J. Physiol. (1972), **227**, *pp.* 709–727
With 7 text-figures
Printed in Great Britain

INTRACELLULAR POTENTIALS FROM INTRAFUSAL MUSCLE FIBRES EVOKED BY STIMULATION OF STATIC AND DYNAMIC FUSIMOTOR AXONS IN THE CAT

By P. BESSOU and B. PAGÈS

From the Laboratoire de Physiologie, U.E.R. de Sciences Médicales, Université Paul Sabatier, 133, route de Narbonne, 31–Toulouse, France

(*Received 18 May 1972*)

SUMMARY

1. Membrane potential changes of single intrafusal muscle fibres were intracellularly recorded in spindles of cat's tenuissimus muscle on stimulating single static or dynamic fusimotor axons.

2. One third of responses elicited on stimulating static fusimotor axons were action potentials while the remainder were junction potentials. Repetitive stimulation of static axons eliciting junction potentials produced summation, facilitation and in some instances the appearance of propagated potentials.

3. All the responses evoked on stimulating dynamic fusimotor axons were junction potentials which summated but never produced propagated potentials during repetitive stimulation.

4. Most of the impalements leading to junction potentials were located in the transition zone between intra- and extra-capsular regions of spindle poles.

5. The relation between extracellular and intracellular potentials elicited by stimulation of a fusimotor axon (static or dynamic) makes it possible to assign a physiological nature to the junction potentials recorded intracellularly and to exclude effects attributable to injury.

6. A coupling between junction potential and local contraction is indirectly inferred from the frequencygrams which have been previously obtained during single shock stimulation of dynamic fusimotor axons.

INTRODUCTION

Intrafusal muscle potentials evoked by stimulation of single fusimotor axons were first recorded in the cat tenuissimus muscle by Kuffler, Hunt & Quilliam (1951). These authors were unable to decide whether the potentials were propagated events or electrical changes localized at the

intrafusal neuromuscular junctions. Using the same muscle, Eyzaguirre (1960) considered that the polyphasic intrafusal potentials elicited by stimulation of single fusimotor axons were propagated. Later, Bessou & Laporte (1965*a*) showed that this type of potential was evoked by stimulation of static fusimotor axons and that stimulation of dynamic axons elicited potentials whose shape and time course suggested they were non-propagated. In all these investigations spindle potentials were recorded with extracellular electrodes from the tenuissimus muscle surface. Interpreting such results is obviously limited on account both of the volume conductor recording and of the complexities of the spindle structure. The following factors have to be taken into consideration: (i) a single fusimotor axon possibly innervates several intrafusal muscle fibres; (ii) motor endings are scattered in many places along an intrafusal muscle fibre; (iii) intrafusal muscle fibres are of different diameter and length; (iv) intrafusal fibres lie within a fluid filled periaxial space, i.e. Sherrington's lymph space. An analysis with intracellular micro-electrodes, as has been done in the frog by Koketsu & Nishi (1957*a*, *b*), may yield more information about potential changes evoked in intrafusal muscle fibres by stimulating single fusimotor axons.

The present work has been performed in order to determine the intracellular responses of single intrafusal muscle fibres to the stimulation of a single γ fusimotor axon functionally identified as a static or dynamic axon. Responses possibly elicited by β axons (Bessou, Emonet-Dénand & Laporte, 1963, 1965), that innervate both intrafusal and extrafusal muscle fibres, have not been examined. Propagated potentials have been observed on stimulating fusimotor axons which produced powerful static effects. Junction potentials have been recorded on stimulating both static and dynamic fusimotor axons.

The results presented here demonstrate that nervous stimulation of intrafusal muscle fibres of cat's spindles results in twitch or in local contractions.

Preliminary reports on this work have been published (Bessou & Pagès, 1969; Bessou, Laporte & Pagès, 1970).

METHODS

Thirty-five experiments were carried out on adult cats under Nembutal anaesthesia (40 mg/kg). In each experiment muscle fibres of a spindle located in the distal third of a tenuissimus muscle were impaled and their responses to the stimulation of a single functionally identified fusimotor axon have been recorded. The tenuissimus muscle is a convenient one in which to localize spindles. Dark field illumination underneath its distal part makes it easy to see the spindle during the excision of the overlying bed of extrafusal muscle fibres and facilitates the impalement of intrafusal muscle fibres.

The distal half of the tenuissimus muscle was mounted together with the tenuis-

simus nerve and a part of the sciatic nerve in a flat chamber which was at its proximal extremity in close contact with the posterior region of the semiflexed thigh of the cat. With this arrangement the connexions of the tenuissimus muscle with dorsal and ventral roots were preserved. In order to mount the neuromuscular preparation in the chamber the vascular pedicles joining the popliteal and the sural portion of the tenuissimus muscle were ligatured and sectioned. The distal half of the tenuissimus muscle receives its blood supply from the vessels situated in the gluteal region and in the thigh. The efficacy of the vascularization was checked under a binocular microscope. The muscle lay flat on the glass bottom of the chamber, its distal end attached to a magnetic puller. Mammalian Ringer solution equilibrated with a gas mixture (95% O_2, 5% CO_2) was slowly flowing through the chamber at a temperature of 36–37° C.

The left hind limb and hip region were extensively denervated leaving the innervation of the tenuissimus muscle intact. The dorsal and ventral roots L7 and S1 were cut at their entry into the spinal cord. A single Ia afferent fibre and several single fusimotor axons innervating a spindle located in the distal portion of the tenuissimus muscle were prepared by splitting dorsal and ventral roots. The static or dynamic action exerted by single fusimotor axons was identified by observing the effects produced by repetitive stimulation at 100/sec of an axon on the response of the primary ending to phasic stretch (Matthews, 1962). After identifying the axons, the distal half of the tenuissimus muscle was placed on the glass bottom of the chamber and held in place by small metal springs attached to its sides and its distal extremity.

The spindle was located by electrical and mechanical stimulation of its primary ending under visual observation (Bessou & Laporte, 1965*b*) using a binocular microscope and dark field illumination. The layers of muscle fibres covering the spindle were removed without damaging the innervation and the blood supply of this spindle. Then intrafusal muscle fibres were impaled with 3 M-KCl-filled microelectrodes (30–80 MΩ resistance).

RESULTS

Visual observations of the spindle

After the layers of extrafusal muscle fibres covering the spindle are removed some structural details can be observed with a binocular microscope Wild (magnification × 50), namely the periaxial space, the intrafusal muscle bundle passing through it and the spindle nerve supply (a nerve twig given off by the main trunk running axially inside the muscle). The point where this nerve twig reaches the muscle bundle in the periaxial space is used as the reference point for locating the distal and proximal extremities of the periaxial space and the sites of impalements. From this reference point the intrafusal muscle bundle can usually be traced for 2–3 mm on both sides. However, outside the periaxial space, it becomes progressively more difficult to follow the intrafusal muscle bundle and to distinguish it from the extrafusal muscle fibres even with a very good dark field illumination, the outlines of this bundle becoming obscured by the extrafusal muscle, connective tissue and nerve or blood vessels which run across it.

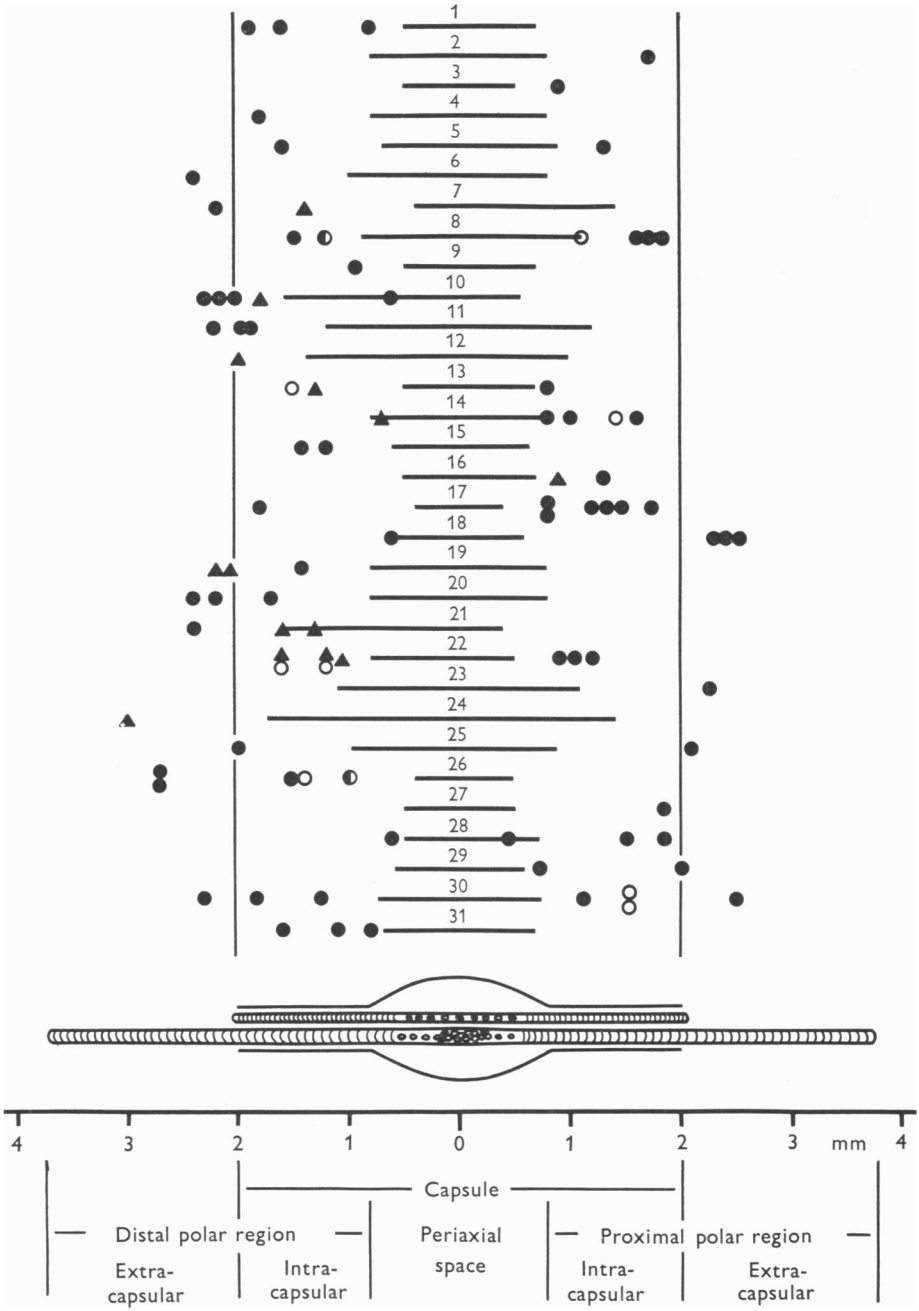

Fig. 1. For legend see facing page.

The mechanical effects produced by repetitive stimulation of each fusimotor axon can be determined by observing the intrafusal muscle bundle under a binocular microscope. At $\times 50$ magnification when a dynamic fusimotor axon is repetitively stimulated (100/sec) it is not possible to see a movement whatever portion of the intrafusal muscle bundle is being examined. At the same magnification, during similar repetitive stimulation of a static fusimotor axon, one can always observe a displacement of the reference point which moves either towards the distal pole or towards the proximal one. Usually the contracting part presents an immobile portion on both sides of which the other portions of the pole are attracted. If a static axon supplies both poles the direction of the displacement of the reference point is determined by the resultant of the contractions developed by each pole.

The observation of intrafusal movements elicited by stimulation of static fusimotor axons serves different purposes: (i) rapidly detecting a spindle previously located in the tenuissimus muscle by electrical stimulation of its group I fibre and by mechanical activation of its primary ending; (ii) distinguishing the intrafusal muscle bundle from the surrounding extrafusal muscle fibres and also enabling with more safety the removal of the overlying layers of extrafusal muscle fibres; (iii) determining the pole in which the chances of recording intracellular potentials after stimulation of a given axon are the greatest; (iv) implanting the micro-electrode in the immobile portion of the bundle. It is easier to penetrate an intrafusal muscle fibre in this region and to maintain the micro-electrode inside the fibre for a relatively long time.

Fig. 1. Map of micro-electrode impalements leading to intracellular recording of intrafusal muscle potentials evoked by dynamic or static fusimotor stimulation for thirty-one spindles. The reference point for each spindle (see text) is in line with the zero point on the millimetre scale below the map. *Horizontal bars:* periaxial space length for each spindle; polar regions are not drawn because it is usually impossible to see with certainty how far from the reference point they end. *Filled triangles:* intracellular recording sites of muscle potentials elicited by stimulation of single dynamic fusimotor axons. *Circles* (*open, half-filled, filled*): intracellular recording sites of muscle potentials evoked by the separate stimulations of the different static fusimotor axons innervating the same spindle; in spindles nos. 17, 22, 26 and 30, several impalements were made at the same distance from the reference point but in different intrafusal muscle fibres. At the bottom of the map: standard mammalian muscle spindle with only one nuclear bag and one nuclear chain muscle fibre based on histological data (Boyd, 1962) and showing length relationships of the two types of intrafusal fibres with the capsule.

Map of the impalements

During the very slow downward motion of the tip of the micro-electrode, single stimuli are applied every second to each of several isolated fusimotor axons, except if the response to one particular axon is sought for. Potentials are recorded extracellularly when the tip of the micro-electrode is in the vicinity of a motor nerve terminal or of an intrafusal fibre innervated by this fusimotor axon. The impalements yielding intracellular potentials elicited by stimulation of fusimotor axon are called successful impalements.

In Fig. 1, the length of the periaxial space of thirty-one spindles studied is represented by the length of a horizontal line. The position of the reference point on this line is marked by a number which corresponds to the spindle number. The periaxial space symmetrically stretches on both sides seventeen times and asymmetrically fourteen times. The mean total length of the periaxial space is 1·6 mm.

In the polar region of the spindle the limit between the intracapsular and the extracapsular part of the muscle bundle never could be established because the conditions of our observations made it impossible to distinguish the spindle capsule in this region. Using the histological data of Boyd (1962) it has been possible in Fig. 1 to locate approximately the proximal and distal boundaries between the intra- and extra-capsular part of the muscle bundle observed *in vivo*. It is justifiable to do this since the mean length of the periaxial space of the spindles taken into account in this figure fits very well with the mean length of the same spindle region measured in the histological preparation. These supposed mean boundaries have been indicated to give more information of mapping impalements.

Ninety-nine successful penetrations were made on thirty-five spindles. The impaled intrafusal muscle fibre usually gave responses to the stimulation of only one single fusimotor axon. However, in two experiments, by separately stimulating two static fusimotor axons, responses were elicited in the same intrafusal fibre. During a successful penetration, responses were never elicited to both static and dynamic fusimotor axons stimulated separately.

Ninety-three penetrations made on thirty-one spindles are plotted in Fig. 1. Only 8% of the penetrations were made in the periaxial space. Tentative impalements of muscle fibres inside the periaxial space were frequently unsuccessful because the thick capsule in this region breaks the micro-electrode and the mobility of the muscle fibres encountered here permits them to slip past the tip of the micro-electrode during the impalement. The majority (92%) of the penetrations were made in the polar region. Most of these impalements were located close to the periaxial space in the intracapsular polar region. At sites farther from the periaxial

space, successful impalements were less frequent, probably because the intrafusal muscle fibres are less numerous in the extracapsular polar region and the farther they extend from the equatorial region the smaller their diameter and the greater their scattering.

Eighty-seven single fusimotor axons were prepared in thirty-five experiments (twenty dynamic and sixty-seven static). The stimulation of twenty-eight fusimotor axons (six dynamic and twenty-two static) produced no response, either because the micro-electrode did not impale the intrafusal muscle fibres innervated by these axons, or because the point of penetration of the micro-electrode was not suitably placed with regard to the neuromuscular junction (see Discussion). Of the ninety-nine successful penetrations, twenty were associated with potentials evoked by stimulation of fourteen dynamic fusimotor axons while the remainder were associated with responses from forty-five static fusimotor axons. The ratios of the numbers of prepared dynamic axons to penetrated intrafusal muscle fibres and prepared static axons to penetrated intrafusal muscle fibres are roughly similar, so it seems that muscle fibres synaptically connected with dynamic fusimotor axons are as easy to impale as those connected with static axons.

The map of the recording sites in Fig. 1 displays a particular distribution according to the type of fusimotor axon stimulated. The impalements leading to intrafusal muscle potentials evoked by stimulation of static fusimotor axons are evenly distributed on the poles of the spindle. On the contrary, the impalement sites from which post-synaptic potentials have been recorded by stimulating dynamic fusimotor axons are all but one in the distal polar region.

Intrafusal muscle potentials elicited by stimulation of static fusimotor axons

During successful impalements single stimuli applied to the static fusimotor axons elicited either spike potentials (one third of the static responses), or junction potentials.

Spike potentials

Spike potentials are illustrated in Fig. 2. They have an approximate duration of 1·5–3 msec. Their amplitude ranges between 45 and 70 mV. The overshoot above the zero membrane potential is about 5–15 mV. As indicated in records 1 and 2, a prepotential may sometimes precede the spike potential.

Least stimulus interval to evoke a double response. In some experiments, two shocks (2 × threshold) have been applied to a single static fusimotor axon eliciting a spike potential in order to determine the refractory period

of the neuromuscular preparation. A result of such experiments is illustrated in record 3. When the time interval between two stimuli was about 2·5 msec, the amplitude of the response to the second stimulus decreased. As the time interval between the stimuli was progressively reduced the amplitude of the second spike potential progressively decreased and the latency increased. From 1·4 msec time interval between the two shocks the second stimulus was ineffective. This may result from the refractory period

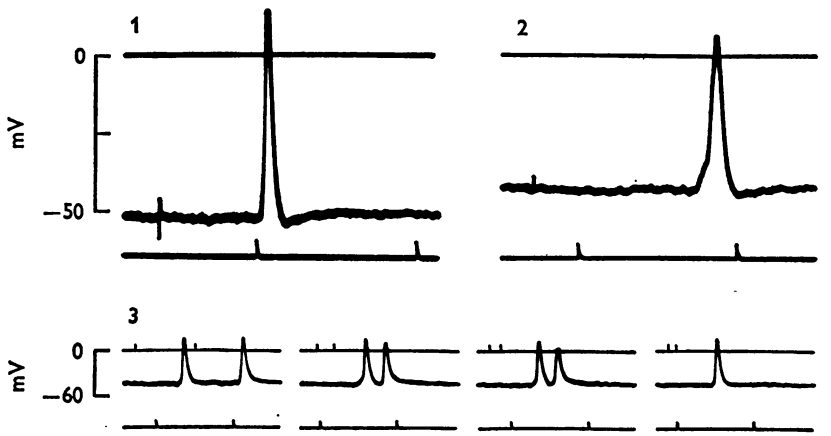

Fig. 2. Intrafusal muscle action potentials evoked by stimulation of single static fusimotor axons. In every recording: *top trace*, zero membrane potential; *middle trace*, intracellularly recorded muscle action potential; *bottom trace*, time marker, 10 msec. Record 1, spindle no. 3, distal pole, impalement 0·90 mm away from the reference point. Record 2, spindle no. 17, proximal pole, impalement 1·35 mm away from the reference point. Records 3, spindle no. 11, distal pole, impalement 2·20 mm away from the reference point; double-shock stimulation with decreasing time interval.

either of the intrafusal muscle fibre, or of the motor terminals themselves. In this experiment spikes were always preceded by a prepotential. Since in the last record the second shock failed to evoke a local potential, this suggests that the refractory period of the intrafusal muscle fibre cannot account for the suppression of the second response. Since the refractory period of fusimotor axons did not exceed 1 msec when stimulating the nerve trunk running inside the tenuissimus muscle and recording from the ventral roots, at intervals shorter than 1·4 msec the second stimulus had set up a second impulse in the fusimotor axon from the ventral root filament, but this impulse failed to invade the motor endings. Presumably a block has occurred at the transition between the myelinated and the non-myelinated axon, the absolutely refractory period of which is probably longer as has been suggested by Brock, Coombs & Eccles (1953) for pre-

synaptic terminals of motoneurones in the spinal cord. From these results it may be concluded that the ineffectiveness of the second stimulus in the last picture of the record 3 arises from a low safety factor of conduction located at the peripheral terminals of the stimulated axon.

The action of a neuromuscular blocking agent. In three experiments during successful impalements of an intrafusal muscle fibre giving a spike potential on stimulating a static fusimotor axon, the circulation of the bathing solution filling the chamber in which the tenuissimus muscle was lying was stopped and small quantities of gallamine (Flaxedil Specia) were added to the Ringer solution. The response of the muscle fibre was elicited once every 5 sec on stimulating the static fusimotor axon and the amplitude of these responses has been plotted against time.

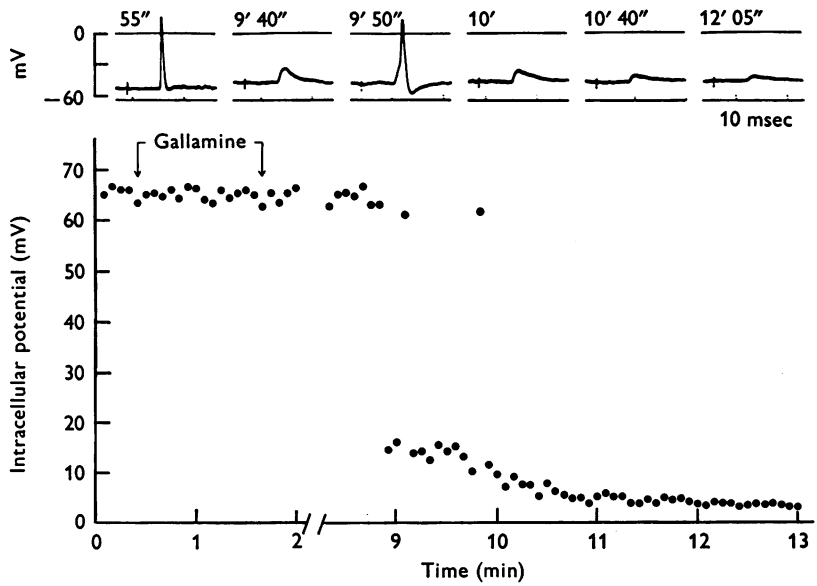

Fig. 3. Effect of neuromuscular block on the response of an intrafusal fibre to single shock stimulation of a static fusimotor axon. Spindle no. 3, distal pole, impalement 0·90 mm away from the reference point. *At the top*, intrafusal muscle potential respectively recorded 55 sec, 9 min 40 sec, 9 min 50 sec, 10 min, 10 min 40 sec and 12 min 05 sec after the impalement; time marker, 10 msec. *At the bottom*, intrafusal muscle potential amplitude plotted against time; between the two arrows, gallamine was slowly added to the bathing solution up to a final concentration of 3×10^{-5} g.ml^{-1}.

Such an experiment is illustrated by Fig. 3 which shows in the upper part some samples of the intracellular records at different times after the impalement and during neuromuscular block, and in the lower part the amplitude of intrafusal muscle potential plotted against time. Seven

minutes after completing the addition of gallamine (final concentration 3×10^{-5} g.ml^{-1}) to the bathing solution, the amplitude and the shape of the response remained unchanged. Shortly after, there was a sudden change in the amplitude and decay of the evoked potential. The generation of the spike potential was suddenly blocked and stimulation of the fusimotor axon only elicited a junction potential. During the next minute blocking action of gallamine was very critical and twice at 9 min and 9 min 50 sec the junction potential leads to a propagated spike. Finally, the neuromuscular block progressed and graded blocking effects of the junction potential may be observed.

Injury caused by impalement of the intrafusal membrane was not responsible for the disappearance of spike potential since washing the preparation in fresh Ringer solution restored transmission to normal levels. Stopping the flow of fluid through the chamber as long as 15 min without adding gallamine was always ineffective in changing the amplitude and the shape of the response owing to the large capacity of the chamber (40 ml.) and the efficacy of the muscle blood supply. Similar reasons may explain the long delay in the gallamine action.

Injury of the intrafusal fibre membrane. The records in Fig. 4 illustrate the signs of injury caused by impalement of the intrafusal membrane and the corresponding modification of the response following stimulation of static fusimotor axons. Record 1 shows the characteristics of the muscle membrane potential (−62 mV) and of the spike potential (amplitude 75 mV, overshoot 15 mV, duration 2·5 msec) immediately after successful impalements. A prepotential may be observed at the beginning of the depolarization phase. Successive records (time interval 2 sec) show the simultaneous decrease of the resting potential and of the amplitude of the spike. Record 2 illustrates an increase in the duration of the spike and the appearance of a positive afterpotential. Subsequent records (3, 4, 5) show the successive transformation of the spike into an abortive spike superimposed on a junction potential. At a certain point (record 6) the abortive spike may be recorded without any change as long as the tip of the microelectrode remains inside the intrafusal muscle fibre.

Abortive spikes are characterized by a short lasting (about 1·5 msec) depolarization phase and a brief (no more than 6 msec) repolarization phase which is not exponential suggesting that the process involved during the return to the resting potential is not a passive one. On stimulating a static fusimotor axon with two shocks at close intervals, the amplitude of the second abortive spike was decreased. Abortive spikes are frequently followed by a small undershoot and repetitive stimulation never evoked a full spike.

Abortive spikes have been observed sometimes immediately after

impaling the intrafusal muscle fibre and on several occasions even while the resting potential (30–50 mV) was as great as the one accompanying fully developed spikes. Injury of the intrafusal muscle membrane is probably not the sole explanation for abortive spikes (see Discussion).

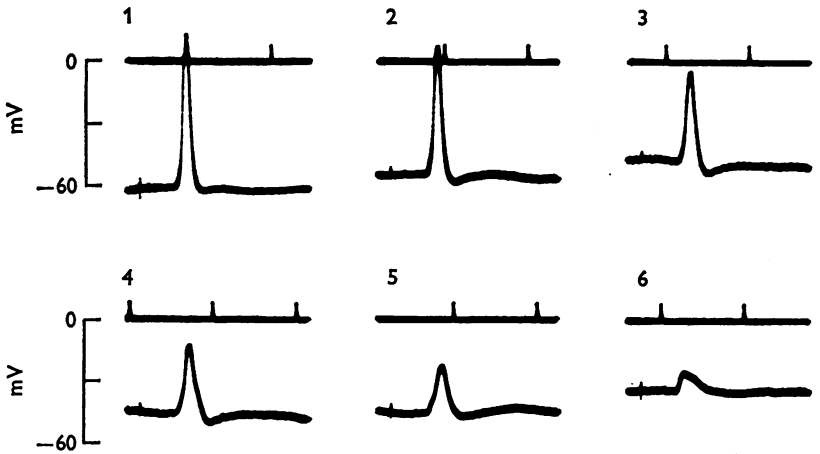

Fig. 4. Changes, occurring after the impalement, in membrane potential and in response of intrafusal muscle fibre to static fusimotor axon stimulation (time interval of single shock: 2 sec). Spindle no. 29, proximal pole, impalement 2·0 mm away from the reference point. *Upper traces*, zero membrane potential and time marker, 10 msec. *Lower traces*, intracellular potentials.

Junction potentials

These potentials usually look like the potentials illustrated in Fig. 3 at the end of curarization. Fig. 5, record 1, shows a junction potential with a steep depolarization phase (1·5 msec) followed by a repolarization lasting 13 msec. Since these potentials have an approximately exponential decay, it can be deduced that the repolarization phase represents merely the passive decay of the electrical change previously set up at the intrafusal muscle membrane by the release of the chemical transmitter. However, junction potentials elicited by the stimulation of single static fusimotor axons show a wide variation in the shapes of both the rising and decaying phase. Records 2 and 3 show potentials characterized by a notch on the repolarization phase. Record 4 illustrates another type characterized by a repolarization phase initially fast then becoming very slow. Summation and often facilitation were observed during repetitive stimulation (record 5). The successive depolarizations build up to a plateau amplitude which depends upon the stimulation frequency (record 6). Beyond 225/sec little further depolarization is added by increasing the stimulation frequency.

At 375/sec (record 7) one stimulus out of two is ineffective. In three experiments, repetitive stimulation produced propagated potentials as illustrated in record 8; spike potentials usually arise from an unchanged resting potential, and this is evidence of some facilitation in the synaptic transmission mechanism.

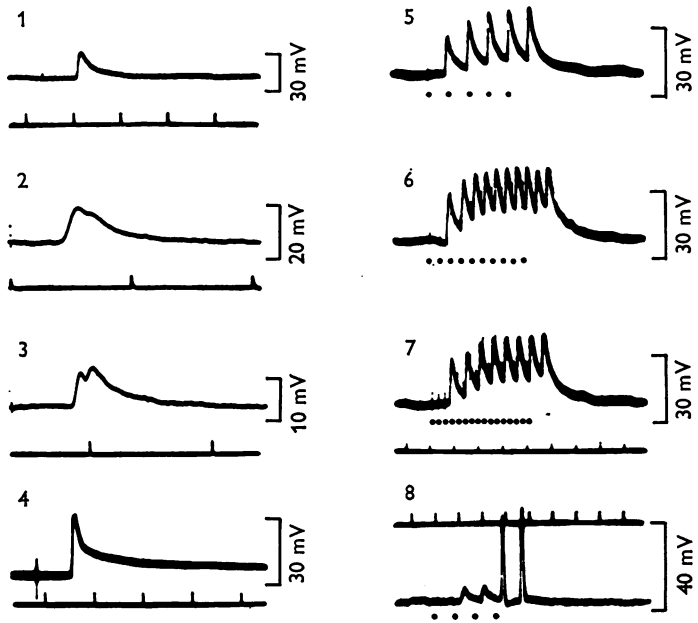

Fig. 5. Junction potentials evoked by static fusimotor axon stimulation. 1–4, Single stimuli; 5–8, repetitive stimulation. 1, spindle no. 30, proximal pole, impalement 1·65 mm away from the reference point. 2, spindle not illustrated in Fig. 1, proximal pole, impalement 2·0 mm away from the reference point. 3, spindle no. 5, distal pole, impalement 1·60 mm away from the reference point. 4, spindle no. 2, proximal pole, impalement 1·80 mm away from the reference point. 5–7, spindle no. 30, proximal pole, impalement 1·15 mm away from the reference point; repetitive stimulation (dots) of the same static fusimotor axon at respectively 120/sec, 225/sec and 375/sec. 8, spindle no. 18, proximal pole, impalement 1·30 mm away from the reference point; repetitive stimulation (dots) of a static fusimotor axon at 113/sec; upper trace, zero membrane potential and time marker; lower trace, intrafusal potential. Time marker, 10 msec.

Intrafusal muscle potentials elicited by stimulation of dynamic fusimotor axons

Stimulation of fifteen dynamic fusimotor axons never produced spike potentials. There were only small transient monophasic depolarizations of 5–20 mV amplitude, the resting membrane potential being 30–40 mV

(Fig. 6). Their shape is regular and notches have never been observed on either the depolarization or the repolarization phase. Their duration ranges from 5 to 30 msec (Fig. 6). The responses are characterized (record 1) by a short depolarization phase and longer duration of the repolarization phase, the decay of which follows an exponential time course. These responses look like junction potentials elicited by stimulating static fusimotor axons. When the dynamic fusimotor axon is stimulated by a double

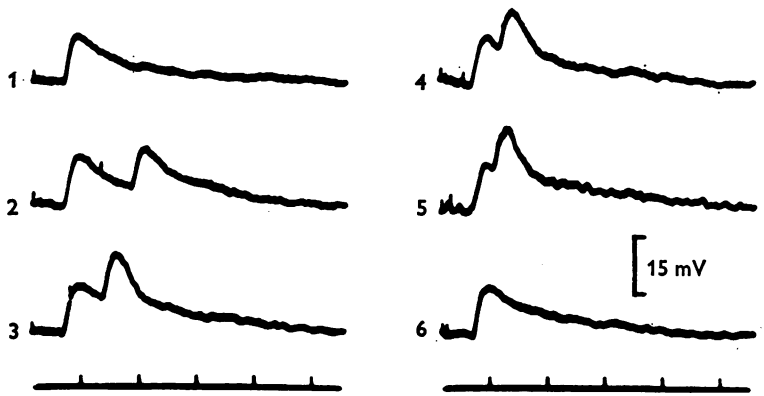

Fig. 6. Intracellular recordings of intrafusal muscle fibre potentials evoked by stimulation of a dynamic fusimotor axon. Spindle no. 12, distal pole, impalement 1·95 mm away from the reference point. 1, single shock stimulation. 2–6, double-shock stimulation (time interval between stimuli: 2, 11·5 msec; 3, 6·6 msec; 4, 4·1 msec; 5, 1·5 msec; 6, 0·6 msec. Time marker 10 msec.

shock (time interval 11·5 msec) the peak value of the second response approximates to that of the first one (record 2). With double shocks successively 6·6 msec, 4·1 msec, 1·5 msec apart (records 3, 4, 5) the onset of the second depolarization approaches more and more the peak of the first response, so that its peak sums with the first peak to achieve nearly double the amplitude. When the interval between the two shocks is reduced to 0·6 msec, the second response disappears (record 6).

Physiological nature of the junction potentials

Repeatedly probing the spindle and impaling intrafusal muscle fibres possibly injures the muscle membrane or the motor terminals, therefore it is necessary to decide between two possibilities: (i) the junction potential is the last electrical event in a normal process of the intrafusal synaptic transmission; (ii) the junction potential appears because of either a damaged generative process of a spike potential or a depressed excitability of the motor nerve terminals.

A study of the extracellular potentials recorded during stimulation of a fusimotor axon before impaling an intrafusal muscle provides an answer to this problem. At first glance one might think that analysing these potentials is difficult because the fusimotor axon which produces synaptic activity in a given intrafusal muscle fibre sends collaterals to several neighbouring intrafusal muscle fibres so the activity of other structures in the vicinity might confuse the recording. Nevertheless, it seems reasonable to assume that if the change of recording conditions from extra to intracellular can be achieved by a minute forward movement of the microelectrode (particularly when the last extracellular potential recorded is at a maximum negativity), then the potentials recorded in the two conditions are due to the activation of the same structure.

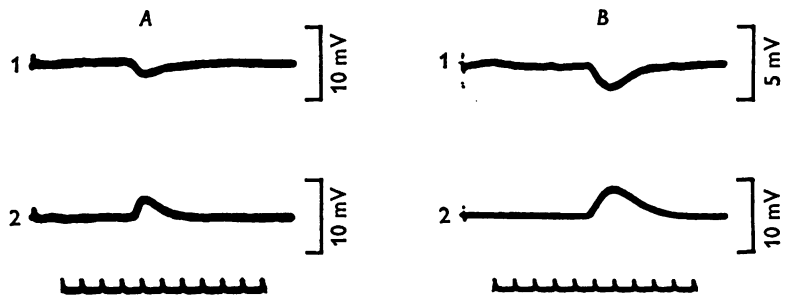

Fig. 7. Comparison between intra- and extracellular recordings of intrafusal muscle potentials evoked by the stimulation of a static fusimotor axon (*A*) and a dynamic one (*B*). *A* 1 and *B* 1, extracellular recordings. *A* 2 and *B* 2, intracellular recordings (zero membrane potential not indicated, membrane potentials respectively 50 mV and 40 mV). Time marker, 10 msec.

Fig. 7 shows the relation between extracellular (upper traces) and intracellular (lower traces) potentials the latter having been recorded immediately after the extracellular potentials. Potentials elicited by stimulation of a static fusimotor axon are represented in column *A*, whereas those evoked by a dynamic fusimotor stimulation are in column *B*. The impalement did not provoke a change in the shape of responses of the intrafusal muscle membrane. The relationship between the intra- and extracellular potential changes is evident.

In eight experiments the conditions were favourable for such a comparison during the stimulation of three dynamic axons and of five static axons. Every time the relationship between the intracellular and close extracellular records was obvious.

In some experiments the junction potential extra- and intracellularly recorded was preceded by a small diphasic spike potential. It is very likely that this spike potential was recorded from a nervous twig terminating on

the intrafusal muscle from which the junction potential was recorded (Hubbard & Schmidt, 1963). By comparing both records in such a situation it is possible to observe that the impalement did not provoke a response change either of the intrafusal muscle membrane, or of the motor terminal. These observations provide evidence that the finest branches of the fusimotor axon have not been struck by the micro-electrode and that a relative depression of the intrafusal neuromuscular junction has not been produced by the impalement.

So, when the extracellular potentials are compared with potential changes recorded intracellularly, a close correspondence is found to exist between them. This makes it possible to assign a physiological nature to the junction potential recorded intracellularly and to exclude effects attributable to injury.

DISCUSSION

The electrical changes elicited in intrafusal muscle fibres by stimulation of fusimotor axons can be divided into two classes: action potentials, and junction potentials showing with repetitive stimuli some degree of facilitation which sometimes elicits spike potentials.

One third of intracellularly recorded potentials are action potentials. From the observations of Bessou & Laporte (1965*a*) it may be thought that most of these potentials are propagated only along a polar region. Such an incomplete propagation possibly explains abortive spikes recorded immediately after impaling the intrafusal muscle fibre. These potentials could be interpreted as an intracellular recording of electrotonic spread of spike potentials generated in the opposite pole of the impaled intrafusal fibre. These abortive spikes look like the blocked spike intracellularly recorded from antidromically activated motoneurones (Brock *et al.* 1953).

Spike potentials were only evoked by static axon stimulation while dynamic axon stimulation never gave propagated potentials even after repetitive stimulation. There are several reasons for believing this to be a reliable statement: (i) the ratio of the numbers of prepared axons to penetrated intrafusal muscle fibres is approximately the same for both dynamic and static axons. So the diameter of the intrafusal muscle fibres innervated by dynamic fusimotor axons is unlikely to be smaller than that of the intrafusal muscle fibres innervated by static fusimotor axons, and consequently will not be more susceptible to damage from the micro-electrode; (ii) the stimulation of fourteen dynamic fusimotor axons never evoked spike potentials while stimulating the same number of static fusimotor axons evoked spikes on several occasions.

Two thirds of intracellularly recorded potentials are junction potentials. The physiological nature of these potentials has been demonstrated by

comparing extra- and intracellular potential changes evoked by fusimotor axon stimulation. Such a method has been successfully used by Fatt (1957*a*, *b*) in the studies either of electrical potentials occurring around a neurone during its antidromic activation or of electrical events occurring during synaptic activation of a motoneurone.

Junction potentials are evoked by stimulating some static axons and all dynamic axons. Most of these local potentials appear to originate in the juxta-equatorial and midpolar regions, i.e. in the transition zone between intra- and extra-capsular polar regions (Fig. 1). Since the neuromuscular junctions found in this region of the spindle may be trail or plate endings (Barker, Stacey & Adal, 1970), it cannot be concluded which of two types of synapse elicits junction potentials or prepotentials initiating spike potentials. So far, dynamic axons have never been demonstrated to innervate trail endings while there is direct evidence that static axons do so (Barker, Emonet-Dénand, Laporte, Proske & Stacey, 1970, 1971).

The extreme diversity in the shapes of junction potentials may be attributed to the morphological variability of trail endings. Junction potentials which look like the frog extrafusal muscle end-plate potentials described by Fatt & Katz (1951) could by analogy be assumed to be restricted to the area that generates the end-plate potential and to originate from synaptic contracts uniformly distributed over a restricted region of the intrafusal muscle membrane. Junction potentials showing notches on their rising or falling phases may be attributed to the uneven distribution of the synaptic contacts upon the intrafusal muscle fibre. Consequently there is an electrotonic distortion and a temporal dispersion of the potential transients. This distortion has been directly demonstrated in frog muscle by Fatt & Katz (1951). The temporal dispersion may arise from small differences in conduction time of the motor terminals or from variations in synaptic delay at the synaptic contacts.

Intracellular recordings confirm the interpretation given by Bessou & Laporte (1965*a*) of spindle potentials recorded from the tenuissimus muscle surface. It is noteworthy that the spindle site from which the junction potentials have been intracellularly recorded on stimulating dynamic fusimotor axons corresponds to the site of extracellularly recorded monophasic potentials. If this site differed for the two modes of recording, it would be impossible to infer the existence of non-propagated potentials. Moreover, the agreement of the two sets of experimental findings make it unlikely that slackening of the intrafusal muscle fibre following the spindle dissection can explain the absence of spikes due to a low output of transmitter from nerve endings (Hutter & Trautwein, 1956).

The mechanism of static or dynamic actions of a fusimotor axon cannot be correlated with a difference in the type of post-synaptic response.

Indeed, static axons evoked spikes or junction potentials and it has been observed in nine experiments that the stimulation of the same static axon could evoke during successive impalements, probably in different intrafusal muscle fibres, spikes or junction potentials.

Multineuronal innervation was found in two instances; this kind of motor innervation could be more frequent taking into account the fact that conduction may be blocked in the equatorial region of the intrafusal muscle fibre and that electrical events may not be recorded when they arise in innervation foci remote from the tip of the micro-electrode.

Since the stimulation of some single static fusimotor axons elicits propagated action potentials the existence of a twitch system in the intrafusal muscle may be inferred. This conclusion fits very well with the high frequencies of stimulation required to obtain a smooth 'frequencygram' during tetanic stimulation of some static fusimotor axons (Bessou, Laporte & Pagès, 1968).

Local contraction is probably associated with junction potentials evoked by fusimotor axon stimulation, even though such a stimulation may not elicit a spike potential following summation. The indirect evidence for a coupling between junction potential and local contraction is the small increase in the discharge rate of spindle primary endings which has been observed many times on the 'frequencygrams' during single shock stimulation of dynamic fusimotor axons (Bessou *et al.* 1968). The magnitude of the mechanical response elicited by junction potentials in the intrafusal muscle fibre is probably roughly related to the potential amplitude. The smooth 'frequencygrams' recorded during repetitive stimulation of dynamic fusimotor axons showed a rising phase becoming progressively shorter and a plateau progressively higher as the rate of the stimulation increased (Bessou *et al.* 1968). Thus the contraction elicited by local depolarization produces a tension which may be graded because the depolarization also can be graded. Such a coupling between local potential and local contraction is found in muscles of some arthropods and in the slow muscle system of the frog. In these cases, the neuromuscular junction is fairly similar in morphology to the trail endings of intrafusal muscle fibres of cat's spindles (see the reviews of Hoyle, 1957; Barker, 1968; Hess, 1970).

Repetitive stimulation of dynamic fusimotor axons never evokes movement of the partly dissected spindle poles (Bessou & Pagès, 1967) and never elicits visible shifts of the reference point under the binocular microscope ($\times 50$ magnification). This feature may be due either to the weakness of the contraction or resulting from the excessive compliance between the contraction site and the region under observation.

Static effects exerted by some single fusimotor axons result from both twitch and local contractions, simultaneously occurring in the intrafusal

muscle bundle, since in nine experiments the stimulation of a single static axon elicited both spike and junction potentials, probably in different muscle fibres. Such findings do not permit the conclusion that static effects can be attributed to either twitch or local contractions of all muscle fibres innervated by an axon. On the other hand, dynamic effects exerted by fusimotor axons are only associated with local contractions in all of the muscle fibres innervated by these axons.

We would like to thank Dr L.-M. Leitner, Dr U. Proske and Dr P. H. Ellaway for valuable advice during the preparation of this manuscript. This investigation was supported by a research grant from the Fondation pour la Recherche Médicale Française.

REFERENCES

BARKER, D. (1968). L'innervation motrice du muscle strié des vertébrés. *Actual. neurophysiol.* **8**, 23–71.

BARKER, D., EMONET-DÉNAND, F., LAPORTE, Y., PROSKE, U. & STACEY, M. (1970). Identification des terminaisons motrices des fibres fusimotrices statiques chez le Chat. *C. r. hebd. Séanc. Acad. Sci., Paris* **271**, 1203–1206.

BARKER, D., EMONET-DÉNAND, F., LAPORTE, Y., PROSKE, U. & STACEY, M. (1971). Identification of the endings and function of cat fusimotor fibres. *J. Physiol.* **216**, 51–52*P*.

BARKER, D., STACEY, M. J. & ADAL, M. N. (1970). Fusimotor innervation in the cat. *Phil. Trans. R. Soc.* B **258**, 315–346.

BESSOU, P., EMONET-DÉNAND, F. & LAPORTE, Y. (1963). Occurrence of intrafusal muscle fibres innervation by branches of slow α motor fibres in the cat. *Nature, Lond.* **198**, 594–595.

BESSOU, P., EMONET-DÉNAND, F. & LAPORTE, Y. (1965). Motor fibres innervating extrafusal and intrafusal muscle fibres in the cat. *J. Physiol.* **180**, 649–672.

BESSOU, P. & LAPORTE, Y. (1965*a*). Potentiels fusoriaux provoqués par la stimulation de fibres fusimotrices, chez le Chat. *C. r. hebd. Séanc. Acad. Sci., Paris* **262**, 1970–1972.

BESSOU, P. & LAPORTE, Y. (1965*b*). Technique de préparation d'une fibre afférente I et d'une fibre afférente II innervant le même fuseau neuromusculaire, chez le Chat. *J. Physiol. Paris* **57**, 511–520.

BESSOU, P., LAPORTE, Y. & PAGÈS, B. (1968). Frequencygrams of spindle primary endings elicited by stimulation of static and dynamic fusimotor fibres. *J. Physiol.* **196**, 47–63.

BESSOU, P., LAPORTE, Y. & PAGÈS, B. (1970). Phénomènes de facilitation temporelle observés au niveau des synapses des fibres fusimotrices statiques, chez le Chat. *C. r. hebd. Séanc. Acad. Sci., Paris* **271**, 2145–2148.

BESSOU, P. & PAGÈS, B. (1967). Enregistrement de mouvements de fuseaux neuromusculaires partiellement disséqués consécutifs à la stimulation de fibres fusimotrices statiques chez le Chat. *C. r. hebd. Séanc. Acad. Sci., Paris* **265**, 351–353.

BESSOU, P. & PAGÈS, B. (1969). Intracellular recording from spindle muscle fibres of potentials elicited by static fusimotor axons in the cat. *Life Sci. Oxford* **8**, 417–419.

BOYD, I. A. (1962). The structure and innervation of the nuclear bag muscle fibre system and the nuclear chain muscle fibre system in mammalian muscle spindles. *Phil. Trans. R. Soc.* B **245**, 81–136.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1953). Intracellular recording from antidromically activated motoneurones. *J. Physiol.* **122**, 429–461.

EYZAGUIRRE, C. (1960). The electrical activity of mammalian intrafusal fibres. *J. Physiol.* **150**, 169–185.

FATT, P. (1957*a*). Electric potentials occurring around a neurone during its antidromic activation. *J. Neurophysiol.* **20**, 27–60.

FATT, P. (1957*b*). Sequence of events in synaptic activation of a motoneurone. *J. Neurophysiol.* **20**, 61–80.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol.* **115**, 320–370.

HESS, A. (1970). Vertebrate slow muscle fibres. *Physiol. Rev.* **50**, 40–62.

HOYLE, G. (1957). Comparative physiology of the nervous control of muscular contraction. *Cambridge Monographs in Experimental Biology*, ed. SALT, G. Cambridge: Cambridge University Press.

HUBBARD, J. I. & SCHMIDT, R. F. (1963). An electrophysiological investigation of mammalian motor nerve terminals. *J. Physiol.* **166**, 145–167.

HUTTER, O. F. & TRAUTWEIN, W. (1956). Neuromuscular facilitation by stretch of motor nerve endings. *J. Physiol.* **133**, 610–625.

KOKETSU, K. & NISHI, S. (1957*a*). Action potentials of single intrafusal muscle fibres of frogs. *J. Physiol.* **137**, 193–209.

KOKETSU, K. & NISHI, S. (1957*b*). An analysis of junctional potentials of intrafusal muscle fibres in frogs. *J. Physiol.* **139**, 15–26.

KUFFLER, S. W., HUNT, C. C. & QUILLIAM, J. P. (1951). Function of medullated small-nerve fibres in mammalian ventral roots. Efferent muscle spindle innervation. *J. Neurophysiol.* **14**, 29–54.

MATTHEWS, P. B. C. (1962). The differentiation of two types of fusimotor fibres by their effects on dynamic response of muscle spindle primary endings. *Q. Jl exp. Physiol.* **47**, 324–333.