J. Physiol. (1978), **285**, *pp.* 59–69
With 5 text-figures
Printed in Great Britain

FURTHER OBSERVATIONS ON BACK-FIRING IN THE MOTOR NERVE FIBRES OF A MUSCLE DURING TWITCH CONTRACTIONS

BY A. J. BULLER* AND U. PROSKE

From the Department of Physiology, Monash University, Clayton, Victoria, Australia 3168

(*Received 23 May 1978*)

SUMMARY

1. The tension developed by a muscle in response to a single supramaximal nerve volley is often the sum of two contractions, the second resulting from re-excitation of some nerve terminals during the first contraction. Re-excitation or back-firing can be prevented if the muscle nerve is stimulated twice, with the second volley arriving in the muscle at a time when muscle fibres are still refractory following the first volley (Brown & Matthews, 1960).

2. Back-firing has been studied here in the medial gastrocnemius muscle of the spinal cat and responses have been recorded, either representing the summed activity of the muscle's motor supply or discharges of single functional motor axons.

3. When stimulating at a distance from the muscle, the second of two shocks is effective in suppressing back-firing over a narrower range of stimulus intervals than when stimulating close to the muscle.

4. The amount of back-firing can be reduced by stretching the muscle. At a length corresponding to the optimum for a twitch little if any back-firing remains.

5. Measurements of threshold to electrical stimulation of single functional motor axons suggests that low threshold axons are more likely to show back-firing. In the majority of cases, most or all of the motor units in the muscle have to be active before back-firing can be observed.

INTRODUCTION

When a single maximal nerve volley is applied to the motor nerve of the gastrocnemius muscle of the cat, the tension produced can frequently be shown to be the sum of two contractions (Brown & Matthews, 1960). The second contraction results from re-excitation of some nerve terminals within the muscle, presumably by electrical activity associated with the first contraction. Re-excitation or back-firing can be prevented by stimulating the nerve twice, at a sufficiently brief interval for the second of the two evoked nerve volleys to arrive in the muscle at a time when muscle fibres are still refractory following the first volley. Whether the second volley prevents re-excitation of nerve terminals by making the nerve endings refractory during the critical period when re-excitation is possible or whether actual collision

* Visiting Nuffield Professor. Present adress: Faculty of Medicine, University of Bristol, Bristol BS8 1TD, England.

occurs between action potentials from re-excited terminals and the second nerve volley remains uncertain.

In experiments concerned with the isolation of single, functional motor axons in filaments of ventral root (Proske & Waite, 1976), it was noticed that in response to stimulation of the muscle nerve, not one but often two, or sometimes even three, antidromic action potentials were recorded in the filament. Use of the double shock test showed that the second and third action potentials represented back-firing. Since not all axons showed the effect, a systematic search was begun, making measurements of electrical threshold of axons, their conduction velocity and tension in the muscle. While unable to decide incontrovertibly between each of several possible mechanisms, our data provide further support for the idea that the gross electrical activity in muscle fibres is responsible for back-firing of nerve terminals. A preliminary account of this work has already appeared (Buller & Proske, 1973).

METHODS

Initial anaesthesia in cats weighing between 3 and 4·7 kg was induced by a mixture of ethyl chloride and ether. The blood supply to the head was stopped by tying the carotid arteries and clamping the vertebral arteries. The spinal cord was sectioned via the dorsal atlanto-occipital membrane and artificial respiration commenced. At this stage the anaesthetic was discontinued. The level of carbon dioxide in the expired air was constantly monitored throughout the experiment and when necessary the stroke volume of the pump adjusted.

A laminectomy was performed to expose the dorsal and ventral roots L7 and S1. These were cut at their entry into the spinal cord and the ventral root was split into fine filaments for the isolation of functionally single motor axons.

The medial gastrocnemius muscle and its tendon were dissected free of surrounding tissue, keeping intact the muscle blood supply. The muscle nerve was separated from the adjacent nerve trunk over a distance of 22 mm or more. All other nerves in the back of the leg were cut. In four animals, before section of the muscle's tendon, markers were inserted to locate the maximum physiological length corresponding to full dorsiflexion of the ankle. A portion of bone was left attached to the tendon and a tension transducer (Statham G1-80-350) was hooked to the tendon at the point of attachment. The tibia was rigidly held at the knee and ankle by means of sharp pins forced into the bone. Skin flaps surrounding the dissected muscle and spinal cord were attached to metal supports to retain pools of paraffin. The temperature of the aluminium table supporting the animal was thermostatically controlled and by this means the temperature in the paraffin pools could be limited to the range 33–36 °C.

The muscle nerve was stimulated with bipolar platinum electrodes while similar electrodes were used to record the antidromic volley in the ventral root. The ventral root was then subdivided, and recording restricted to those portions containing back-firing motor axons. Eventually a single functional axon showing back-firing could be isolated. The conduction velocity of the axon was calculated from the latency of the electrically evoked action potential and from the distance between stimulating and recording electrodes, measured at the end of the experiment. The electrical threshold of the axon and that of its back-response as determined by gradation of stimulus strength were expressed in terms of the stimulus strength necessary to elicit a maximal muscle twitch. The thresholds were also related to the conduction velocity of the axon and for the back-response to the degree of muscle stretch.

RESULTS

In all of the eleven cats used in these experiments, some back-firing of the motor nerve fibres could be detected. In several animals the proportion of tension contributed by the back-firing axons was barely measurable while in others it could account for up to 31 % of the observed tension. In some preparations with a large

back-response, motor axons were seen to be re-excited twice. In nine experiments a total of fifty functionally single motor axons showing a back-response was isolated as well as a further twenty-two motor axons which did not back-fire.

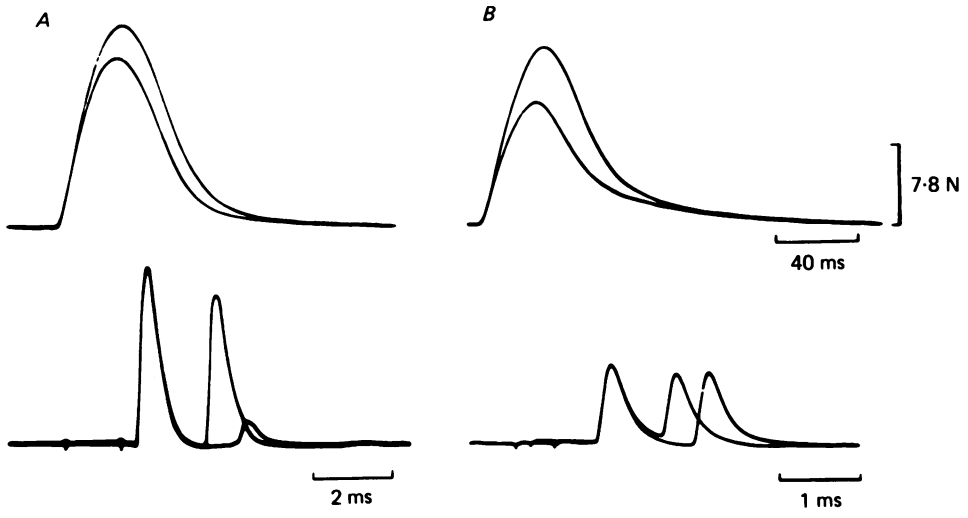

Fig. 1. Examples of back-responses in two preparations, *A* and *B*. Tension is shown in the upper traces and action potentials recorded in the ventral root, below. In *A* (with back-firing representing 15 % of twitch tension) action potentials have been recorded in the whole ventral root while in *B* (back-firing 31 % of twitch tension) the potentials were recorded in a single, functional motor axon. Each set of traces represents two superimposed recordings, the first the response to a single maximal shock, the second the response of two shocks at a sufficiently brief interval to prevent back-firing in the muscle. In *A* the action potential in response to the double shock has two large elevations; the first of the two potentials accurately superimposes on the response to a single shock, the second is the response to the second stimulus. The small potential below it represents the summed activity in back-firing motor axons after the single shock. In *B* the first action potential represents the direct response, then follows the potential to the second of the pair of stimuli and finally, with the longest latency, the back-fired potential.

Examples of preparations with large back responses are shown in Fig. 1 (tension above, nerve action potentials below). In *A* is shown the volley recorded in the whole ventral root and in *B* the discharge in a single, functional motor axon. Pairs of recordings have been superimposed, the first of each pair being the response to a single stimulus, the second the response to two shocks at a sufficiently brief interval to prevent back-firing in the muscle. In *A*, following a double shock the action potential has two large elevations, the first of which accurately superimposes on the response to a single shock, the second representing the response to the second stimulus. The smaller potential below it is the back response in motor axons following the single shock.

It is of interest to note that the tension developed when back-firing occurs is not only greater but its peak value is reached later than for the non-potentiated twitch. This is consistent with the interpretation that the contraction represents two summating twitches. Medial gastrocnemius is composed of several fibre types (Burke, Levine, Tsiaris & Zajac, 1973) and the fast and slow components of the relaxation

phase of the twitch reflect the different contraction times. It may be noted that only the fast component of the relaxation phase of the twitch is displaced as a result of back-firing, suggesting that motor units with long relaxation times may not be back-firing.

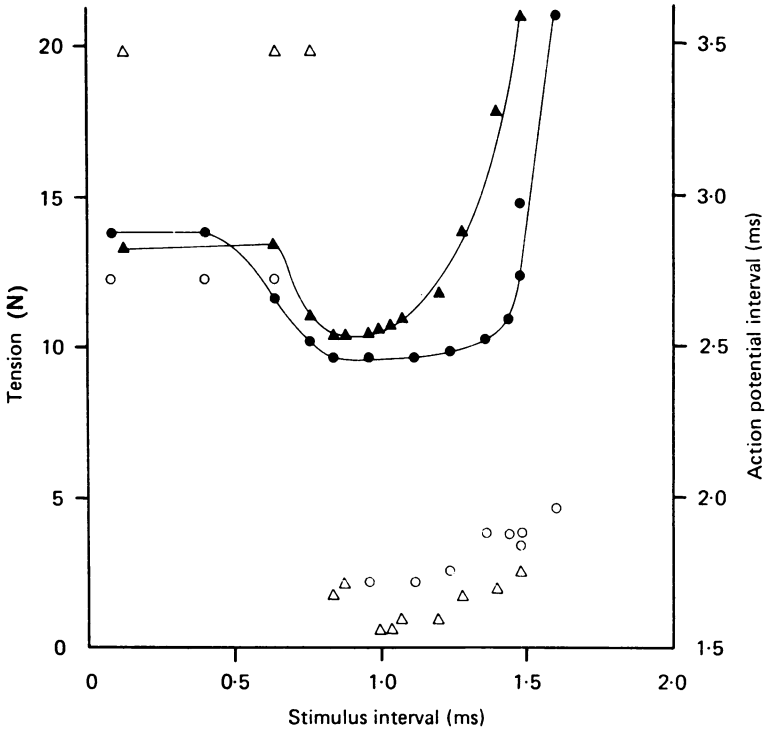

Fig. 2. A plot of the changes in muscle tension (filled symbols) and intervals between potentials recorded in the ventral root (open symbols) in response to double shock stimulation of the muscle nerve over a range of stimulus intervals. The circles represent recordings made with the stimulating electrode on the nerve at its point of entry into the muscle, the triangles when the electrode had been moved proximally 4 cm along the nerve. When the stimulus interval is sufficiently brief for only a single nerve volley to be generated (intervals up to 0·5–0·7 ms) then tension is potentiated and the interval between the two recorded potentials is long (2·7–3·5 ms), the second potential being due to back-firing. Increasing stimulus separation produces two nerve volleys, separated by a brief interval (less than 2 ms), back-firing is prevented and tension falls. The subsequent rise in tension at stimulus intervals greater than 1·2 (1·5) ms is due to summation of twitch tension.

The tension attributable to back-firing in the preparation illustrated in Fig. 1*B* (31 %) is twice as much as in Fig. 1*A*. The responses in the motor axon to two pairs of stimuli have been superimposed, the first pair separated by 0·2 ms, the second pair by 0·7 ms. At 0·2 ms interval only a single nerve volley is generated. This produces the larger twitch and two antidromic action potentials, the first at a latency of 1·0 ms representing the response to the electric shock, the second at a latency of 2·2 ms, the back response. With a stimulus interval of 0·6 ms two nerve volleys are generated as shown by two impulses at 1·0 ms and 1·9 ms latency.

The intervals between the first and second action potentials recorded in the ventral

root and the amplitude of the twitch tension have been plotted over a range of stimulus intervals in Fig. 2. The tension changes observed at different stimulus intervals are similar to those described by Brown & Matthews (1960) and represent a convenient means of detecting a back-response. A further observation made here is that the time course of suppression of back-firing depends in part on the location of the stimulating electrodes.

This is illustrated by the two curves in Fig. 2. The experimental procedure employed was identical except that the stimulating electrodes were first placed on the nerve close to its entry into the muscle (circles) and then moved 4 cm proximal to their previous position (triangles). The stimulus interval/tension plot (filled symbols) shows that with the point of stimulation at a distance from the muscle, the fall in amplitude of the twitch begins later and the time between the end of the refractory period of the nerve and that of the muscle is shorter. Thus summation of twitches begins slightly earlier. A likely explanation is that a motor nerve volley initiated at some distance from the muscle is slightly more dispersed in time on arrival at the muscle than a volley initiated close by and is effective in suppressing back-firing over a shorter period before tension begins to summate.

The open symbols in Fig. 2 represent the interval between the two action potentials recorded in the ventral root. When the two stimuli are less than 0·8 ms apart the second of the recorded action potentials represents a back-response, the interval between it and the direct-evoked potential being 2·7 ms with the stimulating site close to the muscle and 3·5 ms when 4 cm along the nerve. (A longer interval would of course be expected from the proximal stimulation site; for the back-response initiation of a volley 4 cm from the muscle will include the conduction time down to the muscle and back again.) At a stimulus interval greater than 0·8 ms back-firing is prevented and the second recorded potential, now at 1·6–1·8 ms interval, represents that directly evoked by the second stimulus. Any further separation of stimuli (0·8–1·6 ms) results in a progressive increase in the interval between the two recorded potentials. (When the second potential is a back-response, increasing the stimulus separation up to 0·8 ms produces no change in interval between potentials.)

With the stimulating electrode close to the muscle at a stimulus interval of 0·64–0·96 ms only one action potential was recorded. This appeared with a latency appropriate for the response to the first stimulus. The potential due to back-firing seemed to have been suppressed by the second stimulus. Since the technique of double stimulation requires the interval between the stimuli to be sufficiently large to be just outside the refractory period of the nerve, a second antidromic volley ought to have been recorded. The presence of only a single potential can be explained by failure of the second potential to conduct past the stimulating anode. In the example illustrated, the stimulating anode lay proximal to the cathode. For intervals greater than 0·96 ms, again two action potentials were recorded, but now the interval between the two potentials was a little longer than between the stimuli, probably from slowing of the second potential through persisting excitability changes in the axon after the first stimulus.

The effect of muscle stretch

It was noticed during the course of the experiments that back-firing in some motor axons could be abolished if the muscle was stretched up to and beyond the optimum length for a twitch contraction. Fig. 3*B* illustrates an example of the nerve volley

recorded in the whole S1 ventral root in response to a supra-maximal stimulus applied to the nerve, with no resting tension on the muscle. Superimposed on this is the response to the same stimulus with the muscle held at the twitch optimum. At the slack position two secondary peaks are visible in the volley indicating some motor fibres back-firing once, a few even twice. With the muscle stretched, the second of the smaller peaks has almost disappeared while the first peak is greatly reduced in amplitude. Furthermore, at the stretched length the interval between the directly evoked potential and that due to back-firing is slightly longer.

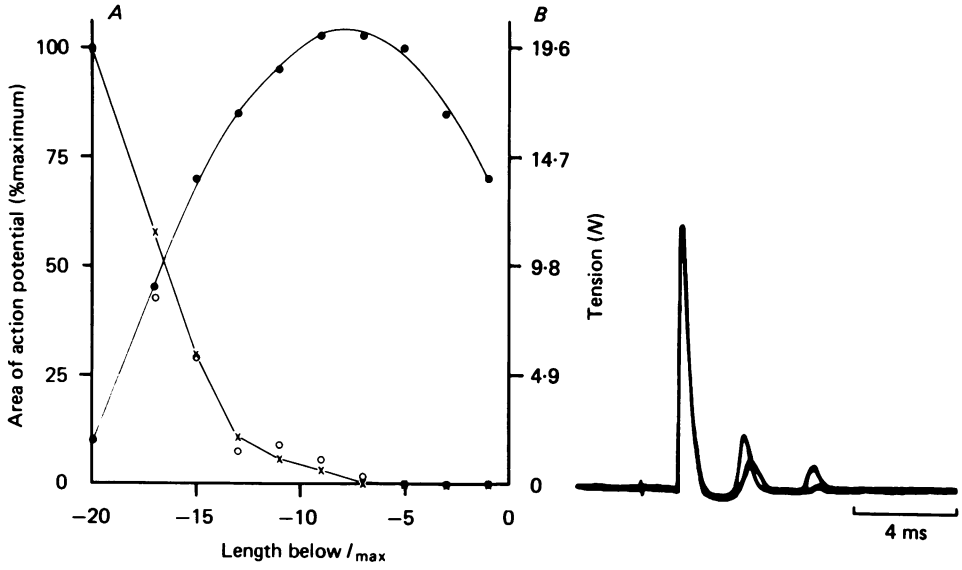

Fig. 3. The effect of muscle length on back-firing. In *B* are shown the potentials recorded in the whole ventral root in response to a single maximal nerve volley. Two pairs of traces have been superimposed; the first represents potentials recorded at a length at which the muscle was slack, the second at a length corresponding to the optimum for a twitch. The large potentials, the direct responses, superimpose accurately while the second and third potentials, representing back-firing, become smaller and occur slightly later at the optimum length. In *A* is plotted the amount of tension attributable to back-firing (crosses), the area of the summed potential of back-firing motor axons (open circles) and the whole muscle twitch tension (filled circles) at a number of different muscle lengths. Muscle length has been represented as mm below maximum body length (l_{max}).

The effect of muscle stretch was studied in detail in four experiments. The amount of back-firing was estimated from measurement of the area of the summed action potential of re-excited axons recorded in the ventral root and from the drop in tension observed with the double shock test. An example is shown in Fig. 3*A*. Open circles represent the area of back-firing action potentials recorded in the ventral root, crosses the tension (as a % of maximum) attributable to back-firing, and filled circles the twitch tension. The muscle length was recorded as mm below maximum length in the body (l_{max}). At $l_{max} - 20$ mm the muscle was slack. The curves show that there is a steep fall in the area of the action potential and the tension attributable to back-firing over the range $l_{max} - 20$ to $l_{max} - 13$ mm. At the optimum length

($l_{max} - 8$ mm) very little tension increment attributable to back-firing remains while at greater lengths none could be detcted.

A difficulty with the measurements of action potential areas was the presence of an elevation attributable to the summed activity in fusimotor fibres. When a stimulus was applied to the nerve at a point close to the muscle, potentials identified by their high threshold to stimulation as coming from fusimotor axons, occurred with a latency which coincided with the latencies of back-responses in alpha fibres. Since with the muscle stretched beyond its optimum length no tension due to back-firing could be detected, it was concluded that the remaining ventral root potential could indeed be attributed to fusimotor fibres. This conclusion was supported by the observation that if the stimulating electrode was moved from a point on the nerve close to the muscle several centimetres proximally, the latency of the gamma potential became shorter while the latency of back-firing became longer. In Fig. 3 the area of potential plotted has had the gamma potential subtracted from it. Thus where the amplitude is shown as zero it had in fact dropped to only 45 % of its original value.

While the example illustrated in Fig. 3 shows no measurable back-response with the muscle stretched to $l_{max} - 7$ mm, in two other experiments a small proportion persisted at all muscle lengths. The effect of stretch was tested on each of a total of forty-nine motor axons showing a back-response. For twenty-seven units the back-response persisted at muscle lengths at which the twitch had increased up to 80 % or more of the optimal value. For the remainder less stretch was necessary, for six of the axons the back-response being abolished at lengths giving a twitch less than 20 % of that at the optimum length. Back-firing in a further four axons occurred only when the muscle lay completely slack and the slightest resting tension caused the response to become intermittent.

Back-responses in single motor fibres

The histogram in Fig. 4*A* shows the conduction velocity distribution for single motor axons showing no back-firing (twenty-two units, stippled area) and those with a back-response (fifty units). The distribution supports the observation made with whole muscle tension recording (Fig. 1), that small motor units are less likely to show back-firing.

A similar result emerged when the threshold to electrical stimulation was compared for axons with and without a back-response (Fig. 4*B*). Axons with a back-response tended to have a lower threshold to stimulation than axons without the response. The distribution of thresholds about the 50 % value is not (quite) significant at the 5 % level (adjusted X^2).

Another variable for back-responses was the interval between the antidromically evoked action potential and that evoked by back-firing. The intervals were measured for twenty-four units and related to the conduction velocity of the axons. Briefer intervals might have been expected for the more rapidly conducting axons since the time corresponding to intramuscular conduction would be less in this group. No simple relation emerged and it must be assumed that this is not an important factor in determining the length of the interval.

The stimulus strength necessary to produce a back-response varied for different motor axons, and as might have been expected, was commonly higher than for stimulation of the axon itself. On several occasions, however, motor fibres were encountered where the threshold for back-firing was lower than the threshold for direct excitation. An example of an axon showing this effect is illustrated in Fig. 5 (right hand panel).

A weak stimulus (20 % of strength necessary for a maximal twitch) evoked an action potential with a latency of 5·1 ms (top trace) which reduced to 3·7 ms (second trace) as stimulus strength was increased. A further increase (third trace) produced one action potential at 3·7 ms latency and a second at 8·2 ms. Finally, (fourth trace) a stimulus maximal for the nerve volley produced an initial action potential (latency 1·6 ms) which represents the electrically evoked potential, followed by two potentials attributable to back-firing. Although not illustrated here, the two later occurring spikes were confirmed as examples of back-firing in the muscle using the double shock test. Two other axons had thresholds for back-firing that were lower than for

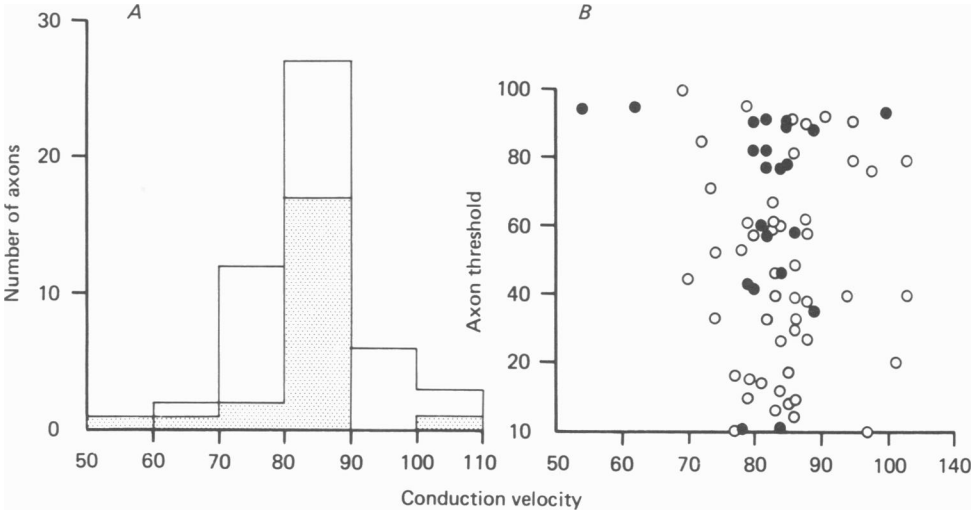

Fig. 4. Back-firing in single motor axons. In *A* is shown the conduction velocity distribution for axons showing no back-firing (twenty-two units, stippled area) and those with a back-response (fifty units, open area). In *B* is plotted the threshold to electrical stimulation, expressed as a % of that necessary to produce a maximal muscle contraction, against axonal conduction velocity. Open circles, axons with back-responses; filled circles, axons without back-responses.

direct stimulation and a further four axons had thresholds for direct stimulation that appeared identical to the threshold for back-firing. No conclusive evidence supporting the possibility of an axon being able to re-excite itself could be obtained. Whenever the thresholds were identical, the values of threshold in terms of the stimulus strength necessary for a maximal twitch were large. Furthermore, when the muscle was stretched to lengths in excess of the optimum body length, the threshold for back-firing in these units rose above the threshold for electrical excitation.

A plot of the threshold of a back-response against threshold for direct excitation is shown on the left in Fig. 5, threshold being expressed as a percentage of the stimulus strength necessary for a maximal muscle twitch. The Figure illustrates that for many axons the nerve volley must excite all of the muscle before back-firing occurs, and that very few axons have a lower threshold for back-firing than for direct excitation.

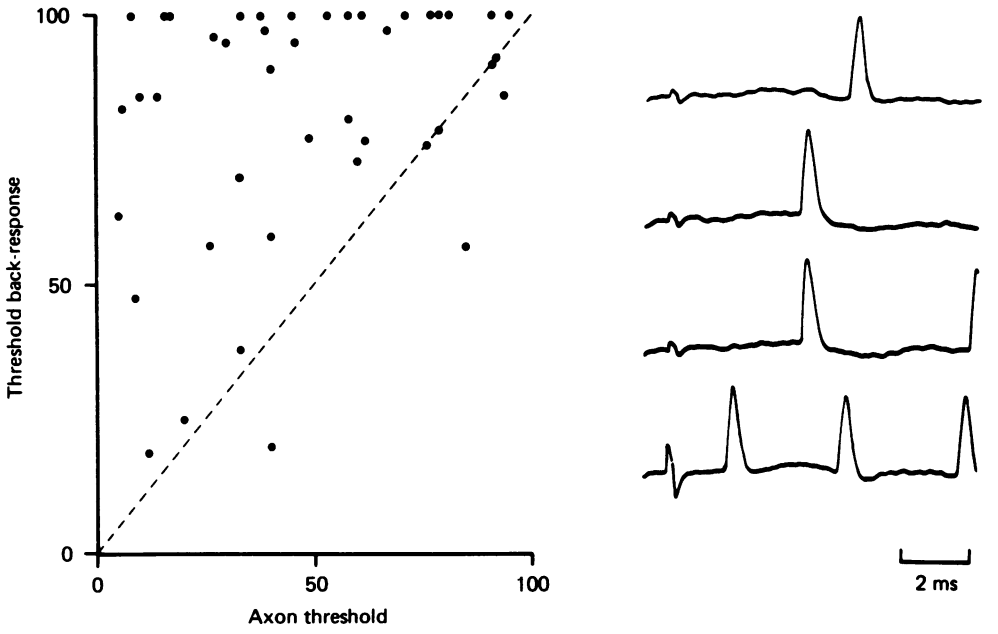

Fig. 5. The threshold for back-firing. Right-hand panel: an example of a single motor axon with a back-response threshold lower than for the direct-evoked potential. From upper trace downwards, increasing stimulus strength produces first a single back-fired potential which reduces in latency (second trace), then two back-fired potentials are produced (third trace) and finally, (fourth trace), the back-fired potentials are preceded by the direct response. Left-hand panel: a plot of the stimulus strength necessary for a back-response, plotted against axonal threshold, threshold being expressed as a percentage of the stimulus strength necessary for a maximum muscle twitch. The dashed line locates points where stimulus threshold and back-firing threshold are identical.

DISCUSSION

The results presented here have demonstrated back-firing in functionally single motor axons. Measurements of the electrical threshold and conduction velocity have included a majority of rapidly conducting, low threshold axons. On the assumption that the threshold for electrical stimulation of axons lying in the nerve bears some relationship to the excitability of axon terminals within the muscle, then if re-excitation results from summed electrical activity within the muscle, the large diameter, low-threshold axons might be expected to be more susceptible to re-excitation. That summed activity in the muscle is necessary is provided by the observation that the stimulus to the nerve must be sufficiently strong to produce a maximal muscle contraction before most axons show back-firing. Nevertheless a few axons had a surprisingly low threshold for back-firing. Brown & Matthews (1960) point out that under normal conditions the muscle never contracts sufficiently synchronously for back-firing to occur. While this is likely to be true for the majority of motor units it is possible that those axons with low threshold for back-firing could be re-excited during normal posture or movement.

When the muscle is stretched from its slack position, the twitch tension rises

while the back-response falls. Thus the amount of back-firing does not simply depend on the gross tension output of the muscle. It was noticed for several motor fibres with a back-response that the interval between the electrically evoked and the back-fired potential became longer by 0·1–0·5 ms when the muscle was stretched. This is also seen for the volley in the whole nerve (Fig. 3*B*). At a muscle length just less than that necessary for abolition of back-firing, the interval between the directly evoked and back-fired potentials was at its longest. While a change in utilization time of the nerve fibre may account for some of the increase in interval, it is possible that in the slack muscle re-excitation occurs at a point further away from the nerve terminals than in the stretched muscle. This may also be part of the explanation for the effect of stretch on back-firing. In the slack muscle nerve axons and muscle fibres lie closer to one another, making re-excitation more likely.

The presence of a back-response in some motor fibres at a stimulus strength subthreshold for electrical excitation of the axon (Fig. 5) excludes in these cases the possibility that muscle fibres innervated by the axon are themselves contributing to the generation of the back-response. Furthermore, as stated earlier, no evidence could be obtained for motor units re-exciting themselves. This is not surprising since it is now known that the muscle fibres of a motor unit lie scattered through one third or more of the muscle (Burke & Tsairis, 1974).

The axon of Fig. 5 was re-excited a second time as late as 5·8 ms after direct stimulation by the electrodes. This would seem to be well past the peak of the summed electromyographic activity of the muscle (Brown & Matthews, Fig. 9). Perhaps it is not the gross electromyogram that is important for back-firing, but activity in certain strategically placed motor units.

Although it remains a widely held view that back-responses are generated by electrical activity in muscle fibres, alternative explanations have been considered. Thus it is conceivable that transmitter released by nerve terminals is able to diffuse across and excite adjacent terminals. It has long been known that administration of anticholinesterases induces repetitive activity in motor nerves (Masland & Wigton, 1940). However, drug-induced discharges and the effect of synchronous nerve stimulation can be differentiated (Werner, 1961) and it is unlikely that they arise from a common mechanism.

It is concluded that although a study at the level of single motor axons does not identify any one hypothesis for the mechanism and site of generation of back-responses, all the observations suggest it is the electrical activity within muscle fibres which is responsible and that this is favoured by particular anatomical arrangements within the muscle.

REFERENCES

Brown, M. C. & Matthews, P. B. C. (1960). The effect of a muscle twitch of the back-response of its motor nerve fibres. *J. Physiol.* **150**, 332–346.

Buller, A. J. & Proske, U. (1973). Re-excitation of motor nerve terminals during twitch contractions. *Proc. Aust. Physiol. Pharmac. Soc.* **4**, 37–38.

Burke, R. E., Levine, D. N., Tsairis, P. & Zajac III, F. E. (1973). Physiological types and histochemical profiles in motor units of cat gastrocnemius. *J. Physiol.* **234**, 723–748.

Burke, R. E. & Tsairis, P. (1973). Anatomy and innervation ratios of motor units of cat gastrocnemius. *J. Physiol.* **234**, 749–765.

MASLAND, R. L. & WIGTON, R. S. (1940). Nerve activity accompanying fasciculation produced by prostigmin. *J. Neurophysiol.* **3**, 229–275.

PROSKE, U. & WAITE, P. M. E. (1976). The relation between tension and axonal conduction velocity for motor units in the medial gastrocnemius muscle of the cat. *Expl Brain Res.* **26**, 325–328.

WERNER, G. (1961). Antidromic activity in motor nerves and its relation to a generator event in nerve terminals. *J. Neurophysiol.* **24**, 401–413.