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A METHOD OF ANALYSING THE RESPONSES OF SPINDLE PRIMARY ENDINGS TO FUSIMOTOR STIMULATION

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SUMMARY

1. The superposition of records of 'instantaneous' frequency of discharge of spindle primary endings after stimulation of single fusimotor fibres leads to the construction of graphs called 'frequencygrams'.

2. The superposition is made on the screen of a storage oscilloscope, the time base of which is synchronized with the stimulus. It is essential that the stimulus be delivered at all possible intervals with respect to the impulse of the resting discharge which precedes it.

3. Frequencygrams obtained by stimulating some static fusimotor fibres by single stimuli display a response with a fast rising phase, a pause, and a slower decreasing phase.

4. Frequencygrams may give some information on the time course of the contraction of intrafusal muscle fibres elicited by stimulating their motor axons.

INTRODUCTION

The method described in this paper was developed with the purpose of obtaining indirect evidence on the mode of contraction of intrafusal muscle fibres when these fibres are activated by their motor axons.

The spindle primary ending is a natural mechanical transducer which has distinct advantages over any instrumental transducer attached to intrafusal muscle fibres through an unknown amount of inactive tissue: (i) its close mechanical linkage with the contractile part of the intrafusal muscle fibres eliminates the distortion of the contraction of these fibres by visco-elastic extrafusal elements; (ii) its sensitivity is high; (iii) the information it gives can be easily collected by recording action potentials from the afferent fibre. The contraction of intrafusal muscle fibres can thus be studied without isolating spindles partially or completely from the surrounding muscle as various authors have recently done (Smith, 1966;

Diete-Spiff, 1966; Boyd, 1966*a*, *b*; Bessou, Laporte & Pagès, 1966*a*; Bessou & Pagès, 1967). The blood supply of the spindles is preserved, and as the complete motor innervation is available it is possible to study the contraction produced by the stimulation of single fusimotor fibres of different types.

However, the rate of discharge of primary endings in resting conditions is relatively low and the transient increase of this rate which sometimes results from a single fusimotor impulse cannot give much information on the time course of the mechanical event responsible for this increase. On records displaying the reciprocal of time interval between successive impulses ('instantaneous' frequency), a single fusimotor impulse, even in the most favourable cases, modifies only the position of two or three points which are separated by more than 20 msec. The random variations of the rate of discharge add to the difficulty of interpreting these responses.

The superposition of records of 'instantaneous' frequency, provided certain conditions are fulfilled, leads to the construction of graphs which we propose to call 'frequencygrams'. It will be shown that while the unitary contributions to the construction of these graphs carry only incomplete and discontinuous information, the frequencygram provides more complete information on the time course of the underlying contraction. Preliminary communications on this work have been published. (Bessou, Laporte & Pagès, 1966*b*, 1967).

METHODS

The experiments were made on spindles of tenuissimus muscles of adult cats anaesthetized with pentobarbitone sodium (Nembutal Abbot Laboratories), given intraperitoneally.

In the first experiments, after the biceps femoris muscle had been removed, the tenuissimus muscle was freed over its whole length from the surrounding muscles, but its nerve and main blood vessels were left intact. The spindles in the distal third of the tenuissimus muscle were used. The proximal end of this muscular segment, about 30–40 mm long, was fixed to a small metallic plate by two clips. Each clip held one side of the muscle so as to leave enough room for the centrally situated nerve and blood vessels. When modifications resulting from stimulation of fusimotor fibres of the response of primary endings to phasic stretch were looked for, the distal end of that segment was attached to a magnetic puller; it was afterwards fixed to a rigid support. Later on, when it was realized that the small random variations in the rate of discharge of primary endings were mostly due to minute movements of the muscular segment, the tenuissimus muscle was not freed from the connective tissue sheath by which it adheres to various muscles of the thigh.

Single Group I fibres and single fusimotor fibres were prepared by the usual technique of splitting spinal roots.

Among single static fusimotor fibres, which were identified by the criteria defined by Matthews (1962), we selected those fibres which produced a distinct increase in the rate of discharge when activated by a single stimulus.

Discharges of primary endings when recorded with an 'instantaneous' frequency meter, appear as a succession of points, each point being produced by a nerve impulse. The ordinate of a point is proportional to the reciprocal of the time interval between this and the preceding impulse. Records of 'instantaneous' frequency of primary endings were superposed on the screen of a storage oscilloscope (Tektronix 564), immediately before and after giving a single stimulus to a single static fusimotor fibre. A large number of traces, thirty to one hundred, were superposed in this way and formed a single picture. The time base of the oscilloscope was synchronized with the stimulus, which always appeared at the same place on the screen. Spindles were submitted to a constant stretch so that primary endings were discharging at a relatively constant rate. The essential condition for constructing frequencygrams (see Results) is that the stimulus must be delivered at all possible intervals with respect to the afferent impulse which preceded it. The simplest way to fulfil that condition was to deliver a large number of stimuli at random with respect to the resting discharge of the ending. The stimuli were applied once every 1–5 sec to single static fusimotor fibres. Photographs of the screen of the storage oscilloscope were taken.

RESULTS

(*a*) *Changes in the rate of discharge of primary endings produced by single impulses in static fusimotor fibres.* The stimulation of some single static fusimotor fibres (approximately one out of five) by single stimulus produces a marked temporary increase of the rate of discharge of primary endings. This effect can be detected on single traces of records of 'instantaneous' frequency. Records 2–5 of Fig. 1 show that the responses which were elicited by stimuli delivered at various time intervals after the preceding afferent impulse differed among themselves, and that the

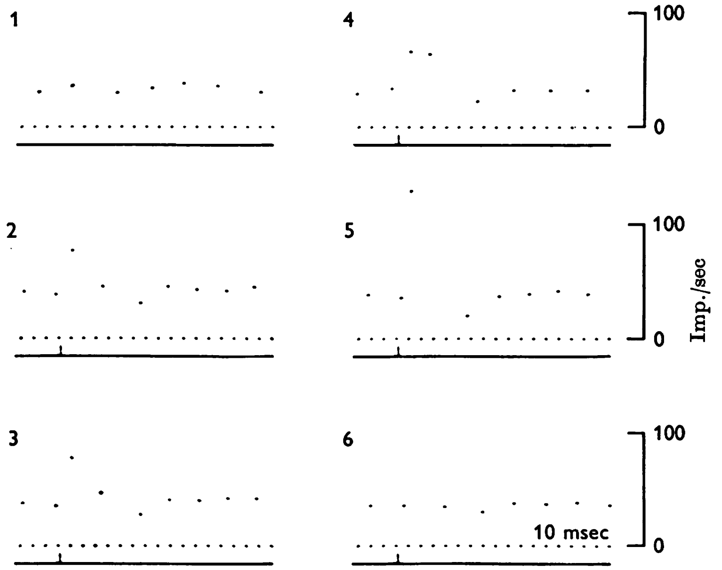

Fig. 1. Increase in the rate of discharge of a spindle primary ending due to the stimulation of a single static fusimotor fibre by a single stimulus. Record 1: no stimulation. In records 2–6 note the different positions of the stimulus with regard to the afferent impulse which precedes it.

responses due to stimuli delivered at almost the same time interval after the preceding impulse were similar (records 2–3). However, no acceleration was observed for one particular time interval (record 6).

Each of the different responses of the primary ending shows that a single motor impulse was able to activate intrafusal structures, but the time course of the frequency increase resulting from this activation cannot be accurately evaluated because the responses are indicated by only two or three points whose position changes for different values of the time interval between the stimulus and the preceding afferent impulse.

(*b*) *Construction of a frequencygram.* The construction of a frequencygram is illustrated by Fig. 2. The rate of discharge of this primary ending, in the absence of any stimulation (record 1) was approximately 35 impulses/sec. Small random variations in the rate of discharge were present. A single fusimotor fibre which had been previously identified as a static fibre was then stimulated 50 times, once every 5 sec. The time base of the oscilloscope was synchronized with the stimulus but as there was no synchronization between the resting discharge and the stimulus the phase between them varied at random.

The response due to each stimulus was superposed on the preceding responses which were stored on the screen of the oscilloscope. In this experiment during each sweep six points were added to the figure stored on the screen.

Comparison of records, 2, 3, 4 obtained, respectively after one, two and three stimuli, is almost sufficient to explain the way the frequencygram is obtained. Essentially it shows that the superposition of the three responses, which differ between themselves, begins to form a curve (record 4). This becomes more evident after a larger number of superpositions as shown by records 5, 6, 7, 8 obtained, respectively, after five, ten, thirty and fifty stimulations.

Five successive parts can be distinguished in a frequencygram such as the one illustrated by record 8.

Part 1, which precedes the response of the ending to the stimulus gives the resting frequency of the ending. The small variations in the resting rate of discharge cause the points to form a band instead of a line.

Part 2 is the rising phase of the response. In record 8 of Fig. 2, it begins after a latency of approximately 12 msec. This latency includes the conduction time in the fusimotor fibre from the ventral root to the muscle (4·2 msec in this experiment), the conduction time in the afferent fibre (1·7 msec) and unknown delays due to intrafusal events.

The duration of the rising phase varies between 5 and 8 msec. The increase in frequency, which in the present example is 35/sec, can be larger (50–60/sec).

Part 3 is a period during which the ending does not generate a nerve impulse. Its duration is approximately 10–20 msec. This pause is not filled even if a much greater number of stimuli is given.

Part 4 is a period during which the frequency decreases. It lasts 30–45 msec.

Part 5 is the phase of return to resting condition (40–50 msec) at the beginning of which the frequency is a little below the initial resting frequency. Grouping of impulses is often observed during this period.

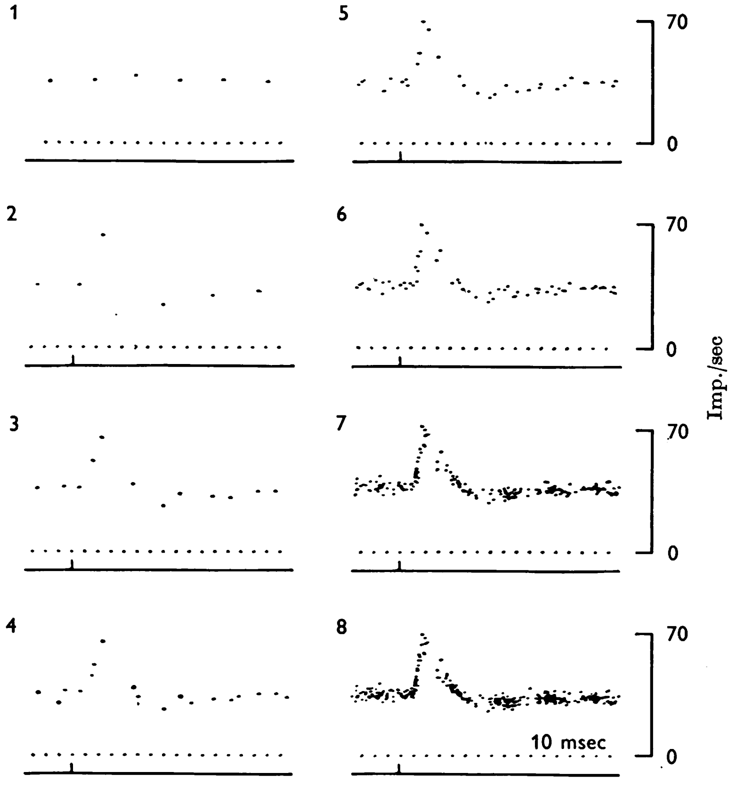

Fig. 2. Successive stages in the construction of a frequencygram (see text).

The number of stimuli necessary to construct a well-defined frequencygram is variable. If the rate of discharge of the ending is relatively high and regular 20–30 stimuli are sufficient. The random variations in the resting rate of discharge of most of the primary endings studied are responsible for the scatter of the points. In favourable cases, especially when precautions are taken to reduce vibrations, the discharge becomes regular and the frequencygrams obtained in these conditions form a thin line. An example is shown by record 2 of Fig. 3. A frequencygram with a large 'noise' activity is shown for comparison in record 1. Although frequencygram 2 is much better defined than frequencygram 1, the same features can be distinguished in both.

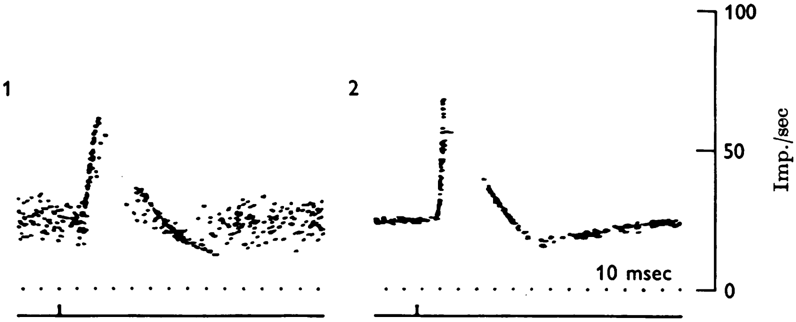

Fig. 3. Effect of random variations in the rate of discharge of primary endings on their frequencygrams. (1) Spindle with irregular resting discharge. (2) Spindle with regular resting discharge.

(*c*) *Frequencygram produced by two successive stimuli.* When the time interval between two successive stimuli was greater than the total duration of the first response, two distinct responses could be recorded (Fig. 4, records 2, 3), the second one being very similar to the first one.

With shorter time intervals (Fig. 4, records, 4, 5, 6) summation of the

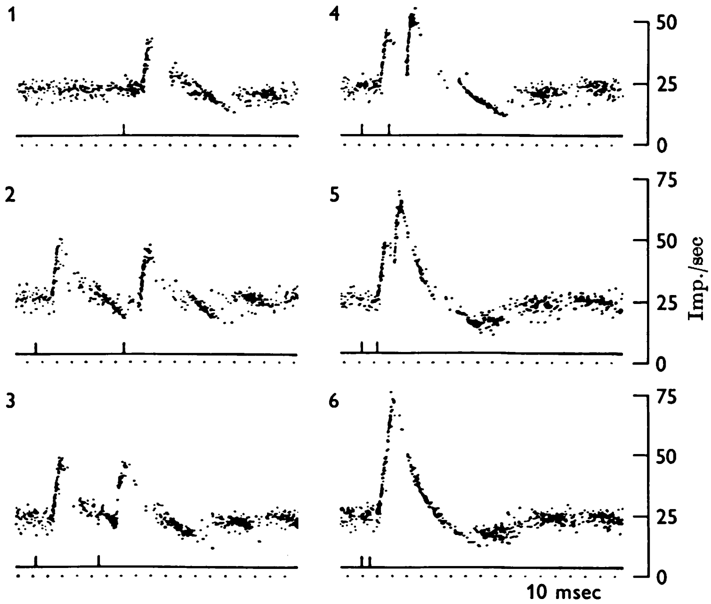

Fig. 4. Summation of the responses by double stimulation.

two responses occurred. Complete fusion was obtained when the interval between the two stimuli was reduced to 6 msec.

DISCUSSION

Frequencygrams are obtained by superposing a large number of records of 'instantaneous' frequency which differ from one another according to the phase relation between the rhythmic discharge of the ending and the stimulus applied to the fusimotor axon. The stimulus is responsible for a transient increase of the generator potential which results from the deformation of the sensory ending by the contraction of intrafusal muscle fibres. To each value of time interval between this transient potential and the preceding impulse corresponds a particular modification of the rhythmic discharge of the ending because the time of initiation of impulses is determined by the value of the generator potential and by the level of excitability of the ending. A transient lowering of excitability, which has been deduced from the 're-setting' of rhythmic discharge after additional activity (Matthews, 1933; Paintal, 1959), is observed after each impulse.

The time course of a frequencygram obtained by single stimuli must be related to the time course of the transient generator potential and consequently to the contraction of the intrafusal muscle fibres. However, no precise deduction on the time course of the contraction can be made because experimental data on the generator potential resulting from the contraction of intrafusal muscle fibres are lacking and also because the state of 'refractoriness' of the ending that follows any impulse initiated in the rising phase of the frequencygram must influence the time of initiation of the next impulse. The pause which is observed in these frequencygrams after the end of the rising phase is likely to be due, partly at least, to refractoriness of the ending. No impulse can be initiated during this period because the generator potential cannot overcome the high threshold of the ending. Another interpretation of this pause was proposed by Richalet (1967), namely that the generator potential produced by a single intrafusal contraction in primary endings, which are sensitive to velocity of stretch, is diphasic. During the positive-going second phase of the transient potential, which is superposed on the constant generator potential responsible for the rhythmic activity, depolarization at the site of initiation of impulse might be too weak to activate the membrane; hyperpolarization might even develop such as the one observed by Katz (1950) after muscle release. It is possible that both the lowering of excitability of the ending and a positive phase in the transient generator potential contribute to the pause. Recording of the generator potential due to the contraction of intrafusal muscle fibres is necessary before any firm conclusion can be reached.

Richalet (1967 and personal communication) who has further suggested that the potential which develops at the site of initiation of impulses is the time integral of the generator potential induced primarily by the deformation of the transducing membrane has obtained simulated frequencygrams similar to experimental ones in experiments with models based on these two assumptions.

An estimate of the duration of the rise time of contraction of intrafusal muscle fibres elicited by these static axons can be made because complete summation of two responses is observed for time intervals of about 6 msec between stimuli (see Fig. 4). Since tetanic fusion of extrafusal muscle fibres occurs when the time interval between successive stimuli is approximately half the rise time of the twitch (Cooper & Eccles, 1930), it is probable that the rise time for intrafusal muscle fibres is about 12 msec. This value fits with the high rate of stimulation, 180–220/sec, necessary to obtain a maximal increase of the rate of discharge of spindle sensory endings when some static axons are stimulated (Harvey & Matthews, 1961; Bessou, Emonet-Dénand & Laporte, 1962).

The method described in this paper has been used to compare the contractions of intrafusal muscle fibres elicited by the stimulation of static and dynamic fusimotor axons (Bessou, Laporte & Pagès, 1968).

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