*J. Physiol.* (1984), **352**, *pp.* 73–90
*With 5 text-figures*
*Printed in Great Britain*



# EFFECTS OF FAST AND SLOW PATTERNS OF TONIC LONG-TERM STIMULATION ON CONTRACTILE PROPERTIES OF FAST MUSCLE IN THE CAT

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(*Received 10 March 1983*)

SUMMARY

1. Different physiological rates of 'tonic' long-term electrical stimulation (rates 5–40 Hz; activity $\geqslant$ 50 % total time) were delivered to the left-side common peroneal nerve of the cat hind limb. The duration of treatment was 8 weeks, and the animals had previously been subjected to a left-side hemispinalization and dorsal rhizotomy. In the absence of stimulation, these operations had no slowing or weakening effects on peroneal muscle contraction.

2. The minimum two-pulse interval that gave a summation of tension (neuromuscular refractory period) was longer for stimulated than for non-stimulated muscles.

3. Twitches of chronically stimulated muscles had become prolonged by more than 100 %. Corresponding changes were found in the tension–frequency relation and in the 'sag'-behaviour of the stimulated muscles.

4. There were no differences between the 'fast' (20 or 40 Hz pulse rates) and the 'slow' (5 or 10 Hz pulse rates) patterns of tonic stimulation with respect to their effects on speed-related muscle properties. Furthermore, during the period of chronic stimulation, the prolongation of twitch contraction time occurred along the same time course for the fast and slow patterns of tonic treatment.

5. All chronically stimulated muscles had become weaker than normal. In comparison to the slow patterns, the present fast patterns of long-term activation caused (1) a smaller amount of decline in maximum muscle force, (2) a smaller twitch: tetanus ratio, and (3) the retention of a normal amount of post-tetanic potentiation of twitch size (decreased by the slow patterns).

6. When tested by a series of 40 Hz bursts, force was better maintained in chronically stimulated muscles than in normal ones. These effects on fatigue resistance were the same for the fast and slow patterns of long-term activation.

7. In peroneus longus muscles *contralateral* to the side of chronic activation, an evident impairment had commonly occurred in the capability to maintain force during tetani at the high rates needed for a maximum tetanic contraction.

8. The results are discussed in relation to problems concerning the long-term effects of motoneuronal activity patterns on the contractile properties of their muscle units.

## INTRODUCTION

The present studies of chronic stimulation were motivated by questions concerning the mechanisms responsible for the matching between contractile properties and usage patterns in muscle.

Within most skeletal muscles, individual motor units may differ considerably from each other with respect to their contractile speed, endurance and maximum force (for references, see Burke, 1981). There is much evidence indicating that such specializations are matched, in a functionally meaningful way, to the manner in which the various units are used by the central nervous system. Thus, for instance, long-lasting postural contractions of moderate strength tend primarily to be executed by those units of a pool that are highly fatigue resistant as well as comparatively slow and weak (cf. Burke, 1981; Henneman & Mendell, 1981). In postural contexts, fatigue resistance is obviously to be desired, slowness contributes to cost-efficiency (Crow & Kushmerick, 1982), and the use of weak units gives a smooth recruitment gradation of force. Contractile speed is not only of relevance for the speed and cost-efficiency of motor acts, but it is also a factor of importance for the way in which motoneuronal impulse trains are decoded by muscle: the slower a muscle or motor unit twitch, the lower are the rates needed for eliciting a given fraction of maximum tension (Cooper & Eccles, 1930; Kernell, Eerbeek & Verhey, 1983*b*). The stable 'primary' range of firing rates of a motoneurone is known to fit well to the steep region of the tension–frequency relation of its muscle unit (Kernell, 1965, 1979, 1983; cf. Eccles, Eccles & Lundberg, 1958). This match of neuronal rate *versus* muscle speed ensures that, in reflex or voluntary contractions, changes in motoneuronal discharge frequency may give rise to marked changes in muscle force.

There is much evidence indicating that various kinds of matching between the usage requirements and the properties of muscle units may arise via long-term influences of activation patterns on the contractile and metabolic machinery of muscle. Thus, tonic stimulation during a number of weeks at rates characteristic for slow motoneurones (5–10 Hz) has been shown to make fast muscles slower, weaker and more fatigue resistant (Salmons & Vrbová, 1969; Pette, Smith, Staudte & Vrbová, 1973; Peckham, Mortimer & Van Der Meulen, 1973; Salmons & Sréter, 1976; for further references, see Salmons & Henriksson, 1981). With respect to, in particular, the long-term slowing effects it is still uncertain, however, to what an extent these actions were caused by the great amount and/or the low rate of the imposed activity. There have been several physiological reports appearing, which have suggested that fast and slow rates of chronic stimulation may have markedly different effects on contractile speed. Salmons & Vrbová (1969) found that long-term stimulation at rates of $\geqslant 20$ Hz were incapable of counteracting the speeding-up effects of tenotomy in the rabbit soleus muscle. Other authors have reported that fast rates were incapable of producing any slowing of fast hind-limb muscles (40 Hz, rabbit, Brown, Cotter, Hudlická & Vrbová, 1976; 100 Hz, cat, Smith, 1978), or that fast rates produced only moderate amounts of slowing (50 Hz, cat, Goldring, Kuno, Nuñez & Weakly, 1981; 40 Hz, rabbit, Hudlická, Tyler, Srihari, Heilig & Pette, 1982). In the rat denervated soleus muscle, very striking differences have been found between the effects on muscle speed of 100 and 10 Hz of chronic stimulation (Lømo, Westgaard & Dahl, 1974; Lømo, Westgaard & Engebretsen, 1980).

The present studies were originally undertaken in order to find out whether there is a continuous relationship between the pulse rate of tonic long-term stimulation and the resulting speed of muscle contraction. The term *tonic* is used here to indicate the presence of a great *amount* of activity per day. The experiments were performed on innervated hind-limb muscles of adult cats, and the rates employed were chosen so as to fall within the known range of discharge frequencies for the cat hind-limb motoneurones (Kernell, 1965, 1979). In this context, rates of 5–10 Hz may adequately be labelled 'slow' because, in the cat, only motoneurones innervating relatively slowly contracting muscle units would normally be capable of sustaining such low frequencies of discharge. Rates of 20–40 Hz may be labelled 'fast' because they would often be utilized by motoneurones of rapidly contracting units. The slowest cat motoneurones would presumably mainly discharge below 20 Hz and they would but seldom be expected to reach a rate of 40 Hz during normal motor activities (e.g. Denny-Brown, 1929; Kernell, 1976).

Much to our own surprise, the present investigations showed that the long-term slowing effects on muscle were about equally marked for the fast as for the slow patterns of tonic stimulation (activity $\geqslant 50\%$ of total time). This equipotence of different patterns of tonic activity with respect to their long-term slowing effects was combined with differences in their long-term effects on various aspects of muscle force.

Part of the present findings have been published in a congress abstract (Kernell, Eerbeek, Donselaar & Verhey, 1981).

## METHODS

*Preparatory operations.* The experiments were performed on adult cats (2·3–4·7 kg). We wished to produce a controlled amount of activity in motor nerves and muscles while causing as little pain and discomfort as possible to the animals. Therefore, we rendered the hind limb in question insensitive by severing the left-side dorsal roots from L1 down to and including at least S2. Furthermore, we wished the cats to make as little spontaneous use as possible of the muscles studied in our experiments. This was achieved by the rhizotomy in combination with a left-side hemisection of the spinal cord at the level of L1. The hemispinalization and rhizotomy were performed in a separate initial operation under general anaesthesia (pentobarbitone, 40 mg/kg I.P.) About 2 weeks later, in a second operation under the same type of general anaesthesia, leads for stimulation and recording were installed and chronic stimulation (if any) was started.

None of the rhizotomized cats showed any behavioural signs of somatic sensitivity or pain in response to pinching of the skin of the left hind limb or electrical stimulation of the left common peroneal nerve. In pilot experiments, electromyographical (e.m.g.) recordings from pretibial flexors (peroneus longus, tibialis anterior) indicated that these muscles tended to be little used by animals that had been subjected to an ipsilateral rhizotomy plus hemispinalization (cf. Goldberger & Murray, 1974). The muscles were not, however, totally inaccessible to the central nervous system; a burst of activity could be produced in the pretibial flexors ipsilateral to the spinal operation by various kinds of manipulation of the animal. The operated cats kept the ankle joint of the experimental limb (always left side) almost permanently in extension. The animals remained lively and playful throughout the post-operative period of 10 weeks.

*Stimulation techniques.* Throughout the present series of experiments, chronic stimulation was delivered to the common peroneal nerve at a level about 20–25 mm proximal to the nerve entry into m. peroneus longus. The stimulating electrodes consisted of Teflon-insulated seven-stranded wires of stainless-steel (outer diameter 0·23 mm; Clark Electromedical Instruments) that were placed under the nerve and fixed to the muscle fascia by silk sutures. Just under the nerve, insulation was removed from a small area. At least three electrode wires were implanted at distances of about 2–3 mm. The electrode pair giving the lowest threshold for muscle activation was used for the chronic stimulation. The remaining wire served as a reserve in case of wire breakage.

Two further wires of the same kind were implanted into tibialis anterior for e.m.g. recordings of muscle activation. All implanted wires were led subcutaneously to the back of the sacrum where they emerged through a small opening of the skin. There, the leads terminated in a connector which could be plugged into various kinds of apparatus, including the portable mini-stimulator which was carried on a saddle at the back of the animal. The mini-stimulator was designed and built by J. R. van Leeuwen and J. M. Volkering at the electronics workshop of the Jan Swammerdam Institute (head, A. A. Meijer). Each stimulator was $19 \times 41 \times 72$ mm in size and it weighed about 90 g (including batteries). Stimulation was controlled via frequency-modulated and digitally encoded signals which were transmitted to the stimulator by means of infra-red light (light-emitting diodes placed on top of each animal cage). The frequency band for signal transmission was different for each one of the sender–receiver pairs used, and there was no interference between the stimulator equipments of different cats. The amplitude, duration and time of occurrence of the stimulus pulses were remotely controlled. Pulse duration was kept at 0·3 ms, and pulse amplitude could be varied in steps of 0·3 V up to a maximum of 9·6 V (maximum current 10 mA).

In all the present experiments, stimulation was given in tonic patterns, i.e. the imposed activity covered a large percentage of the total time per day. Stimulation was given either continuously (5 Hz for two cats of Tables 1–3) or in 1 s bursts interrupted by 1 s pauses (intra-burst rate 10, 20 or 40 Hz for Tables 1–3; three cats for each rate). For each animal, the chosen pattern of stimulation was delivered for 24 h per day during about 8 weeks ($56 \pm 3$ (S.D.) days for the cases of Tables 1–3).

*Control measurements during the course of chronic stimulation.* During a stimulation period, repeated measurements were made of the stimulus intensity needed for a maximal muscle activation. These measurements were performed by aid of e.m.g. recordings as well as by palpation of the contracting muscles. For the eleven stimulated cats of Tables 1–3, the threshold for maximum muscle activation was $1{\cdot}3 \pm 0{\cdot}4$ (S.D.) V at the beginning and $2{\cdot}4 \pm 1{\cdot}2$ V at the end of the 8 week period of treatment. During the periods of chronic activation, stimulus intensity was routinely set to at least 2 times (usually $\geqslant 3$ times) the threshold for maximum activation; in this way we hoped to ensure that all fibres of the peroneal muscles were treated in the same manner. In eight out of the eleven tabulated experiments with chronic activation, the animals had to be re-operated because of breakage of the wires used for stimulation. The point of interruption was then generally close to the exit of the wires through the skin of the back.

During periods of chronic stimulation, frequent external measurements were made of the time course of the twitch of the pretibial flexor muscles (ankle twitch). These measurements were obtained by aid of a force transducer that was pressed against the metacarpal bones of the foot while the common peroneal nerve was activated by single pulses of stimulation. The supporting base of the transducer was held against the tibia of the lower leg. With respect to the contraction time of the twitch, this kind of measurement gave highly reproducible results (see lower curves in Fig. 2*A*; cf. results of Murphy, 1977, as quoted on pp. 176–177 of Mortimer, 1981). The method was not, however, very useful for measurements of muscle force, because it was difficult to control the exact placement of the transducer and its support against the foot and lower leg.

*Final acute experiments.* After the end of the post-operative period of observation and stimulation, the cats were anaesthetized by pentobarbitone (initial doses 40 mg/kg I.P., supplementary doses I.V. as required). The knee was rigidly fixed, the peroneus longus muscle was gently freed from the surroundings, and its tendon was attached to an isometric force transducer. This transducer consisted of a sensitive displacement sensor (Hewlett–Packard, 7DCDT-050) coupled to the muscle via a stiff metal spring. Its resonance frequency was 625 Hz and its compliance was about 0·075 mm/kg. E.m.g. activity was recorded via two fine uninsulated wires thrust into the muscle belly. Muscle and nerves were covered by paraffin oil. Body temperature as well as the temperature of the oil pool were maintained at about 37–38 °C. During the final experiment, the mean arterial blood pressure was generally $\geqslant 120$ (always $\geqslant 100$) mmHg. The same measurements were performed for the peroneus longus muscle of both hind limbs. These measurements were: (1) determination of the force production of the muscle at different lengths. Thereafter, the muscle was permanently kept at the length for which the twitch had its maximum amplitude. Then recordings were made of (2) the 'initial' twitch, caused by single-pulse stimulation at 0·5 Hz (average of ten sweeps), (3) the force responses to double-pulse stimulation at intervals increasing in increments of 0·1 ms from an interval of 0·1 ms up to intervals giving an evident twitch 'summation', (4) the contractions produced by a series of constant-frequency bursts of 1 s at rates

ascending from 4 to 200 Hz (used for determination of sag behaviour and tension–frequency relation), and (5) contractions caused by a series of 40 Hz bursts (used for testing fatigue sensitivity). After the end of the physiological measurements, m. peroneus longus as well as, frequently, a number of other pretibial muscles were removed for later histochemical and histological analysis (Y. Donselaar, O. Eerbeek, D. Kernell & B. A. Verhey, in preparation).

*Experimental material and statistical analysis.* For the analysis of the present paper, we will refer to 5–10 Hz rates of chronic stimulation as 'slow' and 20–40 Hz rates as 'fast' (see Introduction and Tables 1–3). In Tables 1–3, effects of slow patterns are shown for all such cases together (Slow stimulated) as well as, separately, for those of the cats that were stimulated by slow patterns with the same timing as that of the fast patterns (Slow, 50 % time; activation during 50 % of total time).

Measurements of the contractile properties of m. peroneus longus were performed in twenty-nine cats in total, out of which nine served as normal controls, seven had been subjected to hemispinalization and dorsal rhizotomy but no chronic stimulation (experimental controls), and thirteen had been subjected to these same operations plus chronic stimulation (experimental stimulated). Two of the experimental control animals were not included into the final statistical analysis, because their post-operative survival times of 184 and 281 days respectively were considerably longer than those for the animals included in Tables 1–3 ($72 \pm 6$ (S.D.) days). With respect to the time course of the twitch, the muscles from the two long-survival cats were similar to those from other experimental controls (see group Experimental control in Table 1). With respect to the chronically stimulated animals, our own initial expectations were that the slowing effects on muscle contraction would be less marked for fast rates than for slower ones (see Introduction). As will be shown, these expectations were not confirmed by our results. Hence, we were particularly anxious to avoid producing any false bias by the inclusion of artifactually fast muscles within the group that had been treated by slow pulse rates (5 or 10 Hz). Two such doubtful animals were excluded from the final statistical analysis of data for peroneus longus (Tables 1–3). One of these cats had been treated by 10 Hz bursts (1 s on, 1 s off). In this case the final brief contraction time of the left-side peroneus longus (27 ms) might have been caused by an incomplete activation of this muscle during the period of chronic stimulation (partial nerve block?; data excluded from all illustrations). Such an interpretation was, in this case, suggested by our histochemical findings from different muscles of the common peroneal nerve: marked histochemical changes were observed within tibialis anterior and extensor digitorum longus whereas unusually slight alterations were noticeable in peroneus longus (observations in cryostat sections stained for myofibrillar ATPase; tonically stimulated muscles typically acquired a staining pattern similar to that of a homogeneous slow muscle; Y. Donselaar, O. Eerbeek, D. Kernell & B. A. Verhey, in preparation). The second set of somewhat doubtful results came from a case that had been treated by bursts of 5 Hz (1 s bursts alternating with 1 s pauses). During the 8 week stimulation period, the progressive slowing of the ankle twitch was similar to that of other cases (ankle-twitch data included into Fig. 2*A*; final external contraction time of 51 ms obtained 8 days before acute experiment). Due to faulty apparatus, however, this animal received no stimulation during at least 1 day before the acute experiment. In this case, the twitch contraction time of the left-side peroneus longus was 36 ms.

The significance of differences between muscles from different groups of animals was analysed by the *t* test for unrelated samples. Differences between left- and right-side muscles from the same group of animals were analysed by the Wilcoxon matched-pairs signed-ranks test. Unless otherwise indicated, mean values in text and legends are given $\pm$S.D.

## RESULTS

### *Time course of isometric twitch contraction*

Slow as well as fast rates of long-term tonic stimulation caused a very marked slowing of the muscle twitch. In Fig. 1, this is illustrated by recordings from four different animals. Contractions from the non-stimulated right side had a normal time course (Fig. 1, controls). On the stimulated side, the twitches had the same slow time course in the two animals treated by 40 Hz as in the two cats treated by 10 Hz (Fig. 1). A statistical analysis of all the present experiments confirmed that there were no

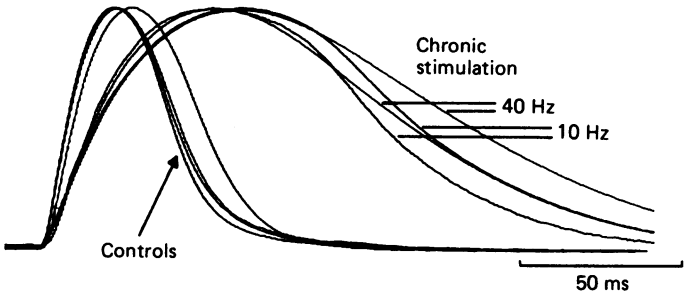


Fig. 1. Twitches recorded from eight different peroneus longus muscles of four different cats. Records labelled chronic stimulation were from left-side muscles subjected to long-term activation at pulse rates of 40 Hz (two cases) and 10 Hz (two cases) respectively. Records labelled controls were from the right-side muscles of the same animals. All records are averages of ten sweeps each. In order to facilitate comparisons of time course, the twitches are displayed at a common time scale but with normalized amplitudes.

TABLE 1. Changes in speed-related muscle properties after different patterns of chronic stimulation

| | Experimental control | | Fast stimulated | | Slow stimulated | Slow, 50% time |
|---|---|---|---|---|---|---|
| Contraction time (L/R) | 0·92±0·04 | * | 2·40±0·25 | N.s. | 2·44±0·45 | 2·47±0·47 |
| Half-relaxation time (L/R) | 0·99±0·26 | * | 3·12±0·53 | N.s. | 3·26±1·01 | 3·05±0·90 |
| Mid-force interval (L/R) | 0·93±0·06 | * | 3·30±0·77 | N.s. | 3·56±1·21 | 3·13±0·32 |
| Mid-force rate (Hz) | 36·1±6·6 | * | 12·0±1·9 | N.s. | 10·4±2·7 | 11·3±1·2 |
| Sag interval/contraction time | 1·11±0·23 | * | 1·97±0·25 | N.s. | 1·96±0·75 | 1·61±0·17 |
| *n* | 5 | | 6 | | 5 | 3 |

Means±S.D. for peroneus longus muscles from chronically treated cats. Experimental control: animals subjected to left-side dorsal rhizotomy and hemispinalization, but no chronic stimulation. Fast stimulated: animals subjected to 20 or 40 Hz patterns of chronic stimulation to left-side common peroneal nerve; stimulation 50% of total time; preceding operations as for experimental control. Slow stimulated: chronic stimulation, 5 or 10 Hz patterns; stimulation 50–100% of total time; otherwise as fast stimulated. Slow, 50% time: slow stimulated animals treated 50% of total time (10 Hz). Contraction time measured from start to peak of twitch. Half-relaxation time measured from peak to half-maximum force of twitch. Mid-force rate: the stimulus rate needed for eliciting a half-maximum force (*mean* force measured in non-fused contractions). Mid-force interval: reciprocal value of mid-force rate. Sag interval: briefest stimulus interval (ms) associated with a sag; same test rates as those used for tension–frequency relation (cf. Fig. 3*A*). L/R: ratio between values for left-side and right-side peroneus longus muscle (i.e. experimental/control). In this and succeeding Tables, the presence of a statistically significant difference between corresponding values of adjoining columns is indicated by * (at least $P < 0·05$), whereas N.s. indicates lack of significant difference (at least $P > 0·05$). Values for mid-force rate and sag interval/contraction time refer to left-side muscles.

differences between the fast and slow patterns with respect to their long-term effects on the time course of the twitch (Table 1; concerning the properties of two excluded animals, see Methods). On average, the twitch contraction time was more than doubled by the tonic stimulation and the half-relaxation time was increased by a factor of about three. The ratio between half-relaxation time and contraction time was significantly greater ($P < 0·01$) for the chronically treated muscles (1·06±0·14, $n = 11$) than for their contralateral controls (0·82±0·09, $n = 11$) or for muscles from

normal animals ($0{\cdot}76 \pm 0{\cdot}08$, $n = 9$). There were no statistically significant differences between normal muscles and the contralateral control muscles of experimental animals with respect to the time course of their twitches.

*Effects of double-pulse stimulation*

It is well known that a single-pulse activation of a muscle nerve may lead to a repetitive discharge in some motor axons and their muscle units (back response; Brown & Matthews, 1960). This complication may be avoided by stimulating the nerve by two pulses at a brief interval (generally about 0·6–1 ms). In the case of single-pulse-evoked back-firing, an appropriately spaced pair of stimuli will cause a weaker contraction than that found after a single pulse (Brown & Matthews, 1960). In the chronically treated animals of the present investigation, such an effect of double-pulse stimulation was seen in none of the experimental (left-side) muscles and in only three out of twelve investigated contralateral control muscles (one from experimental control, one from animal stimulated by fast rate and one from animal stimulated by slow rate). In the three positive cases, the decrease in contractile force was 5, 9 and 19 % respectively whereas the associated decrease in contraction time was in all cases unexpectedly small (about 1 ms). All the present recordings were obtained with the muscle at its optimum length for a twitch contraction (see Methods); at such lengths, the back response is known to be very slight or absent in normal muscles (Buller & Proske, 1978).

Besides for the blocking of possible back responses, we also used double-pulse stimulation for determining the briefest interval that would give rise to an increase of contractile force. This neuromuscular refractory period was found to be significantly longer ($P < 0{\cdot}01$) in muscles subjected to chronic stimulation ($1{\cdot}68 \pm 0{\cdot}23$ ms) than in contralateral control muscles ($1{\cdot}06 \pm 0{\cdot}11$ ms, $n = 9$). There were no significant differences ($P > 0{\cdot}5$) between muscles treated by fast and slow rates with respect to their neuromuscular refractory period (means $1{\cdot}72 \pm 0{\cdot}12$ and $1{\cdot}64 \pm 0{\cdot}33$ ms respectively).

*Time course of development of changes in twitch contraction time*

The 8-week effects of chronic stimulation on twitch speed (Fig. 1 and Table 1) might conceivably have been caused by influences that had been developing along different time courses for different patterns of activation. Hence, it was important to try to ascertain how the contractile speed of the treated muscles changed *during* the period of chronic stimulation. In most of the experimental animals of the present study, the alterations in contractile properties were assessed by aid of frequent external measurements of the ankle twitch, which was evoked by single-pulse stimulation of the common peroneal nerve (see Methods). Samples of recorded ankle twitches are illustrated in Fig. 2*B* and *C*, and average changes in ankle-twitch contraction time are displayed in the diagram of Fig. 2*A*. No differences were found between the time courses of development of the effects produced by the slow (circles) and fast (triangles) patterns of chronic stimulation respectively. The final contraction time measured externally ($54 \pm 5$ ms) was very similar to the corresponding value obtained from the peroneus longus muscle in subsequent acute experiments of the same animals ($53 \pm 4$ ms). When obtained from the non-stimulated muscles of experimental control

animals, the externally measured contraction times were stationary with a variation of about $\leqslant 3$ ms around the mean (Fig. 2*A*, lower curves).

*Tension–frequency relation*

The diagram of Fig. 3*A* shows the relationship between peak contractile force and stimulus frequency for the muscles of Fig. 1. The values for the non-treated muscles (contralateral controls) were normal whereas those for the chronically activated ones

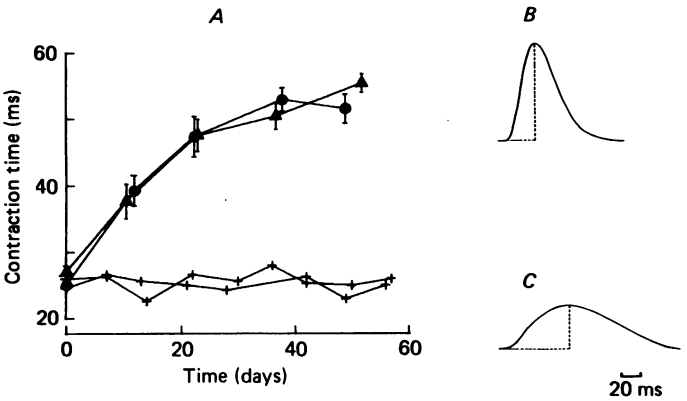


Fig. 2. *A*, upper curves: plot of mean values for externally recorded contraction time of ankle twitches (ms) *versus* time after start of chronic stimulation (days) for animals treated with fast (triangles; data from six cats) and slow (circles; data from four cats) pulse rates respectively. Consecutive values joined by straight lines. Vertical bars give $\pm$S.E. of mean. All stimulated animals of this illustration were treated by the same time-amount of chronic activation per day (50% of total time). For the various individual animals, the final contraction times were 51, 60, 48, 51, 60, 60, 54, 56, 48 and 52 ms after treatment with pulse rates of 5 (one case), 10 (three cases), 20 (three cases) and 40 Hz (three cases) respectively. Lower curves: as for upper curves, but from two experimental control animals (no chronic stimulation). In these cases, the abscissa gives number of days after the installation of stimulation and recording wires in the left hind limb. *B* and *C*, samples of ankle twitches obtained from same animal before onset of chronic stimulation (*B*) and after 54 days of treatment with 20 Hz pattern (*C*). Isometric recordings, arbitrary force scale, common time scale. Twitches traced from photographs of representative single sweeps (repeated sweeps gave practically identical time course of consecutive twitches during a given recording session). Base line and time to twitch peak indicated by interrupted lines.

were shifted toward slow rates of test activation. For the physiological gradation of muscle force, it is of particular importance to know over which frequency range a change in stimulus rate produces a marked change in force. In the analysis of Table 1, the position of the steep region of the tension–frequency curve is indicated by values of mid-force rate (or interval), i.e. the stimulus rate (or pulse interval) producing a half-maximum force. In non-fused contractions, the measurements referred to *mean* force. Whether treated by fast or slow patterns, the mid-force rate of chronically activated muscles had shifted from the normal values of around 36 Hz to typically slow frequencies of about 10–12 Hz (Table 1).

*Sag behaviour*

The recordings of Fig. 3*B* and *C* show partly fused contractions caused by test bursts at stimulus intervals of about 1·5 times the twitch contraction time of the respective muscles. In the control muscle (*B*) the force rises to an early maximum whereafter it sags to a lower plateau. In the chronically stimulated muscle (*C*) no such sag is seen, but the force shows a continuous rise throughout the test burst. In the present chronically treated muscles, no sag was seen at rates exceeding 12 Hz,

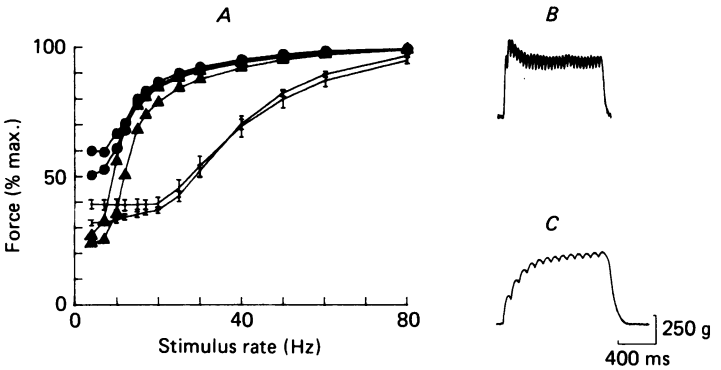


Fig. 3. *A*, tension–frequency relations of muscles subjected to different kinds of long-term treatment. Contractile peak force (percentage of maximum peak force) plotted *versus* rate of test stimulation (Hz) for the same chronically activated muscles as in Fig. 1. Measurements obtained from isometric contractions evoked by series of 1 s constant-frequency bursts at rates ascending from 4 to 200 Hz (data for rates above 80 Hz not shown). Triangles and circles indicate data from muscles subjected to long-term treatment with 40 and 10 Hz pulse rates respectively. At higher rates, values from different muscles overlap (same test rates used for each muscle). Average tension–frequency curves shown for two groups of muscles that had not been subjected to chronic stimulation (curves with vertical bars indicating ±S.E. of mean). One of these latter curves was obtained from data for the four contralateral control muscles of Fig. 1 and the other curve came from data for nine muscles from normal animals (lower-most control curve at low test rates). *B* and *C*, force records from control (*B*, right side) and experimental (*C*, left side) muscles of cat subjected to chronic stimulation at a pulse rate of 40 Hz. The illustrated contractions were evoked by test bursts of 30 (*B*) and 12 (*C*) Hz respectively. For each muscle, the pulse interval of the illustrated test burst was about 1·5 times the respective twitch contraction time. Note presence of sag in *B* but not in *C*.

whereas the normal muscles of the present study still showed a sag at test rates of $\geqslant$ 30 Hz. In relation to twitch contraction time, the minimum stimulus interval producing a sag was almost twice as long for chronically stimulated muscles as for those from normal animals (Table 1). No significant differences were found between the sag behaviours of muscles treated with the fast and slow patterns of chronic stimulation respectively (Table 1).

*Force of tetanic and twitch contraction*

All the chronically activated muscles were weaker than their contralateral controls (cf. Tables 2 and 3). This left–right difference was not caused by a compensatory

TABLE 2. Changes in force-related muscle properties after different patterns of chronic stimulation to own muscle nerve

| | Normal | | Experimental control | | Fast stimulated | | Slow stimulated | Slow, 50% time |
|---|---|---|---|---|---|---|---|---|
| Weight-corrected tetanic tension | 746±209 | N.s. | 652±132 | * | 320±79 | * | 219±43 | 212±56 |
| Twitch:tetanus (%) | 37±3·1 | N.s. | 35±4·3 | * | 26±3·2 | * | 48±8·5 | 47±11·5 |
| Fatigue index (%) | 56±6·4 | * | 45±5·5 | * | 91±3·2 | N.s. | 91±2·3 | 93±2·1 |
| Tetanic endurance (%) | 96±3·1 | N.s. | 93±7·7 | N.s. | 99±0·2 | N.s. | 99±0·2 | 99±0·2 |
| Post-tetanic twitch potentiation (%) | 120±9·3 | N.s. | 121±15·3 | N.s. | 120±4·8 | * | 109±2·9 | 111±2·0 |
| *n* | 9 | | 5 | | 6 | | 5 | 3 |

Means±S.D. for left-side (experimental) peroneus longus muscles. Weight-corrected tetanic tension: the maximum tetanic force (g) divided by animal weight (kg; see legend of Table 3 for control values of force and weight). Twitch:tetanus, ratio between initial twitch and maximum tetanic force. Fatigue index calculated from 40 Hz fatigue test (see Fig. 4) by taking the reciprocal ratio between the maximum obtained peak force and the peak force 2 min later. Tetanic endurance index calculated by taking the ratio between the maximum attainable mean force and the maximum attainable peak force, as elicited by 1 s test bursts at rates from 4 to 200 Hz (cf. Fig. 3*A*); mean force then measured during latter half of the 1 s test stimuli. Post-tetanic twitch potentiation, the ratio between the first post-tetanic twitch and the initial twitch (see text for details). Other abbreviations as in Table 1. Listed number of observations (*n*) valid for all data except for fatigue index; then the *n* values were 4 for normal as well as for experimental control animals.

TABLE 3. Force-related muscle properties after different patterns of contralateral chronic stimulation

| | Normal | | Experimental control | | Fast stimulated | | Slow stimulated | Slow, 50% time |
|---|---|---|---|---|---|---|---|---|
| Weight-corrected tetanic tension | 746±209 | N.s. | 654±71 | N.s. | 730±101 | N.s. | 737±80 | 704±71 |
| Twitch:tetanus (%) | 37±3·1 | N.s. | 35±4·5 | N.s. | 39±4·0 | N.s. | 38±4·1 | 39±5·7 |
| Fatigue index (%) | 56±6·4 | N.s. | 54±0·8 | N.s. | 49±11·0 | N.s. | 47±5·7 | 46±7·9 |
| Tetanic endurance (%) | 96±3·1 | N.s. | 98±0·3 | * | 80±13·3 | N.s. | 91±6·9 | 90±8·5 |
| Post-tetanic twitch potentiation (%) | 120±9·3 | N.s. | 128±7·0 | * | 108±8·8 | N.s. | 117±12·7 | 113±1·5 |
| *n* | 9 | | 5 | | 6 | | 5 | 3 |

Data as in Table 2, but for right-side peroneus longus muscles of the same animals. Normal values same as in Table 2. For all the twenty-five muscles of this Table, the maximum tetanic force was 2·2±0·7 kg, and the cats weighed 3·0±0·6 kg.

strengthening of the muscles contralateral to the side of rhizotomy and long-term stimulation: in relation to body weight, the right-side control muscles produced practically the same maximum tetanic force as that found in peroneus muscles from normal animals (Table 3, weight-corrected tetanic tension). The weakening of the stimulated muscles was significantly greater for cats treated with the slow patterns than for those treated with fast patterns (Table 2). Furthermore, a marked difference was found between the different patterns of chronic stimulation with respect to the effects on twitch size. The twitch : tetanus ratio was significantly *larger* than normal after treatment with slow patterns, and it was significantly *smaller* than normal after treatment with fast patterns (Table 2, in both cases, $P < 0{\cdot}01$).

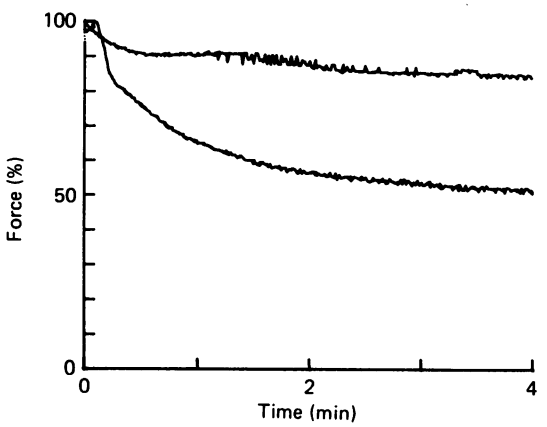


Fig. 4. Diagram showing the peak contractile forces evoked during tests for fatigue resistance in muscle subjected to chronic stimulation at 40 Hz (upper curve) and in its contralateral control (lower curve). During the fatigue test, each muscle was activated by a series of 40 Hz test bursts of 0·33 s, repeated once a second. For each muscle, force is given as a percentage of the maximum force generated during the same fatigue test.

*Endurance*

Contractile endurance was tested by a method similar to that introduced by Burke, Levine, Tsairis & Zajac (1973) for studies of motor units: the decline in force was measured during a series of 0·33 s test bursts at 40 Hz, recurring once a second for several minutes. The diagram of Fig. 4 shows the peak forces produced by such a sequence of stimulus bursts in two peroneal muscles from the same cat. Compared to the contralateral control muscle (lower curve), the chronically stimulated muscle clearly showed a much better resistance to contractile fatigue (upper curve). For quantitative comparisons, we used measurements such as those of Fig. 4 for calculating a fatigue index similar to the one introduced by Burke *et al.* (1973; cf. also Kernell, Eerbeek & Verhey, 1983*a*). This index gives a measure of the relative amount of force production remaining after 2 min of repeated burst stimulation. There were no significant differences in mean fatigue index between the muscles treated with the fast and slow patterns of chronic stimulation respectively (Table 2). Muscles contralateral to the side of chronic activation had normal values of fatigue index (Table 3, $P > 0{\cdot}05$ for comparisons of stimulated groups to normal).

Besides in prolonged fatigue tests such as those of Fig. 4, differences in contractile endurance were also evident when the muscles were tested by short-lasting stimuli at the very high rates needed for a near-maximum contraction. In response to such tetani of 1 s duration, the force was typically very well maintained in muscles that had been subjected to chronic stimulation (Fig. 5*B*, left) whereas a small but evident decline was noticeable in muscles from normal animals or from animals that had become rhizotomized and hemispinalized but not chronically stimulated (Fig. 5*A*,

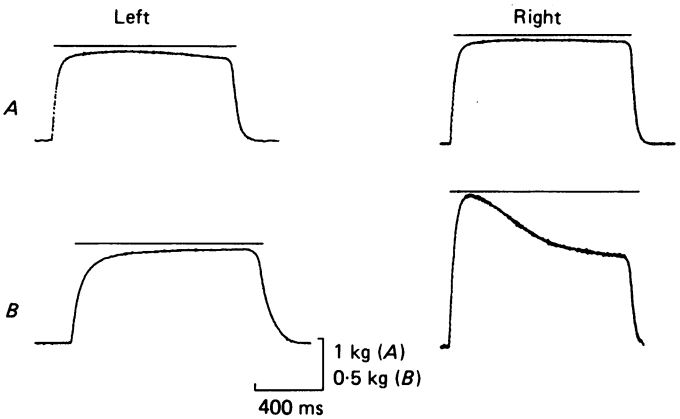


Fig. 5. Isometric contractions evoked by 100 Hz test bursts in left- and right-side muscles of experimental control animal (*A*, no chronic stimulation) and in cat treated by long-term left-side stimulation at a pulse rate of 40 Hz (*B*). Horizontal reference lines drawn above each record.

left). Unexpectedly, however, in the chronically activated cats, the maintenance of tetanic force was not only higher than normal on the experimental side (left), but it was also often much *lower* than normal on the contralateral side (Fig. 5*B*, right). In Tables 2 and 3 (Tetanic endurance) these kinds of effects have been subjected to a statistical analysis. One consequence of the decline in tension during high-rate stimulation (e.g. Fig. 5*B*, right) was that the maximum attainable maintained tetanic force of such a muscle (i.e. average force during the latter half of a 1 s constant-frequency burst) became much lower than its maximum attainable peak force. For each muscle, an index of tetanic endurance was calculated by taking the ratio between the maximum maintained force and the maximum peak force that the muscle would attain in response to the series of tetani used for determining the tension–frequency relation (cf. Fig. 3*A*). Such index values are shown in Tables 2–3 (tetanic endurance) for groups of differently treated muscles. The index of tetanic endurance was significantly *greater* than normal ($P < 0{\cdot}01$) for the eleven muscles that had been subjected to chronic stimulation, and it was significantly *lower* than normal ($P < 0{\cdot}02$) for the eleven muscles contralateral to those that had received the long-term stimulation. There was no statistically significant difference between the values for muscles contralateral to those treated with fast and slow patterns respectively (Table 3).

During 100 Hz test tetani of 1 s, a somewhat variable relationship was found

between the evoked force and the associated compound electromyographic spikes. In chronically activated muscles, the force as well as the e.m.g. amplitude was generally well maintained during the test tetani. In some other muscles, however, a rather marked decline in e.m.g. would occur without a corrresponding drop in force, and vice versa.

*Post-tetanic twitch potentiation*

The twitches used for the calculations of the twitch:tetanus ratios of Tables 2–3 were the initial twitches that had not been intentionally subjected to potentiating activity. The sensitivity of the various muscles to post-tetanic potentiation was studied by measuring the effect on twitch size of the whole of a standardized series of 1 s bursts at rates ascending from 4 to 200 Hz (sixteen bursts, one rate per burst, burst interval 6 s, test twitch at 2 s after final burst). In Table 2 the amount of potentiation is expressed as the ratio between the amplitude of the first post-tetanic twitch and the 'initial' one. The potentiation was lower than normal in muscles subjected to slow rates of long-term stimulation ($P < 0{\cdot}05$). In the muscles treated with fast patterns, the sensitivity to potentiation remained normal (Table 2). In the latter animals, however, the muscles contralateral to the side of chronic stimulation showed a subnormal amount of potentiation (Table 3; $P < 0{\cdot}05$ for post-tetanic potentiation of normal *versus* fast stimulated).

*Non-stimulated muscles of operated animals (experimental controls)*

These animals had been subjected to a left-side dorsal rhizotomy and hemispinalization at about 10 weeks before the final acute experiment ($72 \pm 6$ days, see Methods). With respect to most of their properties, the experimental (left-side) muscles of these non-stimulated animals were not significantly different from the corresponding muscles of normal cats (true for contraction time, half-relaxation time, mid-force stimulus rate, maximum rate associated with a sag, maximum tetanic force, non-potentiated twitch:tetanus ratio, tetanic endurance index and post-tetanic twitch potentiation; see Tables 1 and 2). The neuromuscular refractory period of left-side muscles from experimental control animals ($0{\cdot}97 \pm 0{\cdot}15$ ms) was not longer than that of the respective contralateral control muscles ($1{\cdot}13 \pm 0{\cdot}06$ ms, $n = 3$). Thus, with respect to these various properties, the changes found in stimulated muscles were indeed caused by the long-term activation and not by the preceding operations of the dorsal roots and spinal cord. This was also clearly the case with respect to fatigue resistance, tested as in Fig. 4: the fatigue index was significantly lower than normal in experimental control muscles whereas it was higher than normal in the muscles subjected to chronic stimulation (Table 2).

## DISCUSSION

The present findings have shown that, contrary to our own initial expectations, the fast and slow patterns of long-term tonic stimulation led to about the same degree of slowing of contractile properties (Figs. 1–3, Table 1). Irrespectively of the pulse rate of the chronic stimulation, the treated muscles acquired properties that were even slower than those of normal slow motor units of peroneus longus (Table 4; cf. Kernell

*et al.* 1983*a*, *b*). This was true for the time course of the twitch as well as for the tension–frequency relation. In the chronically stimulated muscles, about 90 % of the maximum tension would be produced already by a stimulus rate of 30 Hz (Fig. 3*A*, circles, triangles). In such muscles, no marked variation of force would be produced by changes in firing rate at test frequencies exceeding 30 Hz. Thus, muscles that had been chronically activated by the fast rate of 40 Hz (Fig. 3*A*, triangles) had clearly *not* become adapted to a rate modulation of force by discharge rates similar to those of the long-term treatment. In hind-limb muscles of the cat, the normally occurring speed match between motoneurones and their muscle units (see Introduction) is apparently not simply caused by an 'automatic' adaptation of muscle speed to the prevailing rates of muscle activation, irrespectively of the amount of this activity.

TABLE 4. Comparisons between chronically stimulated muscles and normal muscles and motor units with respect to their speed-related contractile properties

| | Tonically stimulated mm. | | Slow-twitch units | | Fast-twitch units | | Normal mm. |
|---|---|---|---|---|---|---|---|
| Contraction time (ms) | 54·4 ± 8·2 | * | 29·6 ± 5·8 | * | 18·3 ± 2·7 | * | 22·7 ± 1·9 |
| Half-relaxation time (ms) | 58·5 ± 15·9 | * | 39·8 ± 9·1 | * | 19·2 ± 3·6 | N.s. | 17·3 ± 2·0 |
| Mid-force rate (Hz) | 11·3 ± 2·3 | * | 19·4 ± 4·7 | * | 39·8 ± 7·4 | N.s. | 35·8 ± 3·8 |
| *n* | 11 | | 15 | | 62 | | 9 |

Means ± S.D. Tonically stimulated mm.: muscles subjected to tonic long-term stimulation (same animals as fast stimulated plus slow stimulated of Table 1). The slow-twitch and fast-twitch units were all from normal muscles (Kernell *et al.* 1983*b*), and units failing to show a sag when activated at pulse invervals close to 1·25 × contraction time were classified as slow (cf. Burke *et al.* 1973). Other abbreviations as in Table 1. All data from m. peroneus longus.

For a further understanding of these matching problems, it becomes essential to know how the fast motoneurones themselves might become affected by long-lasting tonic activation (work in progress; for long-term stimulation effects on slow motoneurones, see Czéh, Gallego, Kudo & Kuno, 1978).

Fast as well as slow patterns of chronic stimulation changed the sag behaviour of peroneus longus toward that of slow hind-limb muscles and motor units (Fig. 3*C*; cf. Cooper & Eccles, 1930; Burke *et al.* 1973; Burke, 1981; Kernell *et al.* 1983*a*). The sag behaviour is of considerable methodological interest, because this reaction has been shown to be of use as an aid for the fast/slow classification of hind-limb motor units (Burke *et al.* 1973; Burke, 1981). For such comparisons, the test rate of activation must be specified (e.g. in relation to contraction time; cf. Table 1, sag interval/contraction time), because all types of hind-limb motor units may display a sag if activation rate is low enough (Burke *et al.* 1973).

Physiological evidence for a slowing effect of fast rates of long-term stimulation has recently been reported by Hudlická *et al.* (1982; 40 Hz), who found a prolongation of twitch contraction time by about 40 % but no consistent changes in half-relaxation time. The effects found in the present experiments were much more pronounced; on average, twitch contraction time increased by 140 % and half-relaxation time by 212 % (Fig. 1, Table 1). Such quantitative differences between our results and those

of Hudlická *et al.* (1982) are probably caused by differences in the amount of fast-rate stimulation given per day in the respective series of experiments: we stimulated during 50 % of the total time whereas Hudlická *et al.* (1982) delivered their fast-rate stimulation during only about 8 % of each 24 h day. Still lower amounts of fast-rate stimulation have been reported to produce very little (17 % change, Goldring *et al.* 1981) or no (Brown *et al.* 1976) prolongation of the twitch of a fast hind-limb muscle.

In the experiments of Lømo *et al.* (1980), 100 Hz stimulation made the denervated soleus muscle faster, 10 Hz could make it slower, and no marked changes of contractile speed were found in the complete absence of long-term activation. The differences in frequency dependence between these results and those of the present experiments might, to a considerable extent, have been caused by differences between the utilized species of muscle (rat denervated slow muscle *versus* cat innervated fast muscle). It is interesting to note that, in the experiments of Goldring *et al.* (1981), 50 Hz stimulation caused a speeding up of the cat soleus but a slight slowing down of the gastrocnemius.

The present results indicate that the slowing down of a fast muscle after cross-innervation with a slow nerve (Buller, Eccles & Eccles, 1960) is largely caused by the fact that the slow motoneurones normally tend to be comparatively easily recruited (Henneman & Mendell, 1981) and hence they will be more persistently active than fast motoneurones (cf. Denny-Brown, 1929). Monster, Chan & O'Connor (1978) actually observed that, among human muscles, there was a significant positive correlation between the percentage of type I fibres (slow) and the percentage of time taken up by e.m.g.-recorded activity during an 8 h working day.

In spite of the fact that the present fast and slow patterns were equipotent in their effects on isometric speed (Table 1), their long-term influences differed with respect to a number of aspects of muscle force (Table 2). The differential effects of fast and slow rates on the twitch:tetanus ratio were qualitatively similar to those recently reported by Lømo *et al.* (1980) and by Hudlická *et al.* (1982). In normal peroneus longus muscles, the non-potentiated twitch:tetanus ratio is about the same for fast and slow motor units (Kernell *et al.* 1983*a*). Thus, the marked changes of twitch:tetanus ratio after chronic stimulation (Table 2) do *not* represent adaptations toward the characteristics of normal fast and slow units respectively. The changed twitch:tetanus ratios after tonic treatment might perhaps represent transitional states as has, in fact, been found to be the case for the changes in twitch:tetanus ratio after the cross-innervation of a fast muscle with a slow nerve and vice versa (rat, Close, 1969).

Differences in twitch:tetanus ratio might, for instance, reflect variations in the completeness of single-pulse activation of the contractile elements (cf. Close, 1972). The completeness of contractile activation after a single pulse would, of course, be of importance for the degree to which twitch amplitude might become increased by a preceding tetanus (cf. Close, 1972). Hence, it is not very surprising that the muscles with a high twitch:tetanus ratio showed a comparatively small amount of post-tetanic potentiation (Table 2; cf. Lømo *et al.* 1980).

In the present series of experiments, all muscles became weaker than normal after 8 weeks of tonic stimulation. Such a decline in maximum force has also been found to occur in other studies of the effects of similarly great amounts of long-term stimulation (Salmons & Vrbová, 1969; Salmons & Sréter, 1976). Within mixed

hind-limb muscles of the cat, the muscle fibres of slow units have a smaller mean diameter than those of fast units (Burke & Tsairis, 1973; McDonagh, Binder, Reinking & Stuart, 1980; Burke, 1981). Furthermore, indirect evidence suggests that, within mixed muscles, slow fibres might have a smaller specific force than that of fast fibres (Burke & Tsairis, 1973; McDonagh *et al.* 1980; Burke, 1981). If such differences between fast and slow motor units were activity dependent, then a considerable weakening would actually be expected to occur more or less in parallel with the slowing effects of long-term stimulation. It should be stressed that, in the present context, a decline in maximum tetanic force is not *a priori* to be regarded as a pathological phenomenon, but it might well represent the normal reaction of muscle fibres to large amounts of activity (cf. Salmons & Henriksson, 1981).

It is well known that the resistance to contractile fatigue may be considerably enhanced by low-rate tonic activation (Peckham *et al.* 1973; Pette, Ramirez, Müller, Simon, Exner & Hildebrand, 1975; Salmons & Sréter, 1976). When the present project was started, it was still uncertain whether such effects might be produced by fast pulse rates (e.g. 40 Hz) of chronic stimulation as well (cf. Brown *et al.* 1976). That this is indeed the case is shown by the present results (Fig. 4 and Table 2, fatigue index) as well as by those recently published by Hudlická and co-workers (1980, 1982; see also Lømo *et al.* 1980).

One of the most unexpected findings of the present study was the observation that certain effects of chronic stimulation appeared *contralateral* to the side of treatment (Fig. 5 and Table 3). The contralateral effects on post-tetanic twitch potentiation are likely to have resulted from the tetanic fatigue developing in these muscles during test stimulation at high rates (Fig. 5*B*, right); the post-tetanic twitch was evoked 2 s after such a tetanus. Further experiments are needed for a clear identification of the cause and nature of long-term stimulation-evoked changes in contralateral tetanic endurance. Whatever the explanation, however, the results of Fig. 5 and Table 3 stress that one should never *a priori* assume that one-sided manipulations of the motor system leave contralateral muscles and motoneurones in their normal state (for other examples of contralateral effects, see Guth & Yellin, 1971; Srihari, Seedorf & Pette, 1981).

Buller & Lewis (1965) have shown that, under normal circumstances, the neuromuscular refractory period is longer for slow (soleus, $1{\cdot}61 \pm 0{\cdot}14$ ms) than for fast (flexor hallucis longus, $1{\cdot}03 \pm 0{\cdot}17$ ms) muscles of the cat hind limb. These normal values are very similar to those obtained in the present study from chronically stimulated ($1{\cdot}68 \pm 0{\cdot}23$ ms) and contralateral control muscles ($1{\cdot}06 \pm 0{\cdot}11$ ms) respectively. For slow rates of long-term treatment, a similar prolongation of refractory period has previously been reported by Salmons & Vrbová (1969; increase by 78%). The present findings show that, for tonic patterns of activation, these long-term effects were not dependent on the pulse rate of chronic stimulation. The stimulation-evoked changes in neuromuscular refractory period suggest the presence of alterations in the properties of muscular and/or neuronal membrane components.

The intracellular changes responsible for the long-term contractile effects of different patterns of stimulation (Tables 1 and 2) will be further discussed in relation to our histochemical and morphological findings from the respective muscles (Y. Donselaar, O. Eerbeek, D. Kernell & B. A. Verhey, in preparation).

The present project was partly supported by the Foundation for Medical Research FUNGO which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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