This had a capacity of about 20 ml. and the temperature could be controlled as accurately as that of the larger bath.

In order to reduce the possibility of stimulus spread, the ventral roots were cut close to the cord to give the maximum length for stimulation. The roots were split longitudinally with fine forceps along their whole length and placed on the anode of the stimulating electrodes. The cathode was placed on back muscles, stimuli of 100 μsec and 100–200 mV were delivered from an isolated stimulator. Twitches were displayed on a storage oscilloscope and the arrangement of roots was not accepted unless there was no increase of tension detectable (less than 2%) when the stimulus intensity was increased to ten times the just-maximal value. Except where stated, during measurement stimulus intensity was set at 1·5–2 times just-maximal.

Muscle tensions were measured by an unbonded wire strain gauge transducer (Ether UF2-16) the amplified output of which was digitized and analysed by a digital computer. Maximum tensions were calculated from the mean of values over a 5–10 msec period on either side of the single greatest value. This procedure ensured that the result should not be based on a single possibly spurious point, but as a further precaution the digitized values were displayed on a storage oscilloscope (cf. Figs. 1 and 3) with a mark superimposed to indicate the programme estimate of maximum value. The voltage change produced by switching a calibration resistor across the dynamometer bridge before each stimulus was also measured by the programme and, together with a calibration factor, allowed the tension of the muscle contraction to be calculated and typed out to three significant figures. Under stable conditions of the preparation results were consistent to a level better than 1%. The programme allowed an average of responses to be calculated and this enabled the arithmetic sum of two dissimilar responses to be estimated directly. Tetani were elicited at frequencies of 100/sec or 125/sec to ensure fusion and had a duration of 300 msec (except that twice this duration was used to produce post-tetanic potentiation). In most measurements the muscle was set at the length optimal for tetani.

The electromyogram was recorded from cotton-wool wicks, one was laid along the length of the muscle and the other over inactive muscle. The potentials were amplified with a differential amplifier with 3 db points set at 0·8 Hz and 1 kHz. The amplified signal was sampled at 100 μsec intervals and the programme measured the mean level for 20 msec before the stimulus and summed the absolute values of the differences of potential from this mean value for a period from 1 msec after the stimulus to a time up to 50 msec after the stimulus (the end of the period was set during the experiment). The signal and the integral of the full wave rectified signal were displayed and the value of the integral was typed out in arbitrary units.

RESULTS

Experiments were performed on three groups of kittens. There were thirteen animals aged between 38 and 44 days (6-week kittens'), twenty aged between 12 and 16 days ('2-week kittens') and three aged either 2 or 3 days ('3-day kittens'). In addition three adult animals were studied. In all except three kittens and two adults only one muscle (soleus or FHL) was studied in an experiment.

TABLE 1. Excess of the sum of tensions elicited from two almost equal divisions of ventral roots over tension from the whole root. Corresponding extent of double innervation calculated on assumptions in Appendix. Values are mean ± S.D. (and number of muscles observed). V.R. shows mean ventral root tension as a percentage of tension from muscle nerve

		Tension excess (%)		V.R. (%)	Double innervation (%)
		Twitches	Tetani		
3-day	Soleus	40·4 ± 12·4 (3)	62·2 ± 19·3 (3)	99·7	84·0 (1)*
2-week	Soleus	24·3 ± 9·0 (6)	24·4 ± 11·1 (7)	95	47·0 ± 20·4 (12)
	FHL	18·0 ± 8·7 (9)	12·8 ± 5·0 (9)	95	27·7 ± 10·0 (12)
6-week	Soleus	3·4 ± 4·0 (5)	0·7 ± 1·2 (5)	93	1·3 ± 2·5 (6)
	FHL	10·6 ± 3·2 (6)	1·6 ± 1·5 (6)	94	3·8 ± 3·3 (7)

* Two muscles excluded, since their tension excesses were due in part, at least, to triple or higher order innervation thus invalidating the present method of calculation.

The roots were subdivided so that stimulation of the divisions produced twitches that were not different by more than 10%. Four twitches were measured, the first elicited by stimulating both root divisions simultaneously, the second from one of the root divisions, the third from the other division and finally the two divisions were stimulated together again. This sequence was then repeated but eliciting fully fused tetani. Tension excess was estimated as the difference between the arithmetic sum of the middle two responses and the mean of the first and last responses and was expressed as a percentage of that mean. The mean values of tension excess for each muscle and age group are presented in Table 1. It should be noted

that Brown & Matthews (1960) expressed tension excess in a different way in that they used the smaller of the two submaximal responses as the divisor. Experiments in which the tetanic tension elicited from the ventral roots was less than 90 % of that from the muscle nerve were not used in calculating the first two columns of Table 1. Results were also excluded if the tetanic tension of one root division was more than 50 % greater than that of the other (see Appendix) or repeated if the difference between the

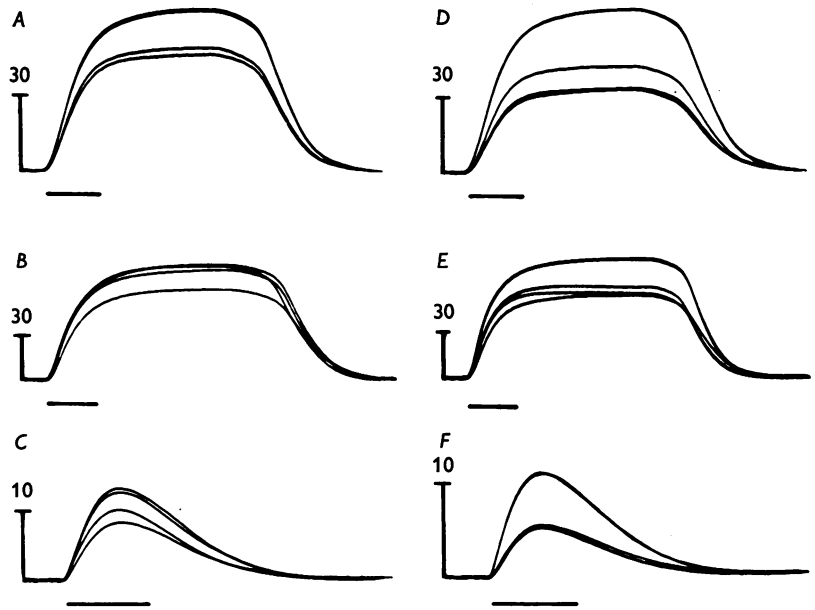

Fig. 1. Isometric myograms from soleus muscles of 3-day kittens to show tension excess. *A* and *D* are tetani from one animal, *B* and *E* and *C* and *F* are tetani and twitches from another animal. The ventral roots have been split into two (*A–C*) or three (*D–F*) divisions. Each panel shows four (*A–C*) or five (*D–F*) myograms superimposed. These were elicited by stimulation of (*a*) all the ventral roots together, (*b*) each of the divisions individually and (*c*) all the roots again (this may be almost coincident with *a* and the two appear only as a thicker line). The tension calibration bars preceding each trace are labelled in mN, the horizontal bars all show 100 msec.

first and last tetanus was greater than 5 %. The latter occurred only once in 41 measurements and the mean difference was +0·36 %. The mean of the absolute value of the difference (that is the larger minus the smaller tension regardless of order) was 1·2 % (S.D. = 1·0 %). The myograms of two experiments are shown in Fig. 1 *A*, *B*, *C*. The mean tension excess was significantly different from zero for tetani in the 3-day kitten soleus muscles

($t = 5{\cdot}6$, $P < 1\%$) in both sets of 2-week kitten muscles ($t = 7{\cdot}8$, $P < 0{\cdot}1\%$) and the 6-week kitten FHL muscles ($t = 2{\cdot}8$, $P = 5\%$) but not in the 6-week soleus muscles ($t = 1{\cdot}2$). Five adult muscles were studied for tension 'excess' and had a mean value of $-0{\cdot}37\%$ (S.D. = $1{\cdot}5\%$) i.e. a tension deficit (but not significantly different from zero) and this is in agreement with the results of Brown & Matthews (1960) and unpublished observations of A. J. Buller & D. M. Lewis. W. Al-Amood and R. Pope (personal communication) have obtained values of $0{\cdot}13\%$ (S.D. = $1{\cdot}0\%$, $n = 11$) in soleus and $-1{\cdot}03\%$ (S.D. = $4{\cdot}04\%$, $n = 5$) in FHL muscles from results selected to match the criteria used above. The tension excess observed in twitches was also significantly different from zero in all except the 6-week kitten soleus muscles. The tension excess for the twitches of 2-week and 6-week kittens were consistently greater than that for tetani. A similar result was reported by Brown & Matthews (1960) who attributed it to non-linear summation of twitches rather than polyneuronal innervation. The differences observed in the kittens were less than those of Brown & Matthews (0–9% here compared with 7–19%) but as noted earlier the methods of calculation were not similar and the smaller difference is also seen in the results of Al-Amood and Pope who found a mean tension excess in soleus and FHL twitches of $-1{\cdot}6$ and $8{\cdot}3\%$ respectively. In the 3-day kitten muscles, the results are different in that the tension excess was less in twitches than in tetani (cf. Fig. 1*B* and *C*). The mean ratio of the twitch excess to tetanus excess was 0·66 (S.D. = 0·062, $n = 3$) compared with the mean ratio in 2-week kitten muscles of 1·24 (S.D. = 0·39, $n = 10$). Neuromuscular failure has been observed in the youngest kitten solei (see also Buller & Lewis, 1965*b*) and this would explain the small ratio if 46% of the end-plates failed in a twitch but not a tetanus, assuming that any non-linear summation was quantitatively similar at the two ages.

Brown & Matthews (1960) suggested that the twitch tension would be greater if one of the root fractions was stimulated a few milliseconds after the other at a time at which muscle contractions sum most strongly. This was tested in four experiments (illustrated by Fig. 2) and the tension was found to be greatest at an interval of between 5 and 10 msec.

The precise variation with stimulus interval (Fig. 2*C*) was not exactly the same as the variation observed when both stimuli were applied to the same group of nerve axons (Fig. 2*D*). The latter points show a step at 0·7 msec which flattens out at intervals between 0·9 and 1·1 msec. This step has been interpreted (Buller & Lewis, 1965*b*) as being due to the recruitment of more end-plates by the second stimulus. The points increased again between 1·2 and 2·5 msec and then decreased in a manner similar to that observed in adult cat skeletal muscle (Buller & Lewis, 1965*a*). The points of Fig. 2*C* show no initial step; this is compatible with polyneuronal innervation for, if two end-plates were sufficiently close in one fibre to interact, the inter-

action would be maximal when the two were excited simultaneously. The points began to increase at 2·4 msec and reached a peak at 6 msec. This second difference between Fig. 2*C* and *D* can be explained by conduction along the muscle fibres. At short intervals action potentials would arise at each end-plate in a polyneuronally innervated fibre and would collide at an intermediate position. At longer intervals

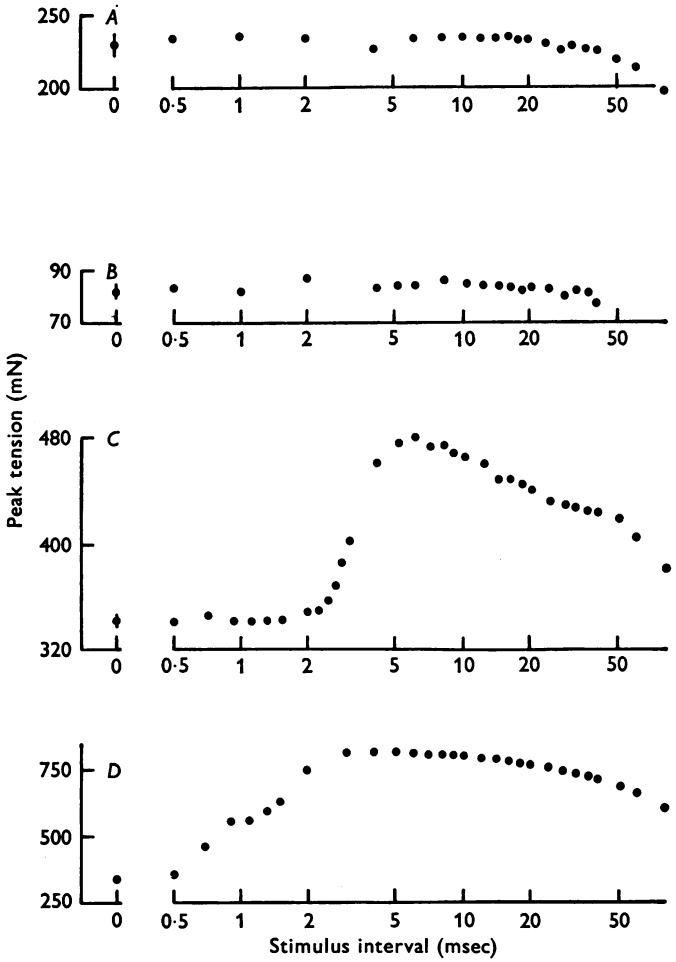

Fig. 2. Interaction of two stimuli in a soleus muscle of a 3-day kitten. The points all show peak active tension plotted against the interval between the stimuli (note logarithmic abscissa). In *D* both stimuli have been applied to the whole ventral root. In *C* one stimulus has been applied to one half of the ventral root and the second to the other half. *B* is similar to *C*, except that two smaller but approximately equal divisions of the root have been used. *A* is the arithmetic sum of two independent twitches elicited from the two divisions of *C*. Here one stimulus was applied at the start of a sweep and the second stimulus was delayed relative to the start of another sweep, the two sweeps were summed in a computer.

one action potential would propagate past the second end-plate at which a second action potential would arise if the stimulus was applied after the muscle refractory period plus the conduction time. Much of the slower rise of the points of Fig. 2*C* could be explained if conduction times between end-plates ranged between a fraction of a millisecond and 4 msec. End-plates less than 30 μm apart have been seen on muscle fibres (Westerman, Lewis, Bagust, Edjtehadi & Pallot, 1972, Fig. 10) but the upper value indicates that large separations also exist. The arithmetic sum of the responses to each of the two root divisions was not independent of stimulus interval, but as seen in Fig. 2*A* it does not vary sufficiently to affect the results. As a final check of these results, two small ventral root divisions were prepared (each about 10% of the total) and they were stimulated at different intervals. The points obtained (Fig. 2*B*) were almost flat as would be expected since the chance of the muscle fibres stimulated by one division being polyneuronally innervated from the other division was low in this case (see Appendix). Similar results were seen in one other muscle from a 3-day kitten. Two experiments in 2-week kitten muscles gave similar results uncomplicated by neuromuscular failure. Electromyograms were also observed in these experiments. The curves of Fig. 2*D* were paralleled by ones of the integrals of the full wave rectified electromyogram except that the initial step was constant for intervals between 0·7 and 2·0 msec. The curve corresponding to that of Fig. 2*C* was complicated by a fall in the integral between 1 and 5 msec due to occlusion of the two action potentials.

It might be suggested that these results could be explained by mechanical non-linear summation not seen in the adult. A further series of experiments in five kittens was undertaken to test polyneuronal innervation by another technique. This depended on the fact that a tetanus will potentiate a twitch (Fig. 3*A*) elicited 10 sec later and that the post-tetanic potentiation decays over a period of about 300 sec (Fig. 3*C* left). The tetani used (600 msec duration at 100/sec) did not produce any change in the integral of the electromyogram. Post-tetanic potentiation occurs in soleus as well as FHL in 3-day and 2-week kittens (cf. Buller & Lewis, 1965*a*). If the tetanus was elicited from one division of the ventral roots and the twitches from the other division potentiation still occurred (Fig. 3*B*). This would be expected if some of the muscle fibres were innervated by nerves from both divisions but it might still be explained by mechanical interaction. However this potentiation also persisted as long as post-tetanic potentiation. The continuous curve on the right of Fig. 3*C* was calculated from the potentiation observed to follow a tetanus in the same root division (left) and from the tension excess measured in tetani of these two divisions. The curve fits the points within the errors of measurement. In the other experiments the first twitch observed after potentiation from the other root divisions was larger than predicted and the initial decay to the second post-tetanic twitch was more rapid. This may be compared with a small post-tetanic increase seen between ventral root divisions in one FHL muscle in an adult cat. This increase decayed over 20–30 sec when true post-tetanic potentiation was 60–70% of its initial value. Two other adult animals showed no post-tetanic effects

between ventral root divisions. It may be concluded that some of the initial increases may be mechanical in origin but that the late crossed post-tetanic effects in kittens are consistent with the existence of polyneuronal innervations.

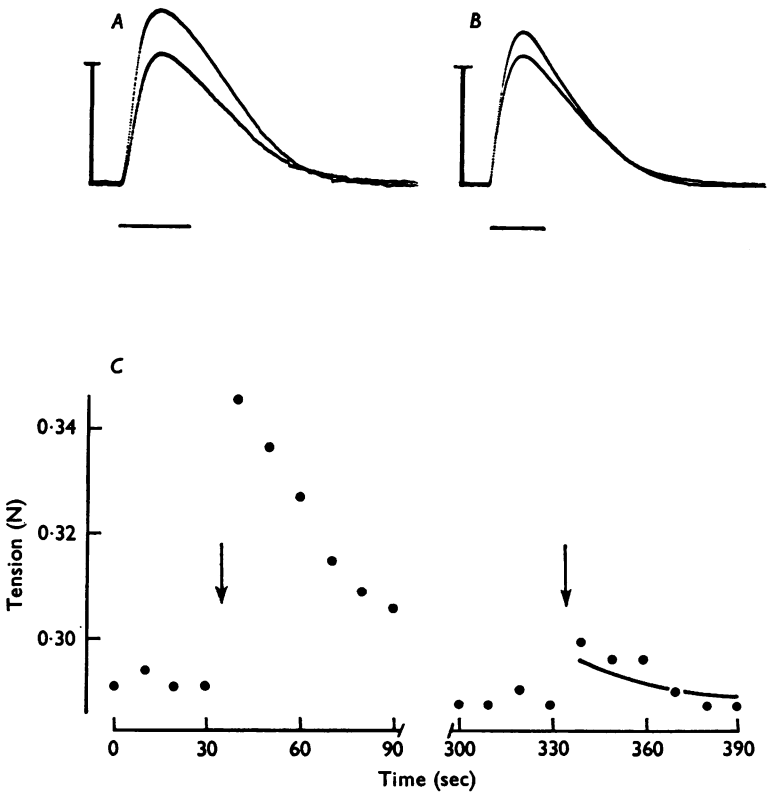

Fig. 3. Post-tetanic potentiation demonstrating polyneuronal innervation in a soleus muscle of a 3-day kitten (*A*, *B*) and a FHL muscle in a 2-week kitten (*C*).

A and *B* are isometric twitch myograms elicited from L7 ventral root, the smaller trace in each pair was obtained after a period of 300 sec of stimulation at 0·1/sec. The larger twitches were elicited 5 sec after the onset of a 0·6 sec tetanus (100/sec), given to L7 root in *A* and to S1 root in *B*. Calibration bars are 30 mN and 100 msec.

In *C* peak active twitch tension elicited from one root division is plotted against time. At the arrows tetani were interposed, the first was elicited from the same root division as the twitches and the second from the rest of the ventral root. The continuous line (340–390 sec) was derived from the time course of post-tetanic potentiation seen between 40 and 90 sec and from the tension excess observed in tetani elicited from the two root divisions (cf. Fig. 1*A*).

It is possible to explain the results if the potentiation were due to an extracellular mechanism such as the accumulation of a metabolite. Evidence against this has been obtained by potentiating a twitch elicited from a small division of the ventral root by a tetanic stimulus applied either to that division or to the whole muscle nerve. No consistent late differences were seen between the degree of potentiation observed in the two situations. Since the potentiation could be increased by using a longer duration tetanus it is difficult to explain the potentiation by a mechanism occurring outside the muscle fibre, after the first 10–20 sec.

If non-linear summation played a part in the observed tension excess it might be predicted that this might vary with muscle length as the arrangement of muscle fibres is different at different muscle lengths. In three experiments tetanic tension excess was measured at a number of muscle lengths greater and less than the optimum by amounts sufficient to reduce the muscle tetanus by more than half. The excess did not vary by more than 8% of that measured at optimum length, and the changes were not systematic with length.

Stimulus spread would also cause tension excess. The precautions to prevent this have been described. However, a further check has been made in five experiments by measuring excess at several intensities. In the experiments reported above the intensity was between 1·5 and 2 times just maximal. Compared with this the excess tension with a 1·1 times maximal stimulus was 1·03 times greater (S.D. = 0·05). If the stimulus was increased to 10 times maximal the excess relative to that observed at the standard stimulus strength was 0·94 (S.D. = 0·07).

If a number of assumptions are made, the most important of which are that either one or two nerve axons randomly innervate muscle fibres and that the discrete and varied size of motor units may be ignored, the degree of double innervation may be calculated from the observed tension excess (see Appendix). In the simplest case in which the two divisions are exactly equal and ventral root stimulation activates all the muscle fibres, the degree of double innervation is twice the observed excess, since half the polyneuronally innervated fibres of one division will, by chance, be innervated only by axons within that division and the other half innervated by one axon from the other division and only this half will produce the observed excess (Fig. 5). The formulae derived in the Appendix have been used to estimate the degree of double innervation in all experiments and the estimates are listed in the last column of Table 1. The values obtained from the more restricted series of column 2 were not significantly different. These values have been calculated as if all the fibres had only one or two end-plates.

Evidence for the number of axons innervating the fibres may be obtained from a further measurement that was made in seven muscles. The ventral roots were split into three divisions producing equal sized twitches, measurements were made by comparing the sum of the tetani of the three divisions with the tension produced by stimulating all three together (Fig. 1, *D–F*). In all cases the excess tension was greater than that seen from two root divisions. The ratio of the excess for three divisions to that for two root divisions was calculated. For six of the muscles the results were similar and the mean ratio was 1·30 (S.D. = 0·085). Both soleus and FHL muscles from 3-day and 2-week kittens were included in the six and an example is shown in

Fig. 1*D*. In the remaining muscle (soleus from a 3-day kitten, Fig. 1*E*, *F*) the ratio was 2·6. It is shown in the Appendix that if no muscle fibres receive more than two axons this ratio should have a value of 4/3. This fits well the mean value observed in the six muscles and it may be concluded that, if all the assumptions made above may be accepted, more than two axons per muscle fibre were uncommon. In the remaining muscle this could not be true and it may be noted that this muscle displayed the largest amount of two root division tension excess (75%), the most neuromuscular failure and the lowest ratio of twitch excess to tetanus excess (0·58).

Another conclusion derived in the Appendix is that the tension excess would be directly proportional to the product of the fraction of axons in the two root divisions. This product will be 0·25 when the division is equal and falls to 0·24 approximately when the tension elicited from one division

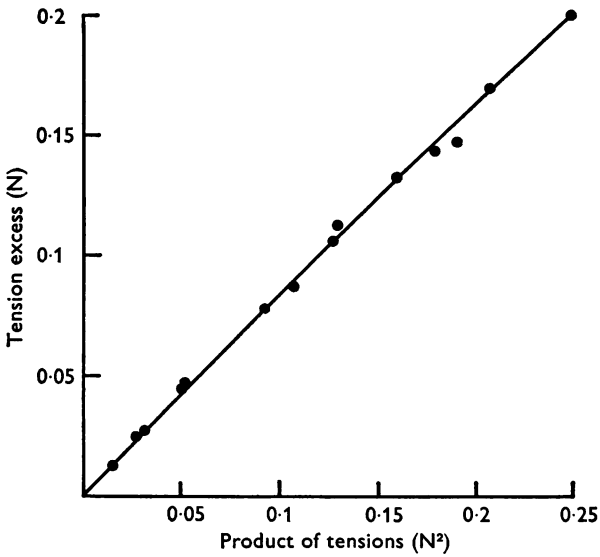

Fig. 4. Overlap between various divisions of the ventral roots seen in isometric tetani of a soleus muscle of a 3-day kitten. The abscissa shows the product of the tensions of two divisions of the roots and the ordinate the excess of their sum over the tension from the whole roots. The filled circles are experimental points. The continuous curve is derived from eqns. (1) and (2) of the Appendix and is scaled to the *Y* co-ordinate of the upper experimental point.

is 50% greater than the other. In eleven experiments the naturally occurring division into two ventral roots was used in addition to an artificial equal division. The results from the latter were used to predict the tension excess expected to be seen with the natural root divisions. The predicted values differed from the measured values by a mean of 0·48% (S.D. = 1·24%).

A more complete test was made in three experiments in which a number of artificial divisions of the roots were made and the results in one are compared with a predicted curve in Fig. 4.

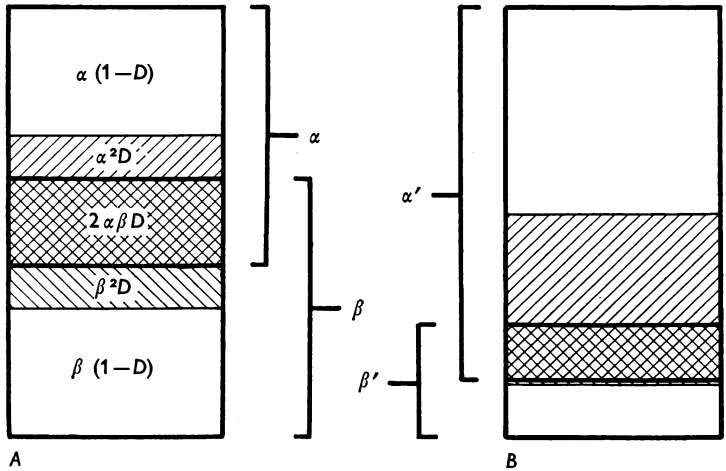

Fig. 5. Diagram of the model of double innervation proposed in the Appendix. The areas are proportional to the number of muscle fibres. It is assumed that α axons in one ventral root division and β axons in the other division innervate muscle fibres randomly in the proportions $\alpha:\beta$. Singly innervated fibres (proportional to $1-D$) are represented as plain areas. Doubly innervated fibres (proportional to D) are oblique-hatched; territories of the two root divisions are indicated by the two different hatchings. The area where the hatchings overlap indicate fibres that will be excited from both root divisions and will be responsible for the excess tension. In *A* $\alpha = \beta$ (cf. Fig. 1). In *B* $\alpha' \neq \beta'$ and it can be seen that the region of overlap is smaller than in *A* (cf. Fig. 4).

DISCUSSION

It is concluded that polyneuronal innervation is common in kittens up to 2 weeks of age in the fast and slow twitch muscle tested. It is just present in 6-week kitten FHL muscle but at this age soleus muscles have reached the adult state. The results in all but one animal may be explained by assuming that few fibres receive more than two axons if a number of assumptions are made and are in contrast with the finding of Redfern (1970) that many neonatal rat muscle fibres receive three or four end-plates. This difference may reflect the relative immaturity of rats at this age or might be explained if a large proportion of the end-plates in the rat result from multiterminal rather than polyneuronal innervation.

It has been suggested (Harrison, 1910) that in the mammal once a motor nerve establishes a connexion with a muscle fibre it inhibits the formation

of further end-plates. Barker & Ip (1966) have observed in adult muscle that occasionally two end-plates may be seen on one muscle fibre but one of these is always abnormal, possibly immature or in a state of degeneration. This may be compared with the state in the kitten where the end-plates are smaller and less specialized than in the adult (Nystrom, 1968) and may fail to produce an end-plate potential large enough to excite the muscle fibre. It might be suggested that both degenerating and immature end-plates fail to exert the postulated inhibitory effect. There is thus a possible link between deficiencies in neuromuscular transmission and the trophic inhibition of multiple end-plate formation, although it may be indirect.

The neuronal control of muscle contraction must necessarily be less precise in polyneuronally innervated muscles. However, the existence of polyneuronal innervation offers the possibility that the adult pattern of motor unit innervation could result from the loss of less useful connexions and be more precisely linked to the demands of the nervous system than it would be if the pattern were established directly. This might be compared with the loss of motoneurones occurring during development in amphibians (Hughes, 1961).

We should like to thank Dr A. Sudbury for helpful discussion and Mrs Lyn Dowsett for technical assistance. This work was supported by the Muscular Dystrophy Group of Great Britain and the computer was purchased by the Medical Research Council.

APPENDIX

It is assumed that the muscle fibres are innervated by one or more axons in a random manner. The number of axons is sufficiently large (100–150) for it to be unlikely that the discrete size of motor units would make this assumption significantly in error but this has not been tested formally. It is further assumed that the mean number of fibres innervated by one axon does not differ between the root divisions, again the number of axons is probably sufficiently large to justify this except when the divisions are small. If axons anatomically close in the ventral roots also innervate adjacent muscle fibres the assumption of random innervation is not justified. A test of this has been made by comparing tension excess between adjacent divisions of the root with that between divisions derived from different roots. No consistent differences have been seen.

The case of muscle fibres receiving either one or two axons may be considered first. The fractions of these may be called $(1-D)$ and D respectively and the proportion of axons in the two ventral root divisions α and β (where $\alpha+\beta = 1$). The number of muscle fibres innervated by these axons has been assumed to be proportional to α and β. On the further

assumption of random innervation the following table of fibre proportions may be set up (cf. Fig. 5).

	Singly innervated	Doubly innervated
From Division 1	$\alpha(1-D)$	$\alpha^2 D$
From Division 2	$\beta(1-D)$	$\beta^2 D$
From both	0	$2\alpha\beta D$

The tension (A) produced by stimulating Division 1 will be proportional to:

$$\alpha(1-D)+\alpha^2 D+2\alpha\beta D$$

or

$$(A) = \alpha+\alpha\beta D$$

and the tension (B) from Division 2 will be

$$\beta+\alpha\beta D$$

and the sum of tensions:

$$\alpha+\beta+2\alpha\beta D\,.$$

The excess tension

$$\begin{aligned}(\Delta) &= (A)+(B)-(A+B)\\ &= 2\alpha\beta D\end{aligned}$$

or

$$D = \Delta/2\alpha\beta. \qquad (1)$$

In the special case where

$$(A) = (B),$$

$$\alpha = \beta = \tfrac{1}{2}$$

and

$$D = 2\Delta.$$

In all cases, the product

$$(A)(B) = \alpha\beta(1+D+\alpha\beta D^2). \qquad (2)$$

Eqns. (1) and (2) have been used to derive the curve of Fig. 4; the only constants that have been used in this calculation are the tension produced from the muscle nerve (which was equal to that from the ventral root within 1 %) and the tension excess measured from two divisions equal within 3 %, i.e. the Y co-ordinate of the upper end of the curve. The last column of Table 1 has been derived from (1) and (2) by successive approximations.

If a proportion (T) of the fibres were innervated by three axons, an estimate of both D and T could be made if one additional observation were made on the tension excess observed from three divisions of the roots. Calculations similar to those above show that the excess tension from two divisions would be

$$\Delta_2 = 2\alpha\beta D+3(\alpha\beta^2+\beta\alpha^2)T$$

and from three divisions (α, β and γ) would be

$$\Delta_3 = 2(\alpha\beta+\beta\gamma+\alpha\gamma)D+3(\alpha\beta^2+\alpha\gamma^2+\beta\gamma^2+\beta\alpha^2+\gamma\alpha^2+\gamma\beta^2+4\alpha\beta\gamma)T.$$

The two special cases in which

$$\alpha = \beta = \tfrac{1}{2}$$

and $$\alpha = \beta = \gamma = \tfrac{1}{3}$$

may be simplified to

$$\Delta_2 = D/2 + 3T/4 \tag{3}$$

and $$\Delta_3 = 2D/3 + 10T/9. \tag{4}$$

The ratio of these two will be

$$\Delta_3/\Delta_2 = \tfrac{4}{3} + 4T/(18D + 27T).$$

If $T = 0$ $$= \tfrac{4}{3}.$$

Of the seven experiments in which the ratio was measured, six gave a value of 1·36 or less (mean 1·30). This suggests that the number of fibres with three axons was negligibly small. Eqns. (3) and (4) were solved for these six experiments and the mean value for D was 0·39 (S.D. = 0·11) whilst that for T was $-0{\cdot}009$ (S.D. = 0·0045). Even if the proportion of triply innervated were determined by chance with a probability equal to that of double innervation (that is $T = D^2$), then the expected value of T would have averaged 0·20, well above the observed value. In contrast the solution for the seventh experiment was $D = 1{\cdot}26$ and $T = 0{\cdot}14$. This impossible result suggests that few fibres were singly innervated. Since the initial assumptions have not been justified, these quantitative conclusions must not be taken too far, but it would seem reasonable to suggest that at or before birth a majority of fibres may be polyneuronally innervated but that in the first few days all except second end-plates may be lost.

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