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MOTOR UNITS IN A SKELETAL MUSCLE OF NEONATAL RAT: MECHANICAL PROPERTIES AND WEAK NEUROMUSCULAR TRANSMISSION

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SUMMARY

1. Isometric twitch and tetanic tensions were recorded from whole muscles and single motor units in isolated fourth deep lumbrical muscles from neonatal rats (most at 3–5 days old) and from older rats of various ages.

2. Whole-muscle time to peak contraction reduced from about 120 ms at birth to about 20–25 ms at 20 days and older.

3. The number of motor units in the muscle was constant with age (eleven on average) and there was no significant branching of motor axons below the common peroneal nerve branching point in the thigh.

4. In the 3–5 days age range, mean twitch:tetanus ratio for whole muscles was 0·299 and for single units was 0·177. As a consequence, mean motor unit size (as a percentage of whole-muscle tension) was greater for tetani (29·7 %) than for twitches (19·9 %). This was not the case in muscles from animals 22 days or older. Evidence is given that the cause of this is low junctional efficacy in some neuromuscular junctions in neonatal muscle. Intracellular recordings supported this view.

5. The relationships of motor-unit size to the contraction time, to the ratio of contraction time:half-relaxation time, and to fatigue index are given. There was no indication of clear segregation of motor units into more than one population, but it is concluded that small motor units are more likely to contain a higher proportion of slowly contracting, fatigue-resistant fibres than large units.

6. The level of overlap by axons in the lateral plantar nerve onto muscle fibres in a single sural nerve motor unit was greater in tetani than in twitches. The results indicate that the distribution of weak and strong inputs was not random, but that there was a tendency for one strong input to accompany a number of weak inputs (on average about two) on each muscle fibre.

7. Intracellular recording indicates that about 12 % of fibres at 3–5 days may be electrically coupled.

INTRODUCTION

In adult skeletal muscle in mammals each muscle fibre receives its motor supply from one motoneurone, and each motoneurone supplies a number of muscle fibres in the muscle. This functional unit of motoneurone and muscle fibres is called the motor

unit (Liddell & Sherrington, 1925), and all the muscle fibres in a particular motor unit have similar histochemical profiles and presumably physiological properties (e.g. Burke, Levine, Tsairis & Zajac, 1973). The homogeneity and fibre properties of the adult motor unit are probably maintained and determined by the motoneurone, largely or exclusively via the activity patterns it imposes on the muscle fibres of the motor unit (see Buchthal & Schmalbruch, 1980).

In neonatal muscle the primary role of the motoneurone in determining motor-unit properties is less clear. At birth, and soon after, each muscle fibre receives several nerve terminals at the end-plate site, each nerve terminal deriving from a separate motoneurone; thus at this stage of development all the muscle fibres are polyneuronally innervated (Redfern, 1970). Subsequently, by a process of synapse elimination, the adult organization is achieved (e.g. Brown, Jansen & Van Essen, 1976).

It is known that muscle fibres are differentiated before birth in the rat, some containing slow myosin while others do not (e.g. Lyons, Haselgrove, Kelly & Rubinstein, 1983). When the level of polyneuronal innervation is high, and motor units overlap extensively in the muscle fibres they contain, are the motor units homogeneous with respect to fibre type (as in the adult) or are they mixed? The answer to this important question relates to such fundamentals of development as the primacy or otherwise of motoneurones in determining muscle-fibre properties, and the importance of, and factors involved in, synapse elimination. In this paper we are concerned with some physiological properties of neonatal motor units, and in the following two papers (Jones, Ridge & Rowlerson, 1987*a*, *b*) with the fibre composition of neonatal motor units and whole muscles at various ages, determined immunocytochemically.

A preliminary account of some of this work has been given earlier (Jones & Ridge, 1985).

METHODS

Experiments were performed on the fourth deep lumbrical muscle of the rat, isolated with its nerve supply, mounted in a small organ bath and superfused with Ringer solution at constant temperature. This muscle was chosen because it is small (less than 1000 muscle fibres in the adult), with a small number of motor units (on average eleven), and the time course of synapse elimination within it is known (Betz, Caldwell & Ribchester, 1979). In addition, the motor axons enter the muscle via two different routes (Betz *et al.* 1979). The majority are from the lateral plantar nerve (l.p.n.), and a minority (sometimes only one) from the sural nerve (s.n.). The sural contribution travels in a small anastomosis from the sural nerve under the achilles tendon to join the lateral plantar nerve at the heel. The muscle nerve branches from the l.p.n. in the foot. The nerve supply was dissected up to the point where the common peroneal nerve branches in the thigh to give the sural and tibial (and hence lateral plantar) nerves (Green, 1955). Most experiments were carried out using a Ringer solution of the following composition (mM): Na^+, 167; K^+, 5; Mg^{2+}, 1; Ca^{2+}, 2; Cl^-, 147; HCO_3^-, 24; $H_2PO_4^-$, 1; D-glucose, 11. The solution was equilibrated by bubbling with 95% O_2, 5% CO_2 (pH 7·2). The temperature in the bath was controlled at 25 °C (±0·5 °C). When motor units were fatigued by repetitive stimulation the Ringer solution contained 5 mM-Ca^{2+} and no glucose. In this case the bath temperature was 30 °C (±0·5 °C). In a few other experiments Ca^{2+} concentration ($CaCl_2$) was raised to 5 or 10 mM in the presence of glucose. In these experiments osmolarity was maintained constant by a compensatory reduction of NaCl concentration.

Rat pups in the age range 3–5 days were killed by decapitation prior to the dissection. We chose this age range because the muscle is still relatively immature (all the muscle fibres are polyneuronally

innervated and myogenesis is still occurring (Betz *et al.* 1979)) but the muscle fibres no longer occur in tight groups surrounded by a basement membrane (Jones *et al.* 1987*b*) as they do at birth. It was important for some of the experiments that electrical coupling should not be very extensive as it is at birth in this muscle (Schmalbruch, 1982). The tight grouping of fibres is likely to be associated with electrical coupling. Here we report that about 12% of fibres are electrically coupled.

A small piece of bone from the fifth digit, where the muscle attached peripherally, was pinned to a Sylgard layer in the bottom of the bath. The tendon at the central end of the muscle was attached to a semiconductor strain gauge (Akers AE 802: see Ridge & Betz, 1984), All tension measurements were isometric. The cut ends of the s.n. and l.p.n. were sucked into the glass tips of two suction electrodes and stimulated with short voltage pulses (usually 0·01 ms and less than 20 V of either polarity). Muscle length for all tension measurements was set for maximal twitch tension in response to supramaximal whole-nerve stimulation. The numbers of motor units in the l.p.n. and s.n. were obtained by counting the number of tension increments occurring in response to single shocks of progressively increasing or decreasing voltage. Whole-muscle twitches were recorded in response to a single shock applied to the s.n. and l.p.n. Care was taken to minimize latency differences in twitch-tension development when stimulating the s.n. and l.p.n. separately, as this could have caused repetitive firing in muscle fibres innervated by both nerves when the nerves were stimulated together. This was achieved either by altering nerve length (and hence conduction distance) or, occasionally, by introducing an appropriate time interval between the stimuli to each nerve. Tetanic tensions were recorded in response to trains of stimuli at 40 impulses/s for 0·5 s (70 or 100 impulses/s in older muscles). This gave fused tetanic tensions in all the muscles and motor units.

Single units

Unit twitches were obtained either from units isolated in a nerve (see below) or from single units obtained by graded stimulation of either nerve (e.g. Fig. 1*C*). It was sometimes possible to stimulate four units separately in one muscle by stimulating each nerve with both polarities. It was not possible, however, to obtain reliable data on unit tetani in this way, and all tetanic data were obtained from isolated single units. Isolated units were obtained in one of the following ways: (1) by their natural occurrence in the s.n., by progressive section of s.n. until an all-or-none response was obtained to a series of single stimuli delivered at threshold voltage, or (2) occasionally, by progressive section of the l.p.n. (in fact there was often some fluctuation in the twitch amplitude of a single motor unit which was therefore not strictly all-or-none; but in spite of this the sporadic appearance of a second motor-unit twitch was obvious and became more regular on raising stimulus strength slightly).

Intracellular recording was via glass micro-electrodes filled with 3 M-potassium acetate, and having resistances of 60–120 MΩ. In some cases the electrode tip was flexibly mounted on a piece of thin silver foil (Heistracher & Hunt, 1969). In all cases the muscle was stretched over a wedge of Sylgard, and penetrations made opposite the nerve entry point into the muscle (which corresponds to the end-plate band running across the muscle).

All mean values are given ± S.D., and the significance of differences between means assessed by Student's *t* test. Measurements of tension and potential differences were made on the face of a storage oscilloscope or from photographs of the display.

RESULTS

Tension development and contraction time in whole muscles

Examples of tension records from a whole muscle are shown in Fig. 1*A*. The data for whole-muscle twitch and tetanic tensions are summarized in Table 1. The data are presented separately for each age as well as in pooled form. The only significant difference discernible with age is that mean tetanic tensions are greater at 5 days than at 3 days. As a consequence the twitch:tetanus ratio decreased from 0·333 (±0·072, $n = 16$) at 3 days to 0·284 (±0·063, $n = 21$) at 5 days ($P < 0·05$), when it reached a value indistinguishable from that of older whole muscles (24–119 days:

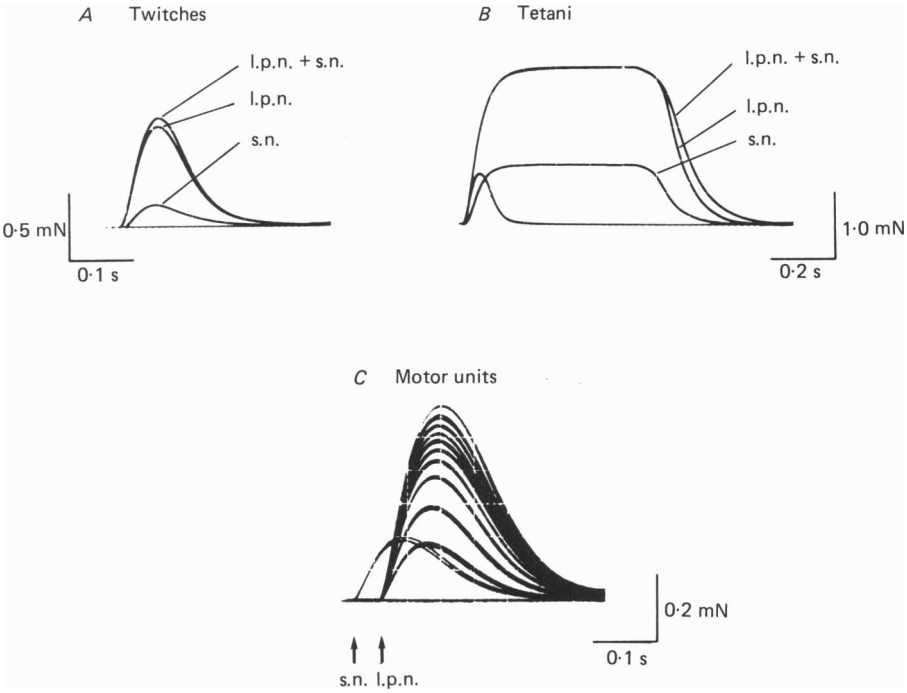

Fig. 1. Examples of isometric tension recordings from a single motor unit (in *A* and *B*) and whole fourth deep lumbrical muscles (*A*, *B* and *C*) from a 4-day-old rat. In *A* (isometric twitches), the smallest twitch is from a single unit obtained by stimulation of the sural nerve (s.n.). Twitches in response to stimulation of the lateral plantar nerve (l.p.n.) and of both nerves together are also shown. There is no complete overlap between the s.n. and l.p.n. In *B* the whole-muscle twitch of the same muscle is shown together with responses to tetanic stimulation of the s.n. and s.n.+l.p.n. There is complete overlap between the s.n. and l.p.n. In *C* the number of motor units in a different muscle is assessed in the l.p.n. (ten units) and s.n. (one unit, displaced to the left) on progressively increasing stimulus voltage. Note small variations in single motor-unit tensions of the s.n. unit.

TABLE 1. Whole-muscle tensions. Results are means ± S.D.

	Tension (mN)	*n*
	Twitches	
3 days	1·264 ± 0·279	16
4 days	1·206 ± 0·262	30
5 days	1·350 ± 0·302	22
3–5 days	1·265 ± 0·279	68
	Tetani	
3 days	3·708* ± 0·546	16
4 days	4·193 ± 0·679	30
5 days	4·733* ± 0·825	22
3–5 days	4·270 ± 0·808	68

*$P < 0·001$

0·268 ± 0·042, $n = 14$), where functional polyneuronal innervation would have disappeared (Betz *et al.* 1979) and contraction time become constant (Fig. 2).

In the 3–5 day age range most of the muscle fibres in the whole muscle are probably activated by a single supramaximal shock to the nerve, since in a different series of

muscles from the same age range that were stimulated by directly applied supramaximal single shocks the twitch tension developed was not greater (1·225±0·300 mN, $n = 29$). However, it should be noted that these muscles had been denervated

TABLE 2. Contraction times of whole muscles and motor units. Results are means±S.D.

	Contraction time (ms±S.D.)	n
	Whole muscle	
3 days	90·0*±8·86	16
4 days	78·9±7·84	30
5 days	76·2*±8·53	20
3–5 days	80·7‡±9·74	67
	Units	
3 days	78·8†±7·9	44
4 days	67·7±7·5	67
5 days	67·0†±13·0	47
3–5 days	70·1‡±11·0	159

* $P < 0·001$; † $P < 0·001$; ‡ $P < 0·001$.

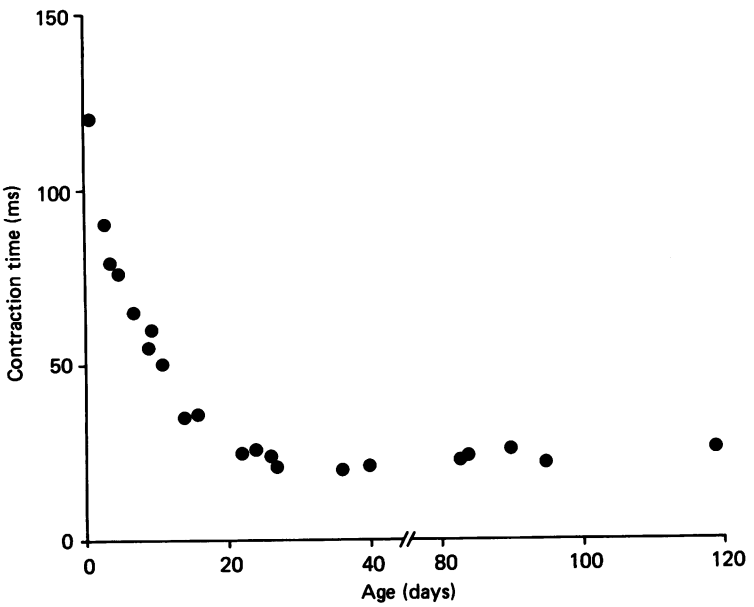

Fig. 2. Graph of whole fourth deep lumbrical muscle contraction time (twitch time-to-peak) with age of the rat. 25 °C; $[Ca^{2+}]_o$, 2 mM. Assuming a Q_{10} value of 2·25 (Ranatunga, 1977) the contraction times at birth (120 ms) and in adults (about 23 ms) would be equivalent to 53 and 10 ms at 35 °C.

chronically 18 h before the experiment in order to prevent activation through the nerve terminals, and this could have caused some fibre atrophy.

The contraction times of whole muscles are given in Table 2. There was marked speeding up over the 3-day period. Changes from birth to adulthood are shown in Fig. 2. Adult contraction times are attained at about 20 days.

The number of motor units in a muscle

The number of motor units in a muscle was assessed as described in the Methods and illustrated in Fig. 1. In the whole sample the mean number per muscle was $10{\cdot}9 \pm 1{\cdot}26$ ($n = 66$). There was no significant change between 3 and 5 days, and the number of motor units per muscle in a sample from 20 to 119 days ($11{\cdot}2 \pm 0{\cdot}94$, $n = 15$) was the same. These values are similar to those given by Betz *et al.* (1979) in the same muscle, and support the view that there is little if any motoneuronal death during post-natal synapse elimination in the rat (Brown *et al.* 1976).

By assessing the number of apparent motor units on stimulating at different points along the nerve length we checked for motor axonal branching. In five muscles we stimulated at three points: the s.n. and l.p.n. separately just below the common peroneal nerve branching point in the thigh, and the whole muscle nerve (a) just below the entry of the s.n. into the l.p.n. at the heel, and (b) close to the nerve entry into the muscle. The muscles had 12, 11, 11, 10 and 9 motor units as assessed at the most central site. The numbers were the same at the middle site, and the same or slightly less (10–12, 10, 10, 9 and 8–9 respectively) at the most peripheral site. The uncertainty and lower numbers counted near the muscle were due to the much narrower range of thresholds encountered here, and the consequent difficulty in separating tension increments. From this there is no evidence of axonal branching peripheral to the thigh. Branching more centrally is not excluded but is unlikely in view of Eccles & Sherrington's (1930) finding that branching of motor axons occurs more commonly in the more peripheral parts of their length.

Do axon latency differences cause repetitive firing in whole-muscle twitches?

It will be seen in Table 2 that the contraction time for whole muscles over the age range 3–5 days was considerably longer (by about 10 ms) than that for the pooled sample of motor units. In only 7 individual motor units (out of 159) were the contraction times longer than those for the whole muscles in which they were situated. Possibly differences in conduction velocity in the axons of innervating motoneurones could lead to repetitive firing in polyneuronally innervated muscle fibres, if differences in conduction time exceeded the refractory period of the muscle fibre. If this were the case, then stimulating the nerve near the muscle would reduce the effect by minimizing differences in conduction time. At the same time twitch amplitude and twitch:tetanus ratio would be reduced. To test this we stimulated the nerves supplying five muscles at three points along their lengths, as described in the preceding section. There was no shortening of contraction time. For the furthest and nearest points of stimulation to the muscle in the five muscles these were: 80–81; 78–77; 74–76; 93–89; 68–71 ms. Also there was no decrease in twitch:tetanus ratio. Values for the same points of stimulation were: 0·44–0·45; 0·36–0·39; 0·31–0·32; 0·28–0·30; 0·27–0·30. We therefore conclude that asynchronous arrival times of motor-axon action potentials is not sufficient to cause significant repetitive firing in the polyneuronally innervated fibres of this muscle. The reason for the difference in contraction times between units and whole muscles remains unknown. Lewis & Rosendorff (1965) made a similar observation on submaximal twitches compared with whole-muscle twitches in cat muscle, and discussed a mechanical explanation involving the series elastic component.

Tension development in motor units

Twitch and tetanic tensions for the pooled sample of motor units are given in Table 3. There were no significant differences within the age range 3–5 days. The mean twitch:tetanus ratio for the motor units was $0{\cdot}177 \pm 0{\cdot}037$ ($n = 67$). This is very

TABLE 3. Twitch and tetanic tensions of single motor units and unit sizes derived from twitch and tetanic measurements (3–5 days). Results are means ± S.D. with the number of determinations in parentheses

Unit twitch tension (mN)	Unit tetanic tension (mN)	Unit size (% w.m. twitch tension)	Unit size (% w.m. tetanic tension)
$0{\cdot}245 \pm 0{\cdot}101$ (160)	$1{\cdot}247 \pm 0{\cdot}517$ (67)	$19{\cdot}9 \pm 7{\cdot}6$* (160)	$29{\cdot}7 \pm 12{\cdot}2$* (67)

* $P < 0{\cdot}001$; w.m., whole muscle.

considerably less than that for the whole muscles (mean = $0{\cdot}299 \pm 0{\cdot}063$, $n = 68$). It compares with a value of $0{\cdot}264 \pm 0{\cdot}046$ ($n = 7$) for a sample of motor units in muscles from rats of 22–119 days, which is the same as the ratio for the whole muscles of the older (5 day) sample (0·268).

One could interpret these data in the following way: in neonatal motor units more muscle fibres respond to tetanic stimulation of the motor axon than respond to single-shock stimulation. However, this is not true when the whole muscle nerve is stimulated, and it is not true for motor units in older animals.

From the developmental point of view it is probably more interesting to express these data as unit size, calculated as a percentage of whole-muscle tension. Mean values calculated from twitches and tetani are given in Table 3. Motor-unit size from twitches is very similar to that obtained by Betz *et al.* (1979) in this muscle at the same age (their Fig. 5), also using twitches. Motor-unit size, derived from tetani, is larger by about a half. At the level of the single unit, in all but one case, unit size was greater when assessed by tetani than by twitches. This contrasts with the small sample of motor units from animals of 22–119 days. Here values from twitch measurements gave a mean motor-unit size of $11{\cdot}4 \pm 6{\cdot}6\%$, and from tetani $10{\cdot}9 \pm 5{\cdot}1\%$ ($n = 7$). These values are very similar to those of Betz *et al.* (1979) for ages over 28 days (their Fig. 5).

We can therefore conclude that in young neonatal rat muscles, but not in muscles from older animals (when most or all synapse elimination has taken place), the average motor unit accounts for a greater proportion of the whole-muscle tension when estimated from tetani than from twitches.

Low efficacy of neuromuscular transmission in neonatal motor units

The results of assessment of motor-unit size can be explained on the basis of low neuromuscular junctional efficacy. Because of this a proportion of the muscle fibres in a motor unit do not respond to a single action potential in the motor axon. However, all the muscle fibres innervated by the axon respond to tetanic stimulation of the axon, due to summation. Also all (or nearly all) the muscle fibres in the whole muscle respond to a single shock to the s.n. plus l.p.n. because of the summations of the several motor inputs on each polyneuronally innervated muscle fibre. Alter-

natively, each muscle fibre has at least one strong synaptic input onto it so that when all the motor axons innervating that fibre are stimulated the fibre will respond.

We tested the hypothesis that some neuromuscular junctions are weak by recording intracellularly in the end-plate region during single and multiple axonal stimulation. There was clear evidence of weak junctions and the results are described later in this paper. One would require a very large sample from such an experiment

TABLE 4. Motor-unit size: a comparison of the effect of tetanizing and raising $[Ca^{2+}]_o$

Age (days)	Motor-unit size (% w.m.tw.t.) 2 mM-Ca^{2+}	Motor-unit size (% w.m.tw.t.) 10 mM-Ca^{2+}	Motor-unit size (% w.m.tet.t.) 2 mM-Ca^{2+}
1	38·8	56·1	57·8
4	19·7	26·2	25·2
5	2·4	4·0	4·6
5	15·0	20·2	21·9
5	21·1	32·2	34·0
5	7·7	11·3	12·3
11	10·1	12·8	12·7
22	7·7	6·7	6·4
22	21·1	19·8	16·9
27	6·8	7·3	6·6

Abbreviations: w.m., whole muscle; tw.t., twitch tension; tet.t. tetanic tension.

to make a comparison with the data from tension recording. Because of this we tested the hypothesis further by measuring the effect on unit size of raising the extracellular calcium concentration ($[Ca^{2+}]_o$). This would be expected to facilitate neuromuscular transmission and convert some or all of the weak subthreshold inputs to effective ones (as found in frog sartorius muscle by Grinnell & Herrera, 1980). It should be noted that raising $[Ca^{2+}]_o$ increases twitch (but not tetanic) tension development in directly stimulated neonatal muscle without neuromuscular transmission (Jones & Ridge, 1984). Therefore in order to see the effect on neuromuscular transmission one has to compare the effect on the motor-unit twitch with the effect on the whole muscle twitch (the assumption being that the direct effect on any one muscle fibre will be the same on average regardless of whether the fibre is or is not in the particular motor unit being tested).

The effect of raised $[Ca^{2+}]_o$ on motor-unit size at different ages

We assessed motor-unit size as a percentage of whole-muscle tension for seven motor units (each from a different muscle) in young animals (1–11 days) and for three motor units in older animals (22–27 days). The size of each motor unit was measured by twitches in 2 and 10 mM-Ca^{2+} and by tetani in 2 mM-Ca^{2+}. Raising $[Ca^{2+}]_o$ to 10 mM did not potentiate tetanic tension in whole muscles or units; often motor-unit tetanic tension was less well sustained than in 2 mM-Ca^{2+}. The data are given in Table 4. In the young group raising $[Ca^{2+}]_o$ increased the apparent size of each motor unit obtained with twitches to, or almost to, that obtained with tetani. This effect is striking. In the older group, however, raising $[Ca^{2+}]_o$ did not increase apparent motor-unit size, and the results obtained with tetani did not give greater unit sizes than these obtained with twitches. In the fourth deep lumbrical muscle virtually all

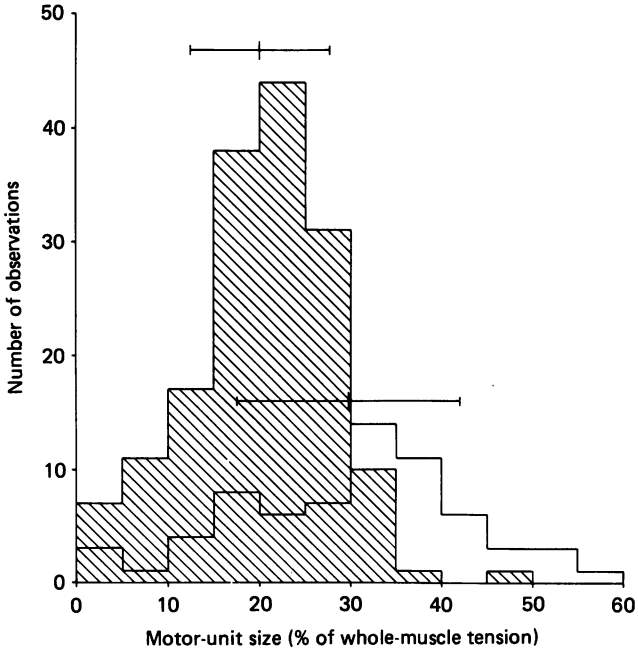

Fig. 3. Distribution of motor-unit size (3–5 days) as a percentage of whole-muscle tension for twitches (hatched areas) and tetani (open areas). Bars are means ± S.D.

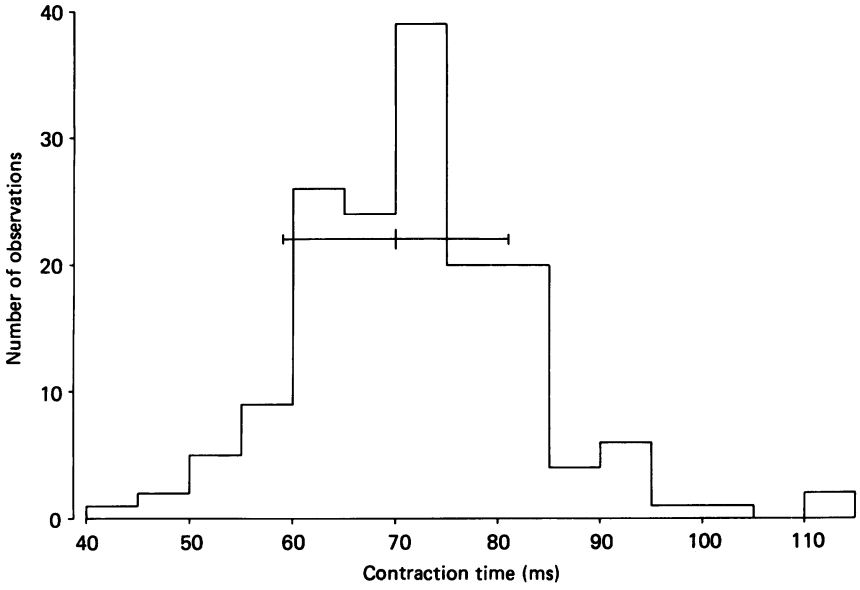

Fig. 4. Distribution of motor-unit contraction times (3–5 days). Bar is mean ± S.D.

the muscle fibres are polyneuronally innervated at 5 days, about 50 % of them at 11 days and about 5 % of them at 22 days (Betz *et al.* 1979), so the effects of tetanizing and raising $[Ca^{2+}]_o$ on motor-unit size parallel the level of polyneuronal innervation rather closely.

Distribution of motor-unit sizes and contraction times

The distributions of motor-unit sizes in the lumbrical muscle, assessed by twitches and tetani, are shown in Fig. 3, and the distribution of contraction times is shown in Fig. 4. Neither of these distributions shows clear evidence of more than one population of motor units, although one should bear in mind that the distribution of contraction times is plotted against the background of a wide range of whole-muscle

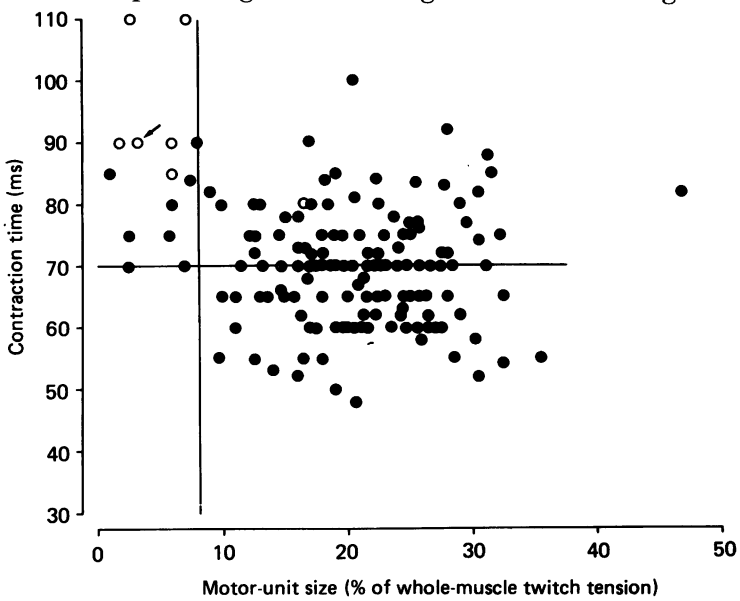

Fig. 5. Graph of motor-unit (3–5 day) contraction time plotted against motor-unit size (as a percentage of whole-muscle twitch). The vertical line is drawn at a unit size of 8%, and the horizontal line is the mean value for the whole sample of contraction times. ○, units in which the contraction time is longer than that of the whole muscle containing the unit; ●, units in which this is not the case. The arrow indicates unit 12 of the following paper (Jones *et al.* 1987*a*). Note that all units with twitch size < 8% of whole muscle twitch had contraction times < 70 ms.

contraction times (62–105 ms, mean 81 ms). As is reported in a following paper (Jones *et al.* 1987*b*) the number of muscle fibres containing slow myosin in adult fourth deep lumbrical muscles is about 9% of the total, which is equivalent to one motor unit of average size. The motor-unit composition of the adult muscle has not yet been determined, but assuming the least possible number of slow motor units (1) we would expect to have encountered about 14 slow motor units in the sample of 160 for which twitches were obtained, if slow motor units had differentiated at this age (assuming unbiased sampling: see Discussion). The data in Figs. 3 and 4 provide no clear evidence of unit differentiation.

In the adult cat gastrocnemius muscle, fast and slow motor units are clearly separable on the basis of motor-unit size (slow units are small), fatigability (slow units are resistant to fatigue) and contraction time (Burke *et al.* 1973). No such comprehensive survey of motor units has yet been made in a predominantly fast muscle in the rat, but in the soleus muscle (which, unlike the soleus in the adult cat,

contains some fast units), Close (1967) found 27 units with a mean time to peak contraction of 38 ms (slow; 35–36 °C) and three in which it was 18 ms. Close (1967) also recorded from motor units in rat extensor digitorum longus, and did not observe any slow units. However, histological study has shown slow-myosin-containing fibres to occur at low frequency in this muscle (3 %; Rubinstein & Kelly, 1981), and Close's total sample of motor units (49) was probably close to the number of units in any one muscle.

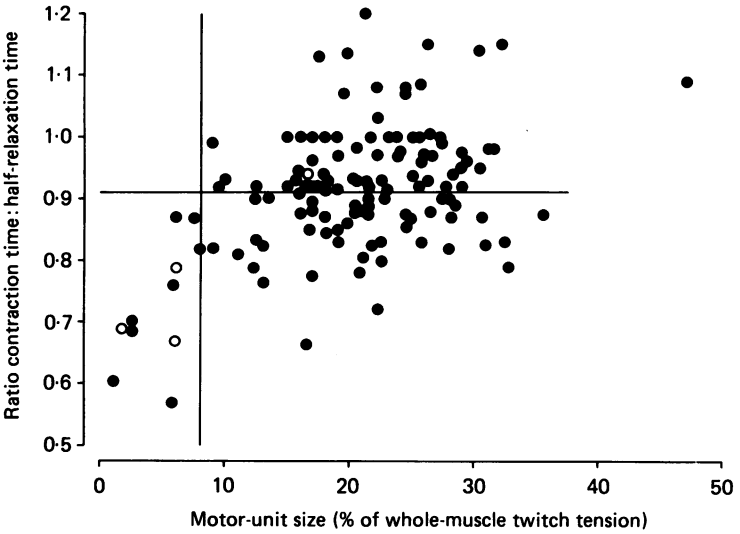

Fig. 6. Graph of the ratio of motor-unit (3–5 day) contraction time: half-relaxation time plotted against motor-unit size (as a percentage of whole-muscle twitch). Symbols and lines as in Fig. 5. Vertical line is drawn at a unit size of 8 % and the horizontal line is the mean value of the ratio for the whole sample. Note that relaxation times are disproportionately long for units < 8 % of the whole-muscle twitch.

In Fig. 5 motor-unit contraction time is plotted against motor-unit size assessed from twitches. There is no clear relationship apparent for the main body of the units but there is a group of small units (14 units less than 8 % whole-muscle twitch; these units were also less than 15 % whole-muscle tetanus) whose contraction times are equal to or longer than the mean for the whole sample (70 ms). In the whole sample of units unit contraction time exceeded that of the muscle in which the unit lay for only seven units as mentioned previously (○, Fig. 5). Six of these fall in the group of small units. Of the fourteen units in the small-unit group seven were from 3-day muscles, two from 4-day and five from 5-day muscles.

The appearance of a group of small, slowly contracting units could be explained if small units are more likely to contain a greater proportion of slow fibres than large units.

Time to half-relaxation of twitches

In fifty muscles and 128 units the time for the twitch to decay from peak tension to half-maximal tension was measured. For ten whole muscles the mean value was $84{\cdot}3 \pm 8{\cdot}7$ ms, and for the units it was $79{\cdot}7 \pm 15{\cdot}7$ (difference is significant at $P < 0{\cdot}05$).

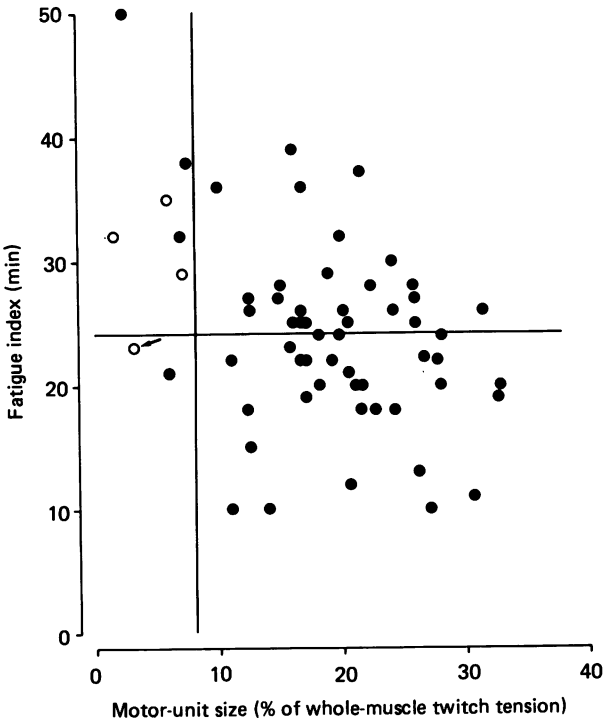

Fig. 7. Graph of motor-unit (3–5 day) fatigue index (time for twitch tension to reduce to half with continuous stimulation at 5 impulses/s plotted against initial motor-unit size (as a percentage of whole-muscle twitch). Symbols as in Fig. 5. The vertical line is drawn at a unit size of 8 % and the horizontal line is drawn at the mean value of the fatigue index for the whole sample. Note that most small units (< 8 % of the whole-muscle twitch) are relatively fatigue resistant.

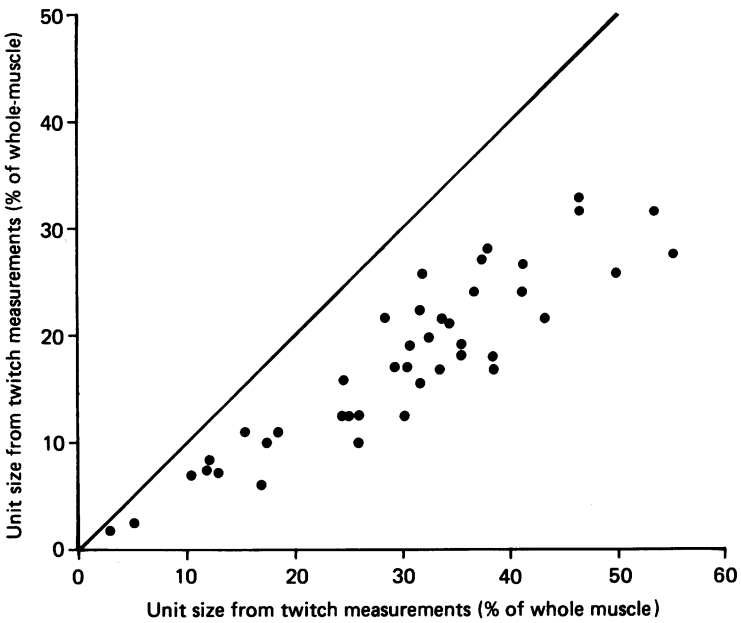

Fig. 8. Graph of motor-unit size (as a percentage of size of whole muscle) as derived from twitch measurements plotted against motor-unit size from tetanic measurements. The line of equality is drawn.

When the ratios of contraction time : time to half-relaxation were compared for whole muscles and motor units they were found to be not significantly different ($0{\cdot}982 \pm 0{\cdot}081$ for muscles; $0{\cdot}908 \pm 0{\cdot}113$ for units). The value of the ratio is plotted against motor-unit size from twitches in Fig. 6. Again the same group of small units stands out. All these units (eleven for which half-relaxation times are available) show values for the ratio of less than the mean value. This means that these small units are not only relatively slowly contracting, but disproportionately slowly relaxing also. This finding is also consistent with the idea that small units are more likely to contain a greater proportion of slow fibres than large units.

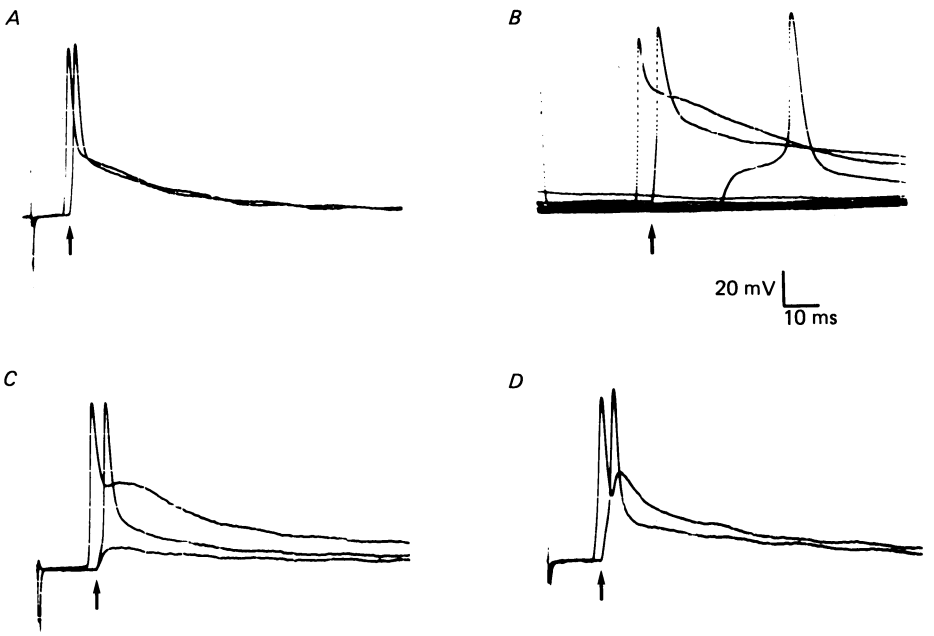

Fig. 9. Examples of intracellular recording in the end-plate region of muscle fibres (3–5 days) on stimulating the l.p.n. and s.n. (arrows; one motor axon only). In *A*, l.p.n. and s.n. input strengths are roughly equal, but in *B–D* l.p.n. input is the stronger (for criteria see text). In *B* there is a spontaneous e.p.p. and action potential and *C* shows a subthreshold and suprathreshold response to stimulation of the s.n.

Motor-unit fatigability

In some of the motor units we attempted to deplete the glycogen reserves of fibres within the single motor unit for subsequent histological identification (Jones *et al.* 1987*a*). We used continuous stimulation of the nerve at 5 impulses/s in Ringer solution containing 5 mM-Ca^{2+} and no glucose, at 30 °C. For each unit we recorded the time taken for the twitch tension to fall from its initial value to half that value. We refer to this time as the fatigue index. The fatigue index for the whole sample of units was $24{\cdot}3 \pm 7{\cdot}6$ min and there was no significant difference with age between 3 and 5 days. The fatigue so recorded probably resulted from several different causes, since we found that the muscle-surface e.m.g. amplitude declined with twitch tension, both tension and e.m.g. showing rapid recovery when glucose was added during the

course of continuous stimulation. Therefore the fatigue may be due to development of incomplete conduction of muscle-fibre action potentials and reduction in the efficacy of neuromuscular transmission, as well as direct fatigue of tension generation.

A plot of fatigue index against motor-unit size derived from twitches is shown in Fig. 7. Five of the eight units that were less than 8 % of the whole muscle twitch and for which fatigue index was measured have fatigue indices longer than the mean for the whole sample. However, mean fatigue indices for units less and more than 8 % of the whole-muscle twitch are not significantly different. The mean fatigue index for these units was $32{\cdot}5 \pm 9{\cdot}1$ min; for the rest of the sample (excluding the seven small units) the mean fatigue index was $23{\cdot}0 \pm 6{\cdot}6$ min. These values do not differ significantly ($P > 0{\cdot}05$).

The small units

From these data we conclude that the units in the total sample are drawn from a continuum with respect to unit size, contraction time, the ratio of contraction time: half-relaxation time, and fatigue index. However there is a group of small units which, though not rigorously separable from the rest, tend to show the following characteristics: (a) long contraction times; (b) disproportionately long half-relaxation times; (c) long fatigue indices (though the difference here is not statistically significant at the 5 % level).

Efficacy of neuromuscular transmission and motor-unit size

There was no evidence from our data that large units contained a higher proportion of low-efficacy junctions than small units, as might be expected if synapse elimination was disproportionately low in small units (see Brown *et al.* 1976). When motor-unit size from twitch measurements is plotted against that from tetanic measurements the data points fall about a straight line rather than about a curve whose slope decreases at higher values (Fig. 8). This line is, of course, depressed below the line of equality, as previously discussed.

Polyneuronal innervation of fibres in single motor units

In those muscles where a single axon entered via the s.n. it was possible to assess roughly the level of overlap of innervation by axons entering via the l.p.n. Here overlap of the s.n. and l.p.n. is given by:

$$\text{overlap } \% = \frac{(T_l + T_s) - T_{l,s}}{T_s} \times 100,$$

where T_l = tension developed on stimulating the l.p.n.; T_s = tension developed on stimulating the s.n.; $T_{l,s}$ = tension developed on stimulating both nerves simultaneously.

For tetani the majority of units were completely overlapped by the l.p.n., and the mean from the sample was $98{\cdot}7 \pm 3{\cdot}6$ % ($n = 22$). However, in all cases overlap was incomplete for twitches, and the mean was $74{\cdot}8 \pm 18{\cdot}9$ % ($n = 23$). An example is given in Fig. 1 *A* and *B*.

One would not expect complete overlap for twitches since, if there were, it would

not be possible to count a separate tension increment for all the units in response to progressively increasing stimulus strengths to the muscle nerve. The shortfall between the true and counted number of motor units would depend on the level of overlap, and the hidden units would be those recruited at the higher voltages. It is unlikely that motor units are hidden in this way at this age, since the number of motor units counted is the same as in adults (Betz *et al.* 1979 and this paper). An alternative explanation, that there is an exactly compensating motoneuronal death rate, seems to us unlikely.

There is another interesting indication from these data. It seems that a strong input from the s.n. is less often associated with a strong input from the l.p.n. than one would predict from a random distribution; that is, a strong input from one axon tends to be associated with weak inputs from other axons onto the muscle fibre. The argument for this is given in the Discussion.

We wondered if the extent of twitch overlap was related to unit size, and especially if small slow units differed in this respect from others. There was no significant relationship between overlap and motor-unit size from twitches or tetani. There were only two units in the sample which were less than 8% of the whole-muscle twitch. These had calculated overlaps of 86 and 73%, which are within the range for the whole sample. However, one should bear in mind that the accuracy of the measurements is less for small units.

Intracellular recording

In order to investigate further the possibility of weak junctional input onto polyneuronally innervated fibres we recorded intracellularly from muscle fibres in the region of the end-plate. At the same time we were able to make an estimate of the proportion of muscle fibres activated by electrical coupling, rather than direct synaptic action, on stimulating a single motor axon. In these experiments we obtained a single s.n. motor unit by tension monitoring and, if necessary, progressive s.n. section. We then recorded intracellularly from as many fibres as possible in the end-plate band (at the level of the nerve entry into the muscle), testing each fibre for s.n. and l.p.n. input with single shocks to each nerve. We penetrated sixty fibres that had s.n. inputs, in eight muscles. All but three of these were in 10 mM-Ca^{2+}, the remaining three being in 5 mM-Ca^{2+}.

Resting potentials

The mean resting potential was $-76{\cdot}5 \pm 5{\cdot}0$ mV. This is higher than that reported by Ward & Wareham (1986) in rat extensor digitorum longus muscle at 5–6 days ($-49{\cdot}6 \pm 1{\cdot}16$ mV). We do not know the reasons for this difference, but would note that all our penetrations were made in raised $[Ca^{2+}]_0$ (we found it very difficult to obtain sustained penetrations in 2 mM-Ca^{2+}, though often penetrations made in 10 mM would be sustained on changing to 2 mM), and we found it necessary to use comparatively high-resistance micro-electrodes of 60–120 MΩ. Our resting potential values were in the range reported by Dennis, Ziskind-Conhaim & Harris (1981) in intercostal muscles of rat embryos at 14 days and older. Dennis *et al.* (1981) also used raised $[Ca^{2+}]$ (4–8 mM) and high-resistance electrodes (80–100 MΩ).

Relative strength of s.n. input

Examples of the intracellularly recorded responses of four fibres to s.n. and l.p.n. stimulation are shown in Fig. 9. Such recordings most frequently showed the s.n. input to be weaker than the l.p.n. input (e.g. Fig. 9*B*, *C* and *D*), by the following criteria: a smaller residual potential (presumably end-plate potential (e.p.p)) after

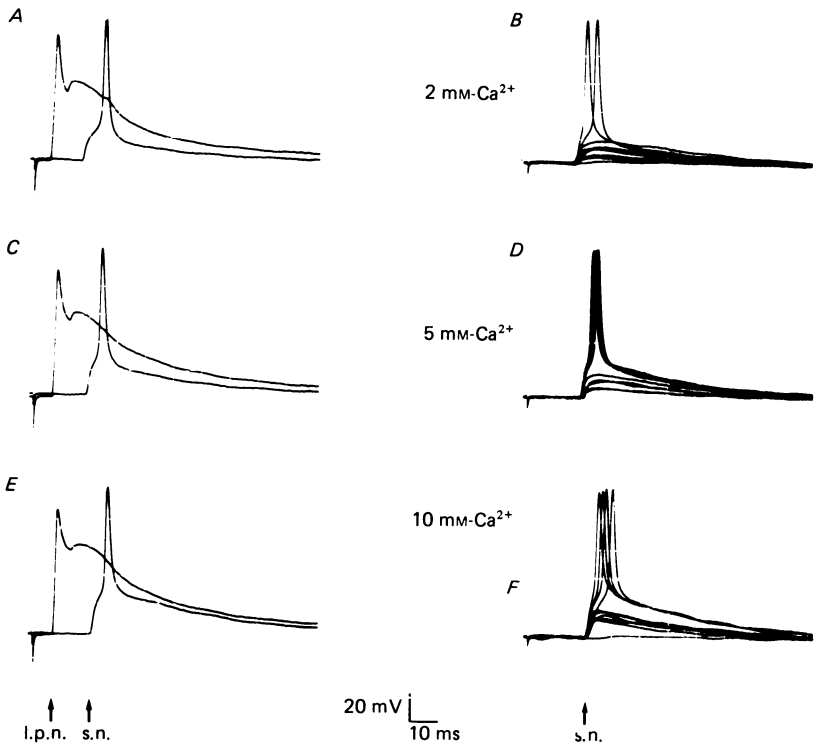

Fig. 10. Examples of intracellular recordings from one muscle fibre (4 day animal) at various Ca^{2+} concentrations in response to single shocks and repetitive stimulation of the sural nerve (s.n.; one axon only) and single-shock stimulation only of the lateral plantar nerve (l.p.n.). Single shocks, *A*, *C* and *E*; repetitive stimulation of s.n. (ten shocks at 0·2 impulses/s), *B*, *D* and *F*. Ca^{2+} concentrations were 2 mM (*A* and *B*), 5 mM (*C* and *D*) and 10 mM (*E* and *F*). The number of suprathreshold responses (action potentials) are two (*B*) five (*D*) and five (*F*).

the action potential and a larger action-potential overshoot presumably because of less increase in membrane conductance in the end-plate region due to acetylcholine. Occasionally input strength was roughly equal (e.g. Fig. 9*A*), and one fibre received input from the s.n. but none from the l.p.n. Two fibres had consistently subthreshold e.p.p.s for s.n. input, but responded with an action potential to stimulation of the l.p.n. Since on these fibres with one s.n. input there are probably two l.p.n. inputs on average (see Discussion), one would expect the l.p.n. input to be the stronger more often than not.

The effect of $[Ca^{2+}]_o$ *on single-unit junctional efficacy*

In order to test our hypothesis that many neuromuscular junctions in neonatal rat muscle have a low efficacy, we wished to examine the effect of changing $[Ca^{2+}]_o$ on intracellularly recorded responses to stimulation of the s.n. and l.p.n. Our aim was to test junctional efficacy in high–low–high $[Ca^{2+}]_o$ in a sandwich configuration. The

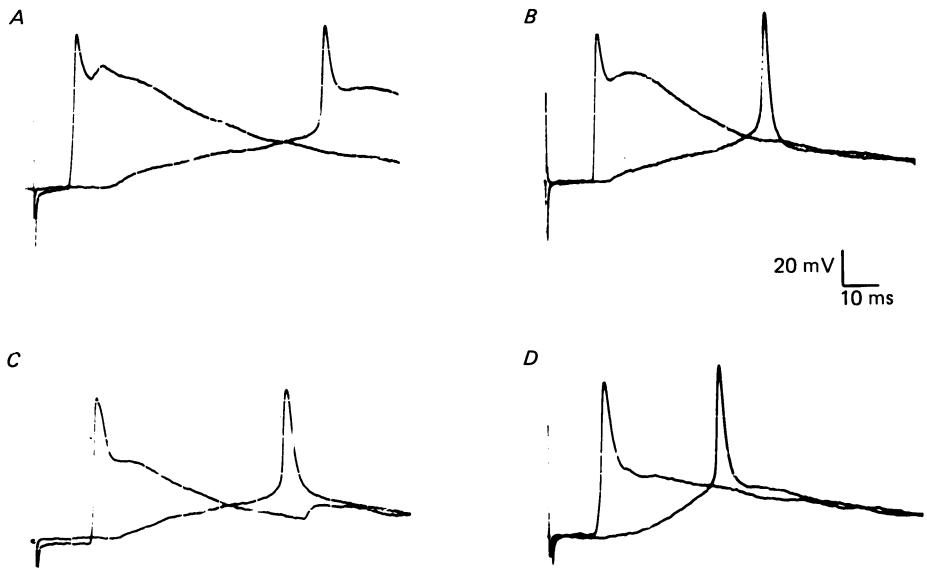

Fig. 11. Examples of intracellular recordings from four different fibres (*A–D*) on stimulating a single sural motor unit (s.n.; longer latency responses) or the lateral plantar nerve (l.p.n.), containing the rest of the motor axons in the muscle (shorter latency responses). The responses to s.n. stimulation are possibly due to electrical coupling between fibres (cf. Dennis *et al.* 1981). The responses to lateral plantar stimulation are synaptic.

main difficulty was to maintain a satisfactory penetration of the muscle fibre with the micro-electrode through the solution changes. We found from tension recording that the Ca^{2+} effect was about 90 % complete within 10 min of changing the solution in our system (this will include bath wash-out time as well as diffusion time into the muscle), and when recording intracellularly we found that a change from 10 mM via 5 mM to 2 mM Ca^{2+} (and the reverse) caused minimal disturbance to the penetration. This procedure gave our best sustained fibre penetration, with 5 min periods between solution changes.

In three muscle fibres with s.n. and l.p.n. input we were able to sustain the penetration long enough to assess changes due to changed $[Ca^{2+}]_o$. In the best sustained fibre we recorded the responses to ten stimuli (at 0·2/s) for six periods in 10 mM-, four periods in 5 mM- and five periods in 2 mM-Ca^{2+}. The number of suprathreshold responses (action potentials) in 10 mM-Ca^{2+} ranged from 10 to 3 with a mean of 7. For 2 mM the range was from 2 to 0, mean 0·8; for 5 mM (but after only 5 min of changed solution) the range was from 6 to 0, mean 4·25. In this fibre, therefore, the expected effect on junctional efficacy of changing $[Ca^{2+}]_o$ was observed.

Examples of records from this fibre are shown in Fig. 10. In the second fibre the effect was much less marked. In 10 mM-Ca^{2+} five out of twelve stimuli (42%), and in 2 mM-Ca^{2+} seven out of twenty stimuli (35%) gave an action potential. In the third fibre the s.n. input was very strong and there were no failures in response to ten stimuli in both 10 and 2 mM-Ca^{2+}, as was the case for all three fibres when stimulating the l.p.n.

Electrical coupling

Of the sixty-eight fibres penetrated and with s.n. inputs, eight (12%) showed strong synaptic inputs on stimulating the l.p.n. but slowly developing depolarizations leading to an action potential on stimulating the s.n. Four examples of these responses are shown in Fig. 11. Similar responses recorded in embryonic rat intercostal muscles were considered by Dennis *et al.* (1981) to be due to electrical coupling between muscle fibres. We have no direct evidence that these responses are due to such coupling but in view of the lack of a normal-appearing e.p.p. and the morphological demonstration of gap junctions at birth in this muscle (Schmalbruch, 1982) we tentatively conclude that such an explanation may be correct.

The fact that we did not observe any such responses on stimulating the l.p.n. is presumably because when there is only one s.n. motor unit virtually all the muscle fibres in the muscle receive motor supply from one or more of the l.p.n. motor axons.

DISCUSSION

In the adult rat, the fourth deep lumbrical muscle contains about 950 fibres (Betz *et al.* 1979) of which about 80 contain slow myosin (Jones *et al.* 1987*b*). The muscle can thus be considered a fast muscle. Thompson, Sutton & Riley (1984) have presented evidence from glycogen depletion and histochemistry that at 8 days the motor units in rat soleus (a predominantly slow-twitch muscle in adults) contain both fast- and slow-twitch muscle fibres. In the next paper (Jones *et al.* 1987*a*) we give similar evidence for the fourth deep lumbrical muscle using glycogen depletion and immunocytochemical labelling. The tension data in the present paper are consistent with this finding, in that there is no homogeneous and separate population of slowly contracting motor units. What we do find, however, is that there is a group of small motor units ($< 8\%$ whole-muscle twitch and $< 15\%$ whole-muscle tetanic tension) that are relatively slowly contracting and relaxing (and may be relatively fatigue resistant). Possibly these units are beginning to differentiate into the pure slow units of adult muscles. Histological data on one of these units are reported in the following paper (Jones *et al.* 1987*a*), and support this idea.

In the adult muscle, with eleven motor units, the mean motor unit size is about 86 fibres, which is very close to the number of slow fibres in the muscle. However, until a survey of the motor units in the adult is undertaken we do not know that there is only one slow motor unit. Betz *et al.* (1979) found a wide range of adult motor-unit sizes (from less than 20 to more than 280 muscle fibres) and therefore there could be two (or more) smaller slow units. In the 160 motor units reported here from 3–5 day rats, there are 14 in the small-unit group. If there is one slow unit present in the adult muscle, and the small units in neonatal muscles will become slow units,

then the best prediction would be that we would find 14·5 units in our sample of 160. The closeness of these numbers (14 and 14·5) may be fortuitous, and in any case it is possible that not all the future slow units are at the same level of differentiation at this age. In this discussion we have assumed that s.n. units are drawn at random from the whole-muscle pool of motoneurones. As far as unit size is concerned this seems to be true (Betz *et al.* 1979) but we need to examine the distribution of slow units between the s.n. and l.p.n. in the adult.

We describe results from measurements of unit twitches and tetani that, compared with whole-muscle tension measurements, are consistent with the view that about a third of neuromuscular contacts in the average motor unit are too weak to induce an action potential in the muscle fibre in response to a single shock (in Ringer solution containing 2 mM-Ca^{2+}, at 25 °C). We have observed these weak neuromuscular junctions, and their potentiation by raised $[Ca^{2+}]_o$, by intracellular recording, which supports this interpretation of the tension data. We cannot say whether these junctions are weak because they are recently formed, about to be eliminated, or for some other reason. Certainly not all junctions that will be eliminated are weak at this stage, since about two-thirds of junctions present will be eliminated, and only one-third are weak. New junctions are forming up to about 10 days in this muscle, on newly appearing muscle fibres (Betz *et al.* 1979). Our data show that the presence of weak junctions is related to the level of polyneuronal innervation of the muscle fibres during the period in which synapse elimination is taking place. It would be interesting to have more data for the age range 10–20 days, when fibre generation has ceased but synapse elimination is proceeding.

The data on the difference in overlap between single s.n. units and the rest of the units (in the l.p.n.) lead to the interesting conclusion that the distribution of weak and strong inputs is not random. This conclusion is arrived at in the following way: about one in three fibres in a sural motor unit which contracts in the single motor-unit tetanus does not contract in the twitch. Let us assume that each other motor unit in the muscle would also be a third smaller in a twitch than in a tetanus if stimulated individually. There are on average eleven motor units in the muscle, and the average motor-unit size at this age on tetanizing is about 30 % of the whole muscle. Therefore, if polyneuronal innervation is divided equally between fibres (from the model of Betz *et al.* 1979, this seems probable; but see Jones *et al.* 1987*a*) each fibre belongs on average to 3·3 motor units; for present purposes let us say that each fibre is part of three motor units. It follows that during tetanic stimulation of the l.p.n. all the fibres in the single sural motor unit will be effectively stimulated; that is, the l.p.n. shows 100 % overlap of the s.n. However, during a twitch the overlap will be less than complete if all the l.p.n. inputs fail on any of the fibres in the unit. The assumption that each muscle fibre is part of three motor units implies that a fibre in the s.n. unit is also part of two l.p.n. units. In each of these l.p.n. units one-third of the inputs will fail during a twitch if the weak junctions are distributed randomly. Therefore, the probability that both fail on any s.n.-innervated fibre is $(1/3)^2 = 1/9$; therefore, in this case the overlap would be reduced by 11 %. However, the *observed* reduction was about 25 % (from about 100 % to about 75 %; see Results), and this is in spite of any spacial summation of weak inputs from the l.p.n. that may occur and would increase the overlap. Therefore, we conclude that there are fewer strong inputs from

the l.p.n. on fibres receiving strong inputs from the s.n. than expected from a random distribution. However, this tendency has not reached the level where strong s.n. inputs are never associated with strong l.p.n. inputs (or effectively summating weak inputs) since, if it had, there would be no overlap between the s.n. unit twitch and the multi-unit l.p.n. twitch. A somewhat similar situation exists in dually innervated muscle fibres of adult *Xenopus laevis*. Here, if the quantal content of the e.p.p. of one ending is large then that of the other is small, the two being reciprocally related (Angaut-Petit & Mallart, 1979).

It would be interesting to know how fixed is the strong input to each muscle fibre during development, or whether its strength is transient as other forces mould the motor-unit organization of the muscle. There is some evidence that activity levels in motor axons have an influence on ultimate motor-unit size (Ridge & Betz, 1984) and it seems unlikely that the process of synapse elimination allows no plasticity in development.

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