

AFTER-EFFECTS OF FUSIMOTOR STIMULATION ON THE RESPONSE OF MUSCLE SPINDLE PRIMARY AFFERENT ENDINGS

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SUMMARY

1. The experiments were performed on the soleus muscle of the anaesthetized cat in which the ventral roots had been cut.

2. A short period of repetitive stimulation of a single fusimotor fibre which influenced a particular spindle primary ending invariably caused a characteristic alteration in the response of the same ending to a subsequently applied ramp stretch of the muscle. The change consisted in the appearance of a burst of impulses at the beginning of the stretch where none had been present before. Occasionally, such an 'initial burst' was spontaneously present; it was then enhanced following fusimotor stimulation.

3. This after-effect of fusimotor stimulation was abolished by a subsequent stretch of the muscle, but otherwise persisted for over a minute.

4. When the muscle was released to below the length at which the spindle had been facilitated and a testing stretch applied from the new initial length there was no burst of impulses at the beginning of stretch. There was, however, a burst as the muscle was stretched through the length at which the fusimotor fibre had been stimulated.

5. These effects are suggested to be due to the persistence of stable bonds between the actin and myosin filaments of the intrafusal fibres, so that their previously activated regions were 'stuck' at the length they were when the fusimotor stimulation was applied.

6. Such effects were produced both by static and by dynamic fusimotor fibres. The effects of the two kinds of fusimotor fibre, however, appeared to be mediated by different intrafusal muscle fibres. This was shown by stimulating one kind of fibre with the muscle slightly stretched, then releasing the muscle a few mm and stimulating the other kind of fibre to the same spindle. A subsequent testing stretch then elicited two bursts, one at the beginning and one in the middle of the stretch.

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INTRODUCTION

In 1951, Hunt & Kuffler described a long-lasting increase in the responsiveness of muscle spindle afferent endings to fusimotor excitation following a preceding period of stimulation of a single fusimotor fibre. It was sometimes accompanied by an increase in the 'resting' discharge of the ending (Kuffler, Hunt & Quilliam, 1951). They named this effect 'post-excitatory facilitation' and because it was abolished by a brief stretch of the muscle they suggested that it depended upon 'some plastic property of the intrafusal fibres' rather than upon any change in the afferent terminals themselves. The present paper describes some new observations on the after-effect of fusimotor stimulation on the response of spindle primary endings to ramp stretches. These suggest that the 'plastic change' after fusimotor stimulation may be the persistence of relatively stable bonds between the actin and the myosin filaments in the striated portions of the intrafusal muscle fibres (cf. D. K. Hill, 1968, for frog's skeletal muscle). In addition, the independence of the after-effects of stimulation of a static and of a dynamic fusimotor fibre to the same spindle favours the idea that these two kinds of fusimotor fibre achieve their different actions by innervating different intrafusal muscle fibres.

METHODS

The experiments were performed on cats anaesthetized with pentobarbitone sodium. The results presently described were obtained from nine preparations which were devoted to the study of post-excitatory facilitation, but confirmatory observations have been obtained in approximately a similar number of further preparations in the course of other work. The soleus muscle was used and the limb otherwise widely denervated. The experimental methods have already been described in detail (Matthews, 1962; Crowe & Matthews, 1964).

The discharge of single muscle spindle afferents were recorded from dorsal root filaments. Those studied were judged to come from primary endings on the basis of having a conduction velocity of over 80 m/sec and on being appreciably sensitive to dynamic stimuli (Matthews, 1964). Functionally single fusimotor fibres influencing the spindle afferent studied were isolated in ventral root filaments. All those studied had conduction velocities between 20 and 55 m/sec; and their stimulation produced no overt contraction (tension < 50 mN; $1\text{ N} \approx 100\text{ g wt.}$). They were initially detected by their excitatory action on the afferent on stimulating them simultaneously with a number of α motor fibres in a relatively thick ventral root filament (Matthews, 1962). They were classified as static or dynamic fusimotor fibres on the basis of their effect on the response of the afferent to a ramp stretch of the muscle of several mm amplitude. One of the single dynamic fibres studied was actually a β fibre which appeared to innervate extrafusal muscle fibres as well as intrafusal fibres (for precautions in identification see Brown, Crowe & Matthews, 1965). It had a conduction velocity of 59 m/sec and produced a tetanic contraction developing about 50 mN tension.

The appropriate dorsal and ventral roots were severed so that the muscle was

functionally isolated from control by the central nervous system. The tendon of the soleus with a piece of its bony insertion was attached to an electro-magnetic servo-controlled 'stretcher'. The maximum extension applied usually stretched the muscle up to approximately the maximum length it could take up in the body when its attachments were intact (i.e. with an angle of about 45° between the foot and the leg). The initial length from which the stretches started was usually 8 mm less.

RESULTS

The initial burst. For some minutes after stimulating a single fusimotor fibre influencing a particular primary ending there was invariably a characteristic change in the response of the ending to a ramp stretch of the muscle. This change was the appearance of an 'initial burst' of action potentials at relatively high frequency at the beginning of the stretch. Typical examples are shown in Fig. 1 *c* and *d*, respectively following repetitive stimulation of a dynamic and of a static fusimotor fibre. The

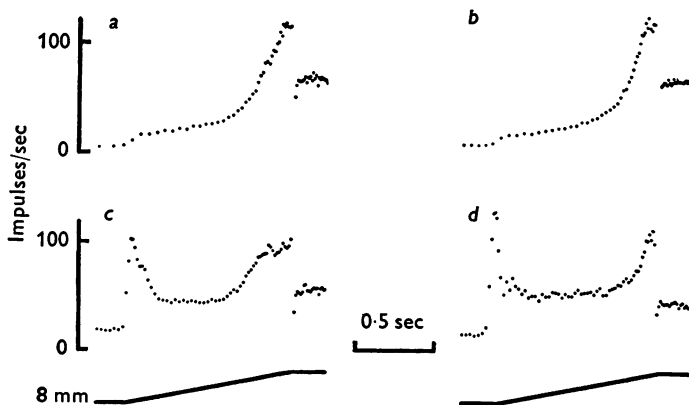


Fig. 1. The development of an 'initial burst' in response to a testing ramp stretch applied after a preceding period of fusimotor stimulation. *a*, *b* responses of a primary afferent ending to a stretch (8 mm at 8 mm/sec) applied 25 sec after a preceding stretch and release of the same size. *c*, *d* responses of same afferent to similar stretches applied about 15 sec after a short period of repetitive fusimotor stimulation. Before *c*, a dynamic fibre was stimulated at 170/sec for 1.7 sec with the muscle at the initial length. Before *d*, a static fibre was stimulated at 150/sec for 1.5 sec. During the fusimotor stimulation the afferent discharge increased to 80 and 105 impulses/sec for *c* and *d* respectively. (The records were taken with a reciprocal pulse-interval display in which each dot represents an action potential and its height above zero gives the 'instantaneous frequency' i.e. the reciprocal of the time interval from the immediately preceding spike. The time calibration in this and subsequent records refers to the dynamic phase of the ramp stretch. When the muscle was at a constant length the time scale was compressed by a factor of 2. The control responses *a* and *b* were recorded just before records *c* and *d* respectively, as the two fusimotor fibres were studied at different times in the experiment.)

control responses of the ending to the same stretch applied without preceding fusimotor stimulation are shown above (Fig. 1*a, b*). These passive responses were obtained 15 sec after releasing the muscle from a similar sized extension, and a single such stretch was sufficient to abolish the facilitatory effect of an earlier period of fusimotor stimulation, at any rate as far as testing stretches of this size were concerned (see later). Sometimes an initial burst was present spontaneously in the response of the unfacilitated ending (Matthews, 1963; Henatsch, 1967; Schäfer, 1967); the frequency with which this occurred seemed to vary between preparations. When a burst was present spontaneously, a period of preceding fusimotor stimulation markedly increased the maximum frequency reached in the burst; however, in general, we preferred to study endings without spontaneous bursts. The after-effect of fusimotor stimulation would presumably have disappeared if sufficient time had been allowed to elapse. In the present experiments we regularly confirmed that the effect persisted without appreciable signs of decay for over 2 min, but we did not extend our observations to longer times. When the muscle was left at a constant length for 5 min or more without fusimotor stimulation there was no appreciable development of an initial burst in those endings which did not normally possess one; those that did, developed it within a few seconds after a preceding release. The time course of the development of the state following fusimotor stimulation which leads to the initial burst was not studied in detail. When the matter was tested an initial burst was found within 1 sec after termination of the stimulation, though it did not reach its full size until the interval was increased to several seconds. It is already known that a stretch applied during repetitive fusimotor stimulation often produces no burst at all, and if a burst is present it is much less marked than those described here and usually consists of only one or two impulses (Matthews, 1962; Crowe & Matthews, 1964; Brown *et al.* 1965). The present effect would appear to be another facet of the post-excitatory facilitation described by Hunt & Kuffler (1951), for both are long-lasting and both are abolished by an intervening stretch. In this they differ from a short term facilitation found in rat spindles by Kidd (1964 *a, b*) which was not annulled by stretch.

In order to obtain facilitation, an appreciable number of stimuli had to be applied to any fusimotor fibre studied, and no observable effect was produced by single stimuli. The precise relationships between the parameters of the stimulating train and the degree of facilitation were not studied. As a rough rule, however, the facilitation produced by any particular fusimotor fibre increased in proportion to the increase in the frequency of discharge of the afferent occurring during the fusimotor stimulation and the same was approximately true for the effects of different fusimotor fibres. Thus, increasing the frequency of a stimulus train applied for a constant period of 1–2 sec progressively increased the facilitation for frequencies of

stimulation up to at least 200/sec, just as it does on the immediate afferent discharge (Matthews, 1962; Andersson, Lennerstrand & Thoden, 1968). On the other hand, increasing the duration of a train of stimuli at constant frequency had little or no effect beyond the 0.5–1 sec required to bring the afferent discharge to a new constant level. Most of the experiments were done with trains of stimuli at about 150/sec applied for about 1.5 sec. Interestingly, after the spindle had been facilitated by a short period of high-frequency stimulation (say at 150/sec) an enhanced initial burst was still present when the muscle was stretched while the same fusimotor fibre was being continuously stimulated at a low frequency (say 30/sec).

The highest frequency discharge developed in the course of an initial burst increased progressively with the velocity of stretching; it follows that so also did the increase in frequency above the pre-existing resting level (cf. Henatsch, 1967).

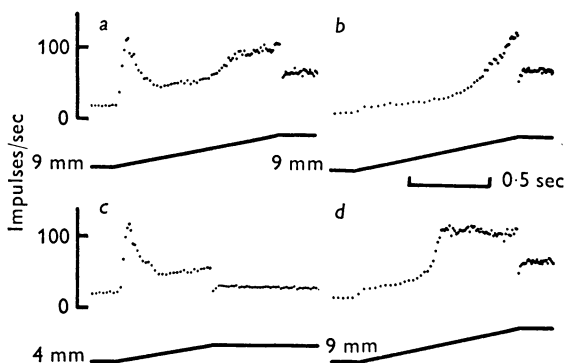


Fig. 2. Records showing that effects of fusimotor stimulation on the response to the final part of a large amplitude test stretch persist in spite of an intervening smaller amplitude stretch. *a*, response to a 9 mm stretch applied after preceding stimulation of a dynamic fusimotor fibre. *b*, response to a 9 mm stretch in the absence of preceding fusimotor stimulation. *c*, response to a 4 mm stretch applied after a period of fusimotor stimulation as in *a*. *d*, response to a 9 mm stretch applied 10 sec after releasing the muscle from the 4 mm stretch shown in *c*. (Same experiment as Fig. 1 but different display sensitivity.)

Similar behaviour has already been illustrated for initial bursts occurring in the absence of preceding fusimotor stimulation (Matthews, 1963; Schäfer, 1967). Some slight 'burst' persisted for velocities down to 1–2 mm/sec, as shown by a small hump on the records of instantaneous frequency against length. The time course of development and decay of the burst was, however, then much slower than at higher velocities as if the burst depended upon stretching through a finite distance rather than upon stretching for a finite time.

Five secondary endings were studied on stimulating single fusimotor fibres, which were presumed to be static ones (cf. Brown, Engberg & Matthews, 1967*a*). Definite initial bursts in response to a ramp stretch were then found for two of the afferents (conduction velocities, 39 and 50 m/sec). The three other endings also showed changes in their discharge pattern at the beginning of stretch, but these were too slight to be considered as 'bursts'; during the progressive stretching the frequency of discharge never showed an initial peak, but rather increased slightly more rapidly than normal as the stretch progressed.

The difference between the response of the afferents to the testing stretch when it was facilitated and when it was unfacilitated was not restricted to the very beginning of the stretch. After the initial burst, the frequency of discharge of the afferent when facilitated tended to be higher throughout the dynamic phase of stretching than when the ending was unfacilitated. This may have been noted already in Fig. 1, but is perhaps more clearly seen on comparing Fig. 2*a* (facilitated response) with Fig. 2*b* (unfacilitated response). Thus the facilitation depends upon some change in the muscle spindle which can continue to manifest itself as the muscle is stretched through many mm. In consequence, it might be expected that a small stretch would not abolish all the facilitation, but would leave some residuum which could be disclosed by a larger stretch. This proved to be so, as illustrated in the bottom half of Fig. 2. The 9 mm stretch of Fig. 2*d* was applied immediately after the 4 mm stretch of Fig. 2*c*, which had been applied when the spindle was facilitated. Thus a stretch of any particular size de-facilitates the ending for further stretches of the same size, but does not abolish entirely the basic change in the spindle properties upon which the facilitation depends. This is readily understandable if the facilitation depends upon a change in the mechanical properties of the intrafusal muscle fibres which is abolished in any particular sarcomere when movement takes place within that sarcomere, but which otherwise persists in spite of a rise in intrafusal tension (see Discussion).

Response at final length. Paradoxically, it was regularly found that the discharge of the afferent at the final length for the first few seconds after completion of the dynamic phase of stretching was slightly lower when the ending was facilitated than when it was not. This effect, which was occasionally as much as 20 impulses/sec for a basic discharge around 40/sec, was more marked when the facilitation was produced by stimulation of a static rather than a dynamic fusimotor fibre. Similarly, near the termination of the dynamic phase of a large stretch the discharge of the facilitated ending was usually slightly below that of the unfacilitated ending. However, when tested some 10 sec or more after completion of the dynamic phase of stretching the discharge at the final length was much more similar in the facilitated and the unfacilitated state, because the unfacilitated ending showed an initial more rapid adaptation in its discharge. Occasionally, the facilitated ending showed an initial reversed adaptation, so that after the abrupt drop in frequency on completion of the dynamic phase of stretching there was a slow small increase in discharge for 1-2 sec. Such effects probably lend themselves to more than one type of explanation. One which is in line with the later discussion is as follows. After fusimotor stimulation the regions of the spindle which have just contracted become more rigid than normal; direct observation (Boyd, 1966) and the recording of spindle potentials (Bessou & Laporte, 1965) both suggest that repetitive fusimotor stimulation elicits localized rather than fully propagated contractions. During progressive ramp stretching of the muscle these rigid regions initially yield less than they otherwise would have done, with the consequence that other regions of the intrafusal fibres (possibly their poles) are forced to yield more than normal and so are relatively over-stretched when the final length is reached. If the normal

adaptation depends partly upon a yielding of the poles of the spindle then this would not occur to the same extent in the facilitated spindle, because the yielding would already have taken place during the progressive stretch. In addition, it is likely that following a localized intrafusal contraction the poles of the spindle remained over-stretched in comparison with what they were beforehand (following a release), and the previously contracted region remained somewhat shortened. Such explanations can be developed more formally in terms of a model of the spindle (cf. Matthews, 1964) which consists of a spring in series with two visco-elastic elements one of which also contains distributed static friction, but such elaboration would contribute little to the preceding qualitative description.

Effect of release on facilitation

The facilitation produced by fusimotor stimulation failed to manifest itself when the muscle was released to below the length at which the facilitation had been produced. This was so even though re-extending the

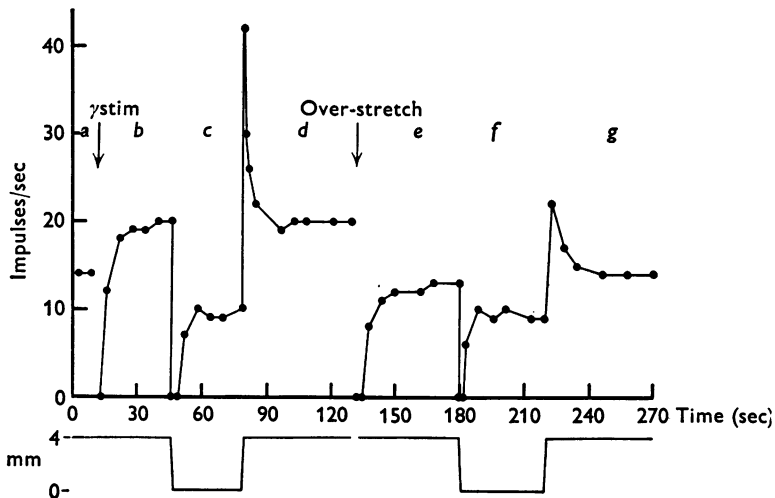


Fig. 3. The after-effect of stimulation of a static fusimotor fibre on the frequency of firing of a primary ending with the muscle at two different lengths. The facilitation of the discharge only manifested itself when the muscle was extended 4 mm, which was the extension at which the fusimotor fibre was stimulated, and there was no overt sign of the facilitation when the muscle was released. The facilitation at 4 mm extension was abolished by the 'over-stretch' which was a brief stretch of a further 4 mm. Further description in text. (Frequency of discharge measured by counting the integral number of spikes in a period of 1 sec.)

muscle to the original length showed that the basic change in spindle properties was still present. The facilitated ending thus behaved rather as if some part of the spindle had 'stuck' at the length it had happened to be when the facilitation had been produced.

Such behaviour was most simply seen in the effects of release on the steady discharge of the afferent when the muscle was at a constant length.

This is illustrated in Fig. 3 which is labelled serially to facilitate description. Period *a* shows the discharge of a primary ending while the muscle was kept extended by 4 mm from the arbitrary zero length. A single static fusimotor fibre was then stimulated at 150/sec for 1.5 sec. During the stimulation the discharge increased to 85/sec (not shown on graph), and this was followed by a period of 'depression' in which the afferent discharge was below its previous level. The discharge then rose to 20 impulses/sec (period *b*), which is appreciably above the previous level of 14/sec at *a*. This increase in the 'resting' discharge has already been described by Kuffler *et al.* (1951) as a part of post-excitatory facilitation, and was regularly seen in variable degree in the present experiments. On release of the muscle by 4 mm the ending was initially silent and then settled down to discharge at 10 impulses/sec (period *c* on graph). This discharge at zero length was the same as that seen earlier when the ending was unfacilitated (see also *f*); control experiments (not illustrated) showed that fusimotor stimulation at this length would facilitate the ending and increase its discharge. Thus no sign of the facilitation was present on release of the muscle by several mm. The muscle was then re-extended by 4 mm, when after an initial adaptation the discharge again settled to the facilitated level of 20 impulses/sec as would be expected if 'stuck' bonds had been pulled tight again (*d* on graph). Next the muscle was stretched a further 4 mm and immediately released again in order to destroy the facilitation by 'over-stretch' (discharge produced by the over-stretch not included in graph). After an initial silence, the discharge rose to a plateau level of 13 impulses/sec (*e*) which was approximately the same as when the muscle was originally at this length and the ending was unfacilitated (*a*, see also *g*). Subsequent release by 4 mm confirmed that the appropriate discharge rate for the unfacilitated ending with the muscle at zero extension was about 10 impulses/sec (*f*), as already stated. The final re-extension (*g*) again confirms that the unfacilitated rate for an extension of 4 mm was 14 impulses/sec. (It may be noted, however, that the frequency for an extension of 4 mm is marginally greater when the muscle has been stretched up to that length than when it has been released from a greater length (*g* compared with *e*.) Behaviour like that illustrated in Fig. 3 was found on all seven occasions on which the matter was investigated.

Frequently, however, stimulation of a single fusimotor fibre produced little subsequent increase in the mean frequency of discharge of an ending even though it caused the characteristic development of an initial burst. Simultaneous stimulation of two separate single fusimotor fibres tended to have rather more effect on the resting discharge than did either alone, and two such experiments are included in the seven quoted above. In earlier unpublished experiments with R. J. Harvey (cf. Harvey & Matthews, 1961) much more prominent facilitatory effects on the resting discharge were found than those described here and the increase in discharge might be as

much as 20 impulses/sec. In the earlier experiments the facilitation was produced by stimulating the muscle nerve with shocks supramaximal for the γ efferents, and which thus excited the whole of the fusimotor supply to the spindle studied along with all the α motor fibres to soleus (the facilitation was not present when the stimuli were supramaximal for α fibres and submaximal for γ fibres). The facilitation so produced behaved identically on release of the muscle to that presently produced by stimulating single fusimotor fibres.

Figure 3 shows another point of interest, namely the slow development of the increase in the 'resting' discharge following the fusimotor stimulation, which was typical. It might be suspected that the slow redevelopment of the discharge was due to a continued slow contraction of the intrafusal fibres following their activation (i.e. a continued 'active' shortening of the region of intrafusal fibre which had just been contracting more powerfully.) This cannot be excluded by the present experiments, but an alternative explanation is that the slow increase in discharge was due to a slow passive shortening of the polar regions of the spindle which had not been contracting, but which had been over-extended by a local contraction of some other region of the intrafusal fibres. In favour of this latter idea is the similarity of the time course of redevelopment of discharge following fusimotor stimulation (*b* on graph) and following the 'over-stretch' which was at the same length of the muscle as the stimulation. The increased final steady discharge in the facilitated state would then result from 'sticking' of the previously contracted regions of the intrafusal fibres at a shorter than normal length. The poles in shortening towards their original length would thus produce a greater intrafusal tension leading to a greater afferent discharge. In line with this explanation was the finding that when it was facilitated an ending was more sensitive to vibration applied at that length of the muscle than it was when unfacilitated (100–300 Hz tested, cf. Brown, Engberg & Matthews, 1967*b*). Increased sensitivity to vibration in the unfacilitated state is associated with the increases in intrafusal tension produced by stretching the muscle or by intrafusal contraction (Brown *et al.* 1967*b*). In addition, the use of vibration showed that the slow recovery of discharge following fusimotor stimulation or stretch was not primarily due to any fatigue or adaptation of the ending dependent upon its just having discharged a number of impulses at relatively high frequency. Similar or greater increases in afferent discharge produced by vibrating the muscle often caused no depression of the resting discharge (cf. Brown *et al.* 1967, Fig. 1); this was so in preparations which showed a slow recovery following stimulation of either kind of fusimotor fibre.

Intermediate bursts. The behaviour just described suggests that the facilitated spindle is 'stuck' at the length at which the fusimotor stimulation was applied. Such an interpretation was favoured by the results obtained by facilitating an ending, then releasing the muscle and subsequently applying a ramp stretch of greater extent than the release. The ending then no longer gave an initial burst of impulses at the beginning of the stretch, but it did give a burst of impulses in the middle of the ramp stretch as the muscle was stretched through the length at which the ending had been facilitated. This is illustrated in Fig. 4, which shows a series of stretches all applied from the same length, but with the preceding facilitation applied at progressively greater lengths of the muscle. Such behaviour was seen in nineteen out of twenty-one times it was sought, and

following stimulation of either a static or a dynamic fusimotor fibre (the two failures to observe the effect followed stimulation of dynamic fusimotor fibres which had only a weak effect at the initial length). The precise length at which the intermediate burst began tended to be a little below the length at which the ending had been facilitated, and more so when the facilitation was applied with the muscle appreciably extended. This is

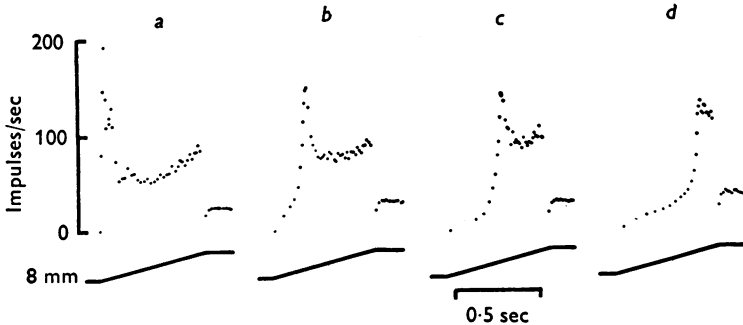


Fig. 4. 'Bursts' produced in the middle of the testing stretch following fusimotor stimulation with the muscle at an intermediate length. *a*, preceding stimulation of a dynamic fusimotor fibre at 150/sec with muscle at initial length from which the test stretch was applied. *b*, the fusimotor fibre was stimulated when the muscle was extended by 3 mm from the initial length shown; the muscle was then released to the original length and the testing stretch applied 10 sec. later. *c*, fusimotor stimulation with the muscle extended 6 mm. and then subsequently released. *d*, no fusimotor stimulation. (Stretch at 13 mm/sec. Similar parameters of fusimotor stimulation throughout.)

what would be expected if the 'stuck' region of the intrafusal fibres is in series with a passive region which shortens slightly when the muscle is released, and by an amount which depends upon the tension in it (*vide supra*).

Independence of facilitation produced by static and by dynamic fusimotor fibres

There is currently some uncertainty as to whether the rather different effects of the static and dynamic fusimotor fibres are mediated by separate intrafusal fibres with different contractile properties (Jansen & Matthews, 1962; Crowe & Matthews, 1964; Brown & Matthews, 1966) or whether by two different kinds of contraction elicited from a single intrafusal muscle fibre by two different kinds of motor ending upon it (Bessou & Laporte, 1966; Barker, 1967). It therefore seemed of interest to study the interaction of the facilitations produced by the two kinds of fusimotor fibre, for the results might be expected to bear upon this problem. On either hypothesis on the mode of fusimotor action the simultaneous stimulation

of a fusimotor fibre of each kind might be expected to produce more enhancement of the initial burst than stimulation of either alone. Not surprisingly, this is what did happen when the matter was tested. However, the two hypotheses lead to rather different expectations of what would happen when the fusimotor fibres are stimulated separately, the first with the muscle stretched, and the second after releasing the muscle a few millimetres. If the two kinds of fusimotor fibre supply different intrafusal muscle fibres, each kind of intrafusal muscle might then be expected to be 'stuck' at a different length, corresponding to that at the time of stimulation of its own fusimotor fibre, so that a single testing stretch should elicit a burst both at the beginning and in the middle. On the other hand, if the same intrafusal muscle fibres were excited by both fusimotor fibres, the intrafusal muscle fibres might be expected to be 'stuck' at the shortest length at which the stimulation had been applied, so that only a single burst at the beginning of the stretch would be expected. This is what always happened in control experiments in which a single fusimotor fibre was stimulated, first with the muscle extended and then again on releasing it. The same result, of a single initial burst, was also obtained in four further control experiments in which two separate fusimotor fibres of the same functional kind were studied in this manner (two pairs of static fibres, one pair of dynamic γ fibres, and one pair consisting of a dynamic fibre of γ velocity and a dynamic β fibre (see Methods)).

When two different kinds of fusimotor fibre were used to facilitate the spindle at two different lengths the regular finding was that two bursts occurred in response to a single ramp stretch. The bottom records of Fig. 5 show such double bursts. Those on the left were elicited by stimulating a dynamic fibre at an intermediate length and a static fibre at the initial length, and those on the right were obtained by reversing the order of stimulation of the two fibres. The various other records show the un-facilitated response of the ending (top) and the single bursts produced after facilitation at the appropriate length by each of the fusimotor fibres alone. It may be seen that the absolute frequency of discharge reached in each of the bursts is approximately the same whether it occurred alone, or as part of a double burst, though of course the increment of frequency in the burst is different in the two cases. Similar results were obtained for four other primary endings on stimulating a pair of fusimotor fibres of either kind. Another two primary endings gave double bursts on using a pair of filaments, one of which contained only a single fusimotor fibre while the other included one or more α fibres as well. In addition, the ending of Fig. 5 gave double bursts when the effect of the dynamic fibre was studied along with a filament containing a different static fusimotor fibre and several α fibres. The concomitant stimulation of the α fibres,

however, always produced less than 1.5 N tension and would not seem to impair the significance of the finding of a double burst. Control experiments showed that the spindle did not develop an initial burst after a contraction of the main muscle had been elicited by stimulating one or more single α fibres without accompanying fusimotor activation. All these results would

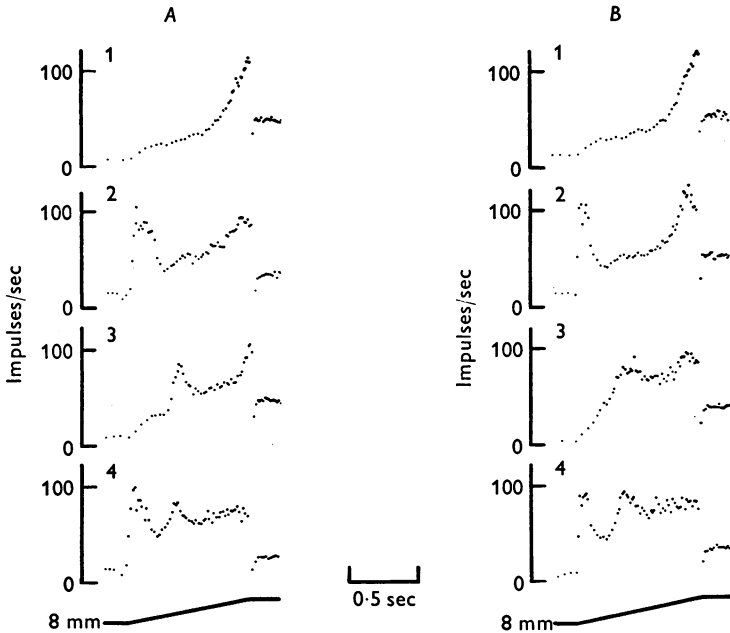


Fig. 5. The appearance of two bursts in response to a single ramp stretch applied after separate stimulation of a static and of a dynamic fusimotor fibre with the muscle at two different lengths. Left hand records (*A* 1-4), static fusimotor fibre stimulated with the muscle at the initial length and dynamic fibre with the muscle at an intermediate length; right hand records (*B* 1-4), vice versa. *A* 1, *B* 1 response of afferent to test stretch applied in the absence of preceding fusimotor stimulation. *A* 2, *B* 2, preceding fusimotor stimulation with the muscle at the initial length showing typical initial bursts (*A* 2 with static fusimotor stimulation, *B* 2 with dynamic stimulation). *A* 3, *B* 3 preceding fusimotor stimulation with the muscle extended by 3 mm, and muscle released before the test stretch; test stretch then evoked intermediate bursts. *A* 4, *B* 4, response to test stretch after preceding stimulation of one fusimotor fibre with the muscle extended 3 mm (as in *A* 3, *B* 3), and then stimulation of the other after releasing the muscle to the initial length (as in *A* 2, *B* 2). The ending then showed burst responses at both the initial and at the intermediate lengths. (The slight drop in frequency at the beginning of the ramp stretch in some of the records is fortuitous. It arose simply from the chance coincidence of the stretch with a slight rhythmic irregularity in the discharge of the ending which was present only at the initial length, and which is sometimes present for unknown reasons).

seem to favour the hypothesis that the static and dynamic fusimotor nerve fibres innervate different intrafusal muscle fibres.

In a further four primary endings studied with a pair consisting of a static and of a dynamic fibre (two with and two without α fibres) a good double burst was obtained when one fibre was stimulated at the intermediate length and the other fibre at the initial length, but was not found when the stimulation was the other way round. A double burst was never absent for both sequences of stimulation. Such asymmetry of fusimotor after-effect was found when the effectiveness of one fibre by itself was appreciably weaker than that of the other, and its explanation seemed to be an occlusion of a rather small intermediate burst by the prolonged tail of a large initial burst. Previous experiments (Crowe & Matthews, 1964; Lennerstrand, 1968) have suggested that there may be more than one pace-maker at which impulses may be initiated in the termination of a single primary afferent fibre, and that the discharge seen in the main afferent fibre at any time is that of the pace-maker with the highest frequency. This could lead to occlusion of the present type, and is suggested as occurring in the present experiments by the finding that the absolute frequency of discharge of the intermediate burst was approximately the same whether or not it was preceded by an initial burst (cf. Fig. 5, i.e. there was normally no summation of the intermediate burst with the tail of the initial burst). Further, occlusion of the intermediate burst was only found when the frequency of discharge in the intermediate burst, occurring alone, was less than that found at the same point on the ramp stretch after producing a burst at the initial length by stimulating the other fusimotor fibre.

In one experiment a slightly different form of asymmetry was seen. On stimulating a pair of fusimotor fibres one way round a double burst was seen; but on stimulating them the other way round the intermediate burst was shifted by 2 mm to the left, so that it occurred at an appreciably shorter length of the muscle than it did when it was produced on its own, though it still did not become confluent with the initial burst. The origin of this shifting is not immediately apparent, but its single occurrence does not appear to favour either hypothesis.

In the experiment of Fig. 5*a*, a β fibre, which supplied both intra- and extrafusal muscle fibres (see Methods) was studied as well as single γ fibres. The β fibre had a dynamic effect when stimulated throughout a ramp stretch, and when stimulated some seconds before a stretch caused the appearance of a typical initial burst. The usual double burst was found when the β fibre was stimulated at the initial length and either of two static fibres at the intermediate length. However, when the β fibre was stimulated at the intermediate length, it produced only a weak intermediate burst, and this was occluded, as described above, when either of the two static fibres was stimulated at the initial length.

It might be suggested that the double bursts arose fortuitously because the particular fusimotor fibres being studied happened to supply different intrafusal muscle fibres, and that this was in no way connected with whether their motor action was static or dynamic. This seems unlikely in view of the regularity of occurrence of double bursts. In addition, in the experiment of Fig. 5, we obtained as many filaments containing fibres of each kind as possible, and stimulated all of these together in place of a single fibre of each kind (two statics and three dynamics, one of which was the β fibre). Typical double bursts were again obtained and it seems unlikely

that this could have arisen by chance. However, the present experiments cannot eliminate the possibility that a single intrafusal fibre is capable of producing two different actions upon the primary ending; but they are far more readily explained on the hypothesis that the static and dynamic effects are due to the contraction of two different sets of intrafusal muscle fibres.

DISCUSSION

The present results find ready interpretation in terms of the sliding filament hypothesis of muscular contraction and the supposition that following fusimotor stimulation a number of rather stable bonds persist between the actin and the myosin filaments in the previously activated regions of the intrafusal muscle fibres. This is in line with Hill's (1968) recent suggestion that a few such stable bonds exist in ordinary resting striated muscle (frog sartorius) where they give rise both to a slight resting tension and to a 'short-range' elastic resistance which gives way on stretching the muscle through any distance. Denny-Brown's (1929) experiments showing a 'preliminary rigidity' on beginning a slow stretch of some muscles of the cat may now also be similarly interpreted. The discharge of the spindle primary afferent may be taken to reflect the deformation of the central regions of the intrafusal fibres, around which the ending spirals. Hence the occurrence of an initial burst in the present experiments may be attributed to an initial over-extension of the central region of the intrafusal fibres relative to their polar regions, and thus taken to show that at the beginning of a stretch the polar regions are relatively more rigid than the central regions, in comparison with their stiffnesses later in the same stretch. The central regions of the intrafusal fibres are unlikely to change their properties as a result of fusimotor activation, for they contain very few myofilaments and are also devoid of motor nerve endings. The more distal regions of the intrafusal fibres have, however, been seen to contract on nerve stimulation (Boyd, 1966). Thus some or all of the intrafusal muscle fibres would appear to have an increased short-range elastic resistance along part of their length following their contraction. The persistence of stable bonds between the actin and the myosin filaments seems the only available explanation for such short range rigidity. The initial burst would occur as these bonds were first stressed, and then ruptured, by the continuing extension. The bonds between myofilaments in different sarcomeres would not, however, all be expected to be ruptured simultaneously at the beginning of a stretch. Movements with rupture of bonds would occur first in sarcomeres which were weakest because they contained fewest bonds. As these initially weak sarcomeres gave way and were extended, their parallel elastic elements would develop a progressively

greater tension, which would be transmitted to all the other sarcomeres of the same muscle fibre. This would in turn rupture the bonds in sarcomeres which were initially the stronger, thus leading to differences throughout the course of a stretch of large amplitude between the responses of an ending when it was facilitated and when it was not, as we observed.

The stability of the postulated bonds is shown directly by the experimental results. The after-effect of fusimotor stimulation persisted for well over a minute. When the muscle was shortened after the bonds had been formed the 'burst' appeared in the middle of the stretch instead of at the beginning, as if the bonds had held the myofilaments in their original position; possibly the 'stuck' intrafusal fibres kinked rather than shortened when the muscle was released (cf. Boyd, 1966). Initial bursts are sometimes seen in the absence of preceding fusimotor stimulation, suggesting that bonds may form 'spontaneously', though in this respect it should be remembered that 'spontaneous' contractions of intrafusal fibres have also been suggested to occur (Granit, Homma & Matthews, 1959; Jahn, 1968). It is also interesting that initial bursts, in the absence of preceding fusimotor activation, tend to be prominent when the length of the muscle is increased in successive steps (cf. Jansen & Matthews, 1962, Fig. 7). The abolition of the initial burst by stretch and release shows that once ruptured, large numbers of bonds do not normally rapidly reform, though a number insufficient to cause a burst may do so. 'Catch' muscles of invertebrates have been suggested to maintain prolonged contractions by the persistence of stable bonds (Jewell, 1959), producing a muscle which can maintain tension but which is incapable of appreciable shortening. Thus, intrafusal fibres would appear to have some similarities with catch muscles as well as with normal striated muscle; indeed, the ease with which stable bonds are formed may well vary considerably in muscles of the same histological type. In the frog sartorius, Hill (1968) found that the total recorded tension only decreased slightly from its initial peak during a progressive stretch which exceeded the elastic limit of the 'short-range elastic component', whereas an appreciable fall in intrafusal tension would be the simplest explanation of the presently observed decline of the burst from its peak. This difference would not appear to be of consequence, and recently one of us (M. C. Brown, unpublished observations) has observed a definite overshoot of tension on stretching slow muscle fibres of the frog which had been 'stuck' by a preceding period of motor stimulation.

If it be supposed that static and dynamic fusimotor fibres supply different kinds of intrafusal muscle fibre (Crowe & Matthews, 1964) then the present results may be taken to show that both the nuclear-bag and the nuclear-chain intrafusal muscle fibres may develop relatively stable

bonds between their myofilaments. On the other hand, it has been suggested that these two kinds of fusimotor fibre produce their different effects by acting on individual intrafusal muscle fibres in two different ways (for example, by contractions which are localized in one case and propagated in the other; Bessou & Laporte, 1966). On this hypothesis, however, the independence of the after-effects of stimulation of static and of dynamic fusimotor fibres would not appear to be readily explicable. Thus the present experiments favour the suggestion that static and dynamic fusimotor fibres innervate different intrafusal muscle fibres.

The present findings are also of interest in debating whether the initial burst should be considered as a true acceleration response (Henatsch, 1967; Schäfer, 1967), or rather as a static friction type of response found only for accelerations starting from zero velocity (Jansen & Matthews, 1962; Lennerstrand & Thoden, 1968). Various points may be noted: firstly, the lability of the initial burst shows that results obtained under a single set of conditions cannot be safely generalized. Secondly, the enhancement of the initial burst after fusimotor stimulation without any contraction of the main muscle strongly suggests that the burst depends upon purely intrafusal mechanisms, rather than partly upon the viscoelastic properties of the extrafusal muscle fibres as recently proposed (Henatsch, 1967). Third, the initial burst tends to be fairly small during continuous fusimotor activity, either when it is induced by electrical stimulation of a single fibre or when it occurs spontaneously in the decerebrate cat (Matthews, 1962; Crowe & Matthews, 1964; Jansen & Matthews, 1962). However, in the present experiments the facilitation left behind after a period of high-frequency fusimotor stimulation persisted during continuous low-frequency stimulation, and such conditions might occur in life. It is therefore premature to conclude that the initial burst is of no functional significance particularly for the higher velocities of stretching when it is most marked.

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