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INTERACTION OF IMPULSE ACTIVITIES ORIGINATING FROM INDIVIDUAL GOLGI TENDON ORGANS INNERVATED BY BRANCHES OF A SINGLE AXON

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SUMMARY

1. Both physiological and morphological studies revealed that cat tail muscles contain at least one pair of Golgi tendon organs innervated by branches of a single axon.

2. Fifteen pairs of such organs were subjected to physiological studies. It was found that, depending on the experimental conditions, two modes of interaction, 'resetting' and 'impulse mixing' may occur between impulse activities originating from individual tendon organs.

3. When a single action potential was elicited from one of a pair of Golgi tendon organs during an interimpulse interval of a train of impulse discharge originating from the partner organ, the subsequent impulses in the train were delayed ('resetting'). Similarly, if both organs were stimulated individually by a mechanical pulse to elicit a train of discharge, then during stimulation of both, only the response of one responding with higher frequency discharge was seen in the parent axon, the impulse activity of the partner organ being completely suppressed during this period.

4. Using the conditioning-test technique it was demonstrated that the initiation of an action potential in one of a pair of Golgi tendon organs caused a significant decrease in excitability of the partner organ to mechanical stimulation.

5. Mixing of impulse discharges originating from individual Golgi tendon organs was shown to occur during stimulation of both by suprathreshold short mechanical pulses.

6. The functional implication of the above results has been discussed.

INTRODUCTION

The Golgi tendon organ, one of the major muscle receptors in the control of muscle activities, has been studied extensively using *in vivo* preparations. It is now well established that the organ is a sensitive tension receptor, particularly in response to a discrete number of motor units whose muscle fibres directly insert into the tendon organ (Houk & Henneman, 1967; Houk & Simon, 1967; Stuart, Mosher & Gerlach, 1972; Reinking, Stephens & Stuart, 1975; Jami & Petit, 1976*a*, *b*; Binder, Kroin, Moore & Stuart, 1977). The study of Golgi tendon organs in response to active and passive forces during stepping cycles of the cat hind limbs (Stuart *et al.* 1972; Goslow, Stuart, Nemeth & Stuart, 1973) has revealed that not only spindle

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Ia and II endings but also Ib endings are activated during both active and passive phases of a stepping cycle. A functional role of tendon organs in the stretch reflex as well as in volitional movements of limbs and intercostal muscles (Newsom Davis & Sears, 1970) has been suggested. The functional significance of the organs in moment to moment control mechanism has become increasingly evident. In spite of the importance of this sense organ, a direct approach to sensory transduction and its control mechanism has been lacking due largely to technical difficulties in isolating functioning tendon organs from limb muscles thus far studied.

It has recently been shown that a living single Golgi tendon organ, with or without muscle fibres attached, can be isolated from cat tail muscles (Fukami & Wilkinson, 1977). The isolated preparation may be kept in a good functional state for many hours *in vitro*. In these muscles we often found a pair of tendon organs supplied by branches of a single sensory axon. This provides an unusual opportunity to examine the interaction between impulse activities originating from individual tendon organs.

Several tendon organs supplied by branches of a single sensory axon have been reported by Cattaneo (1888) in muscles of guinea-pig, cat, dog, rabbit and human and by Dogiel (1906) in cattle eye rectus muscles (for review, Barker, 1974).

Experiments reported here demonstrate that impulse activities originating from one of a pair of organs reset those from the partner, and under certain conditions probabilistic mixing of impulse discharge from individual organs may occur.

METHODS

Preparation

Golgi tendon organs in cat tail muscles were used. Under Nembutal anaesthesia (30 mg/kg, I.P. injection) the tail was skinned. Immediately after being detached at its base from the rest of the body the tail was transferred to a bath containing modified Locke solution (Hunt & Ottoson, 1975; Fukami & Wilkinson, 1977). The thin muscles located in the dorsolateral aspect of the tail were exposed to the solution as quickly as possible by retracting their tendons laterally. Under a dissecting microscope single Golgi tendon organs were identified at musculotendinous junctions. A portion of the muscle containing several such organs was dissected out together with a piece of tail bone from which the muscle originates. The preparation was placed in a specially designed bath for experimentation. Using magnet-based pins the tail bone was firmly fixed to the bottom of the bath, the tendon of the muscle retracted laterally, and the muscle nerve supplying the organ cleaned and isolated as close to the tendon as possible.

Stimulation and recording

Impulse discharge from Golgi tendon organs was recorded by a pair of platinum-iridium electrodes from the isolated muscle nerve lifted into liquid paraffin. Stimulation of the organs either by muscle contraction or by stretching the muscle was avoided because of the mechanical disturbance upon nearby structures. To circumvent this problem direct mechanical stimulation of individual organs was employed: while monitoring nerve activity the tip of a glass rod (0·2–0·3 mm tip diameter) attached to the cone of a loudspeaker was positioned, by use of a micromanipulator, on to a single organ and the speaker cone activated by electrical pulses of various intensities and durations. To obtain a sustained discharge a constant pressure was applied through the tip of a glass rod either by feeding DC current to the loudspeaker or manually by using a micromanipulator. The tip of the glass rod, when examined using a strain gauge (compliance, 50 μm/g; natural frequency, 500 Hz), showed a damped oscillation (frequency, 250 Hz; duration, 20–30 msec) in response to a square pulse activation of the cone. The relation of the input (measured in terms of voltage) *versus* output (the peak amplitude of damped oscillation or the static displacement of the rod tip) was linear (25 μm/V) within the range used

in the present experiments. Nerve activities were amplified differentially by a Princeton Applied Research Model 113 amplifier. The amplifier output together with electrical pulses driving the loudspeaker were recorded on FM analog magnetic tape (Hewlett Packard 3960 Instrumentation Recorder). Intervals between successive impulses (interimpulse intervals) were determined either manually from taped records or by use of an interimpulse interval meter.

Identification of a pair of Golgi tendon organs supplied by branches of a single axon

1. *Physiological.* Under a dissecting microscope, individual organs were stimulated one by one by gentle pressure using a glass rod held in hand. The impulse discharge thus elicited was displayed on a storage oscilloscope to examine the amplitude and shape. A pair of organs whose action potentials showed similar amplitude and shape was selected and subjected to a further test which provided a crucial clue as to whether the selected pair was supplied by branches of a single axon. Using two independent stimulating glass rods a selected pair of tendon organs was mechanically stimulated individually. The intensity of an electrical pulse of 1 msec duration was adjusted to elicit a single impulse from each. If the impulses could be superimposed it was clearly indicated that the two receptors were innervated by separate axons. If not, they were taken to be supplied by branches of a single axon and further experiments were carried out.

2. *Morphological.* At the conclusion of each experiment the distance between the pair of tendon organs was measured and the position of each receptor marked by a piece of string placed on it or a small cut made on the common tendon. The preparation was fixed in the bath with 1 % OsO_4 in 0·2 M-cacodylate buffer for 2–3 hr at room temperature to stain myelinated axons. The preparation was then washed in 0·2 M-cacodylate buffer and cleared in glycerin. Under a dissecting microscope the pair of tendon organs previously subjected to physiological studies were identified and the innervating single axon was traced to the point of bifurcation. In ten out of fifteen pairs the branching point was confirmed (Table 1).

All experiments were performed at room temperature of about 25 °C.

RESULTS

1. *Effects of a single impulse originating from one Golgi tendon organ on the rhythmic impulse discharge from the partner organ.* Following physiological identification of a pair of tendon organs supplied by branches of a single axon, one of them was stimulated by a long mechanical pulse to elicit a train of impulse discharge. The other was stimulated by a suprathreshold short mechanical pulse of 1 msec duration to elicit a single impulse. When a single impulse thus elicited was injected into an interim pulse interval of the train, the timing of occurrence of the subsequent impulses in the train was delayed. This phenomenon known as 'resetting' (Matthews, 1931) occurred in all six pairs thus examined. An example is shown in Fig. 1 where several single-trace records were superimposed. Shown in record *A* is a train of impulse discharge originating from one of a pair of tendon organs. When a single impulse elicited from the other (record *B*) was injected between the first and the second impulses of the train, subsequent impulses in the train were delayed and shifted toward right (record *A* + *B*). The amount of this shift varied in parallel with the timing of the injected impulse relative to the preceding impulse in the train as may be seen in record *C*. These results suggest that the resetting may be due to the decreased excitability or refractoriness caused by the injected impulse in the site where a train of discharge originated (Adrian & Zotterman, 1926).

If an impulse initiated in one of a pair of tendon organs propagated not only orthodromically into the parent axon but also antidromically into the branch innervating the partner organ and reset the impulse activity there, the conduction time needed for an impulse to travel from one to the partner for a distance of 10 mm

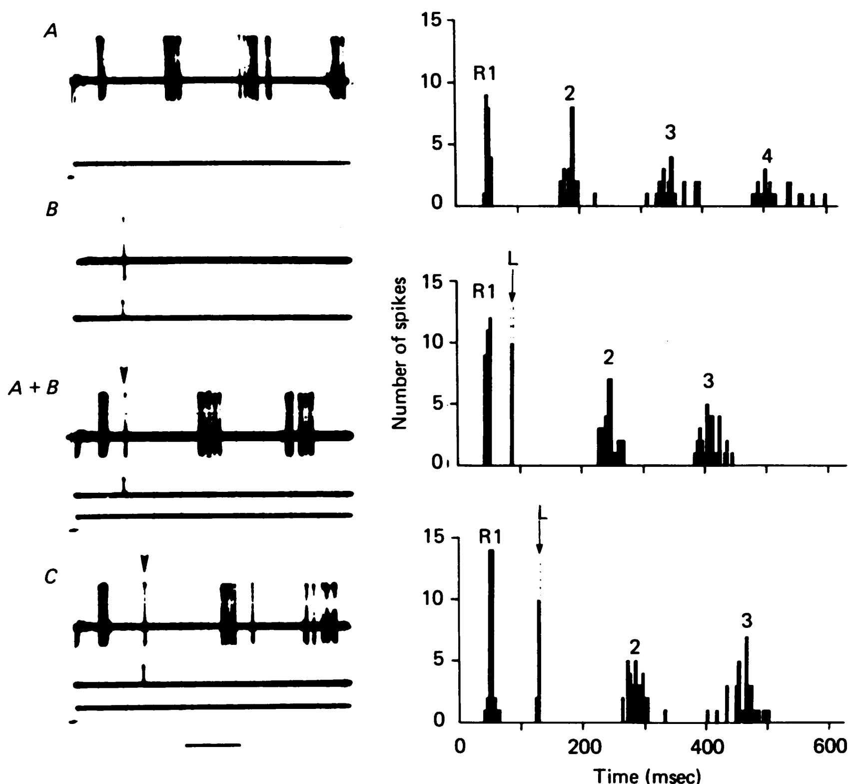

Fig. 1. Effects of a single impulse originating from one Golgi tendon organ on the impulse discharge from the partner organ. Record *A*, a train of discharge initiated from a right-hand side organ (R) by a long mechanical pulse stimulation (lower trace). Twelve traces were superimposed. Record *B*, a single impulse elicited from the partner (L) by a short mechanical pulse of 1 msec duration of suprathreshold intensity (lower trace). Five traces were superimposed. The response to combined stimulation of both organs is shown in record *A* + *B* (fourteen superimposed traces). In record *C* the timing of single-impulse injection was delayed (twelve superimposed traces). Time bar, 100 msec. Tendon organ pair no. 6. Poststimulus time histograms shown on the right correspond, from top to bottom, to record *A*, *A* + *B* and *C*. R stands for the response from R and L from L tendon organ. The top histogram was constructed from twenty-two, the middle from thirty-two and the bottom from thirty-four single-trace records. Bin width, 4 msec. The arrow with a letter L in both the middle and bottom histograms denotes injected single impulses, the number being twenty-two and thirty-two, respectively (truncated). The first and the second interimpulse intervals (R2–R1 and R3–R2) in the top histogram, $135{\cdot}5 \pm 9{\cdot}2$ and $167{\cdot}5 \pm 21{\cdot}5$ msec, respectively. Values for R2–L in the middle and the bottom histogram are $154{\cdot}1 \pm 11{\cdot}5$ and $158{\cdot}2 \pm 14{\cdot}0$ msec which are both significantly larger than the value for R2–R1 in the top histogram ($P < 0{\cdot}001$). Inter-impulse intervals for R3–R2 in the middle ($166{\cdot}4 \pm 9{\cdot}7$ msec) and the bottom histogram ($173{\cdot}6 \pm 22{\cdot}1$ msec) are both not significantly different from the corresponding value ($167{\cdot}5 \pm 21{\cdot}5$ msec) in the top histogram ($P > 0{\cdot}7$ and $> 0{\cdot}3$, respectively).

or less (Table 1) in an axon of 10 μm thick would be a fraction of a millisecond. This suggests that the resetting occurs almost simultaneously with the initiation of an injected impulse, and the chance for collision of an antidromic and an orthodromic impulse to occur in a branch would be extremely small. The possibility that the

TABLE 1

Golgi tendon organ pair no.	a (mm)	b (mm)	c (mm)	Branching
1	—	—	7·0	Confirmed
2	—	—	2·8	—
3	0·2	0·9	1·1	Confirmed
4	—	—	3·5	—
5	—	—	3·0	—
6	3·6	0·9	4·5	Confirmed
7	—	—	4·0	Confirmed
8	—	—	3·0	Confirmed
9	0·2	2·3	2·5	Confirmed
10	—	—	10·0	—
11	—	—	1·0	—
12	2·5	1·8	4·3	Confirmed
13	—	—	5·0	Confirmed
14	0·8	2·2	3·0	Confirmed
15	1·0	7·0	8·0	Confirmed

a, Distance between the left-hand side tendon organ and the branching point of an innervating axon. b, Distance between the branching point and the right-hand side tendon organ. In cases where only the value for c is listed it represents the distance between two tendon organs measured along the common tendon. In others the value for c is a sum of a and b.

point of bifurcation of the axon is the site of resetting seems less likely (see Discussion).

To examine in more detail the above results, post-stimulus time histograms of impulse discharge were constructed before and during impulse injection as illustrated on the right-hand side in Fig. 1. As may be seen in these histograms injection of an impulse did not significantly alter interimpulse intervals of the successive discharge in the train (e.g. R3–R2 in Fig. 1). However, the interval between the injected and the first succeeding impulse in the train (R2–L in Fig. 1) was, in most cases, significantly longer than the first interimpulse interval measured before the injection. The similar finding of an additional delay was noted by Horch, Whitehorn & Burgess (1974) in type I cutaneous mechanoreceptors for interimpulse intervals following either antidromic impulses or short interimpulse intervals seen in orthodromic discharge.

2. *Interaction between trains of impulses originating from individual Golgi tendon organs.* The above results predict that if a pair of tendon organs supplied by branches of a single axon are stimulated together only the higher frequency discharge originating from one of them will be seen in the parent axon: the higher frequency discharge will leave no chance for the partner organ to initiate an impulse during combined stimulation. This prediction was confirmed by experiments. Examples are illustrated in Fig. 2 where successive interimpulse intervals are displayed as dots using an interimpulse interval meter. The response of one of a pair of tendon organs to a mechanical pulse is shown in the top record, whereas the middle record represents a sustained discharge originating from the partner organ during maintained mechanical stimulation. The response during combined stimulation of both shown at the bottom is almost identical to the response shown at the top, the lower frequency background discharge being completely suppressed during this period. Similarly, when the

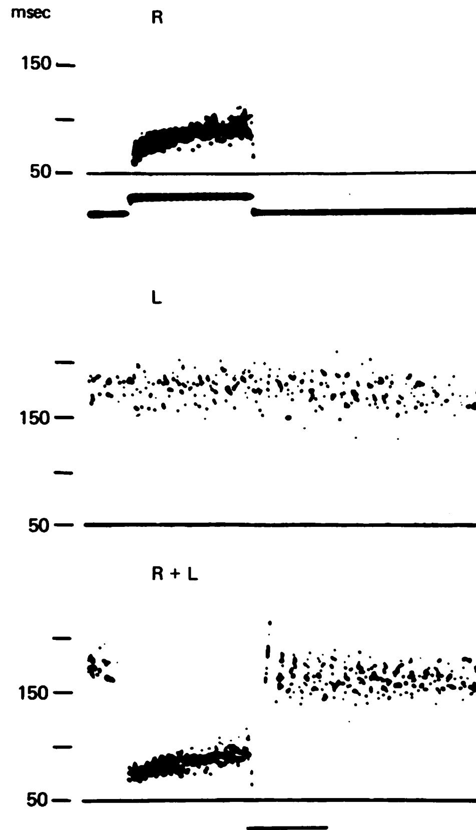

Fig. 2. Interaction between trains of impulses originating from individual Golgi tendon organs. Successive interimpulse intervals were displayed as dots on a storage oscilloscope and photographed. R and L represent respectively the response originating from R and L organ. Top record, response of R to a mechanical pulse (lower trace). Middle record, background discharge originating from L in response to a maintained mechanical stimulus. Bottom record, response during combined stimulation of both tendon organs. Twenty single-trace records were superimposed for each record. Note high frequency 'off' response at the end of mechanical pulse stimulation as well as several groups of discharge following the mechanical pulse seen in the bottom record as a longitudinally aligned pattern. In each record a horizontal line was drawn through 50 msec on the ordinate as a reference level. Time calibration, 1 sec. Tendon organ pair no. 15.

frequency of background discharge was made higher than the response of the partner (not shown) the former suppressed the latter during combined stimulation (reciprocality of resetting).

For further analysis of the above results frequency histograms of interimpulse intervals were constructed as illustrated in the upper part of Fig. 3. The mean ± S.E. of interimpulse intervals of discharge from the left-hand side of tendon organ (shaded histogram) is $173{\cdot}8 \pm 16{\cdot}4$ msec and that from the right-hand side tendon organ (stippled histogram) is $82{\cdot}9 \pm 10{\cdot}9$ msec, whereas the corresponding value during combined stimulation (continuous line histogram) is $82{\cdot}0 \pm 11{\cdot}5$ msec. The latter two values are statistically not significantly different ($P > 0{\cdot}2$, double

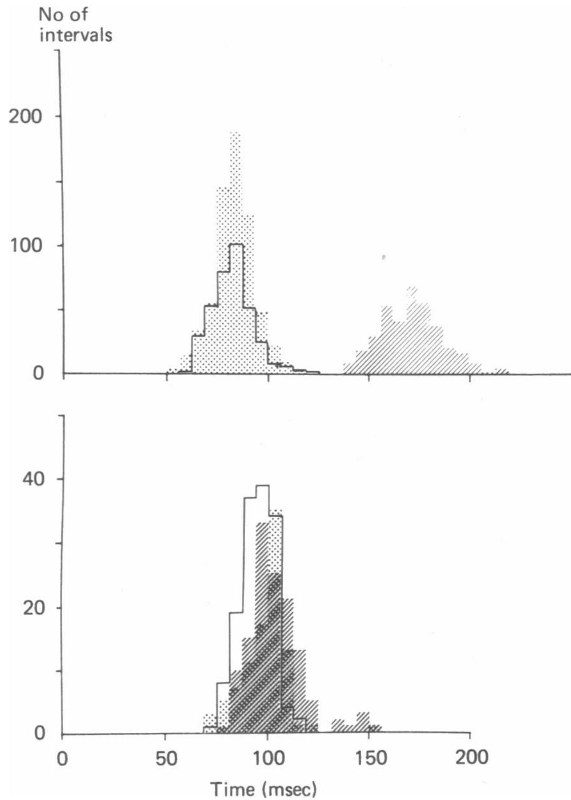

Fig. 3. Frequency histograms of interimpulse intervals. Upper histograms were constructed from records partly shown in Fig. 2. Records for lower histograms were taken from tendon organ pair no. 7. Shaded histograms represent background discharge from one of a pair of tendon organs (L in upper, R in lower), stippled ones the response of the partner to a mechanical pulse and continuous line histograms the response during combined stimulation. The 'off' response was excluded. Bin width, 6·25 msec. The mean ± S.E. of upper histograms (msec), 173·8 ± 16·4 ($n = 377$) for L, 82·9 ± 10·9 ($n = 647$) for R and 82·0 ± 11·5 ($n = 361$) for R + L (□). The difference between mean values for R and R + L is not significant ($P > 0{\cdot}2$ by double tail t test). Coefficient of variation (c.v.) for R and R + L, 0·131 and 0·140, respectively. The mean ± S.E. of lower histograms (msec), 104·3 ± 13·8 ($n = 130$) for R, 96·1 ± 16·1 ($n = 113$) for L and 93·9 ± 8·1 ($n = 144$) for L + R. C.v. for R, L and L + R, 0·132, 0·168 and 0·086, respectively. The test for variance ratio (F test) indicates that the interval distribution of L + R is significantly different ($P < 0{\cdot}001$) from the distribution of either L or R.

tail t test). In contrast, as shown in the lower part of Fig. 3, when the distribution of interimpulse intervals of discharge from individual sources is partly overlapped combined stimulation of both tendon organs caused a significant decrease ($P < 0{\cdot}001$, F test) in the variance of the distribution together with a slight decrease in the mean interimpulse intervals (continuous line histogram). These effects of combined stimulation may be explained in terms of 'resetting': interimpulse intervals longer than the most frequent one in responses of individual tendon organs tend to be suppressed during combined stimulation by higher frequency discharge, resulting in the shift of interimpulse interval histogram toward shorter intervals and less

variability of intervals. The above findings appear to agree with the theoretical prediction by Eagles & Purple (1974).

3. *Reduced excitability of a Golgi tendon organ following initiation of an impulse.* As suggested above, resetting may be accounted for by decreased excitability of one of a pair of tendon organs caused by initiation of an impulse in the partner. Using the conditioning-test technique, excitability of individual tendon organs was measured on

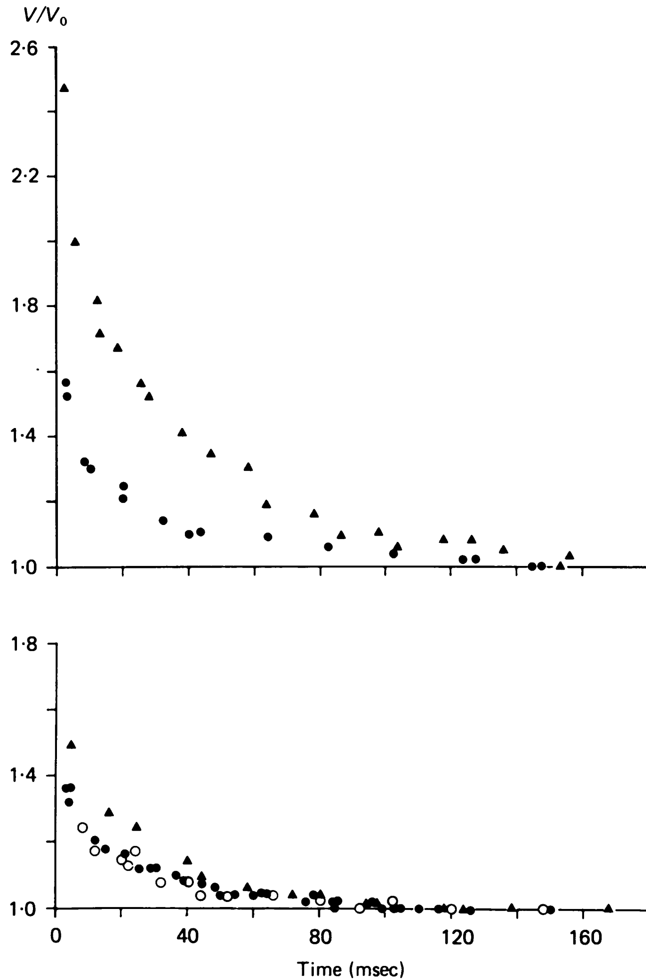

Fig. 4. Reduced excitability of one Golgi tendon organ caused by an impulse initiated from the partner. The excitability of each organ (ordinate) was expressed as a ratio of the threshold stimulus intensity (V) determined at various intervals following a conditioning impulse to that obtained without conditioning stimulus (V_0), and plotted as a function of intervals measured from the conditioning impulse to the test stimulus. In both upper and lower graphs filled circles represent the result obtained by applying conditioning stimuli to R and test stimuli to L, whereas filled triangles were obtained by applying conditioning stimuli to L and test stimuli to R. Open circles in the lower graph were obtained by applying both conditioning and test stimuli to R. Upper graphs were obtained from tendon organ pair no. 9 and those in the lower part from pair no. 13.

six pairs. A conditioning short mechanical pulse was applied to one of a pair to elicit a single impulse. Following the conditioning impulse the partner was stimulated by a mechanical pulse of 1 msec duration at various intervals. The minimum strength of the test stimulus required to elicit an impulse was measured in terms of the voltage of an electrical pulse delivered to the loudspeaker. This value was taken as a threshold (V). The excitability was expressed as a ratio of V to the threshold value obtained without conditioning stimulus (V_0). Similarly, in some cases both conditioning and test stimuli were given to the same organ. In all cases examined initiation of an impulse in one caused a remarkable decrease in excitability of the partner. The degree and time course of this reduced excitability differed not only between pairs but also among individual tendon organs. The period of reduced excitability ranged between 80 and 200 msec. Examples are shown in Fig. 4 where the pair of tendon organs shown in the upper graph exhibits more pronounced and longer lasting decrease in excitability than the one shown in the lower graph. Quite evident from these graphs is that the reduced excitability curves obtained from a pair are rather distinct from each other. The great variability of sensitivity to stretch among individual tendon organs (Fukami & Wilkinson, 1977) may account for this difference. Different muscle tensions under which experiments were performed may also influence the difference in excitability curves obtained from different pairs of tendon organs.

During the present experiments an attempt was made to reveal the 'late supernormal period' reported recently by Gregory, Harvey & Proske (1977) for secondary endings of muscle spindles as well as tendon organs in cat soleus muscles and in lizards. The attempt was without success except in one case where the reduced excitability was followed by a slight increase ($\sim 5\%$) in excitability lasting for about 60 msec. This failure might be due to the difference in experimental procedures; Gregory *et al.* (1977) employed antidromic retriggering of a discharge that had adapted to silence. Their technique might be more sensitive to disclose the 'late supernormal period' than the one employed in the present experiments. In addition, the difference in experimental conditions between *in situ* and *in vitro* experiments has to be taken into account; the late supernormal period might, for example, be highly temperature dependent.

The underlying mechanism for the reduced excitability is not entirely clear; it might be due either to increase in membrane conductance, with or without change in membrane potential, or to long-lasting Na-channel inactivation at the site of impulse initiation. Fig. 5 shows a record taken from experiments designed to record receptor potentials from the nerve of an isolated Golgi tendon organ *in vitro* (Fukami & Wilkinson, 1977). A ramp-and-hold stretch (middle trace) of 10 μm amplitude produced a single action potential (top trace) followed by afterhyperpolarization lasting about 100 msec (top and bottom traces). Since the refractory period of the innervating axon itself is about 5 msec this long lasting afterhyperpolarization seems to be a characteristic feature of the terminal (see Discussion), suggesting its role for regulating the excitability cycle in this sense organ.

4. *Mixing of impulse discharge originating from two sources.* The above results suggest that mixing of impulse discharge originating from individual tendon organs may occur in the following situations. First, when the impulse activity is very low

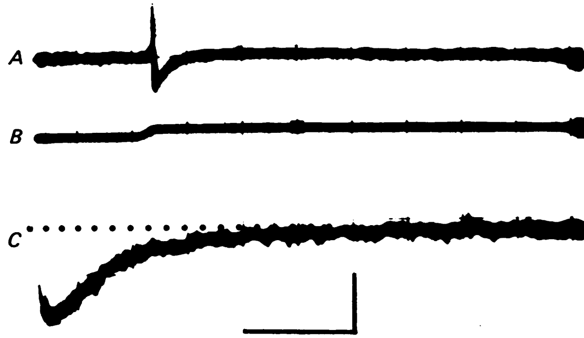

Fig. 5. Response of a single isolated Golgi tendon organ to a ramp-and-hold stretch in an experiment designed to record receptor potentials from the nerve lifted into oil. A stretch of 10 μm in amplitude (middle trace) gave rise to a single action potential (top trace) followed by afterhyperpolarization lasting about 100 msec (top and bottom traces). The bottom trace shows a magnified record of afterhyperpolarization in record *A*. Voltage calibration, 500 μV for *A* and 200 μV for *C*. Time calibration, 200 msec for *A* and *B* and 40 msec for *C*.

(for example, 2–3 impulses per sec) there would be a chance for an impulse from the partner to occur in the interval between successive impulses without any interaction whatsoever. Impulse mixing of this sort was demonstrated by an experiment (not shown) where individuals were stimulated repetitively by short mechanical pulses of threshold strength, at an interval longer than twice the period of reduced excitability described above. When the delay between two sets of stimuli was adjusted so that one set of stimuli applied to one occurred at the middle of the interstimulus interval of the other set applied to the partner, complete impulse mixing occurred during combined stimulation of both tendon organs. When the delay was shortened to be well within the period of reduced excitability the delayed set of impulses was completely suppressed, obviously due to the decrease in excitability left behind by individual impulses in the partner's train. The simple case of the second situation is represented by the above conditioning-test experiments, a demonstration of impulse mixing of a conditioning and a test impulse originating from two different sources. It follows that when short mechanical pulses of suprathreshold intensity are applied to individual tendon organs, impulse mixing may occur during combined stimulation. In either case the impulse mixing is a probabilistic process: in the first case the probability of impulse mixing depends on interimpulse intervals, the longer the interval the more chance of impulse mixing may be expected, whereas in the second case the probability is a function of stimulus intensity. Fig. 6 illustrates an example of impulse mixing occurring in the second situation. While one of a pair of tendon organs (R) was stimulated repetitively by short mechanical pulses at a 203 msec interval (bottom trace of record *B*) the partner (L) was stimulated similarly at a 312·5 msec interval for about 4 sec (record *A* and the middle trace of record *B*). Stimulation of the L tendon organ was repeated once every 10 sec. During combined mixing occurring during this period. Frequency histograms for interimpulse as well as interstimulus intervals during combined stimulation were constructed from

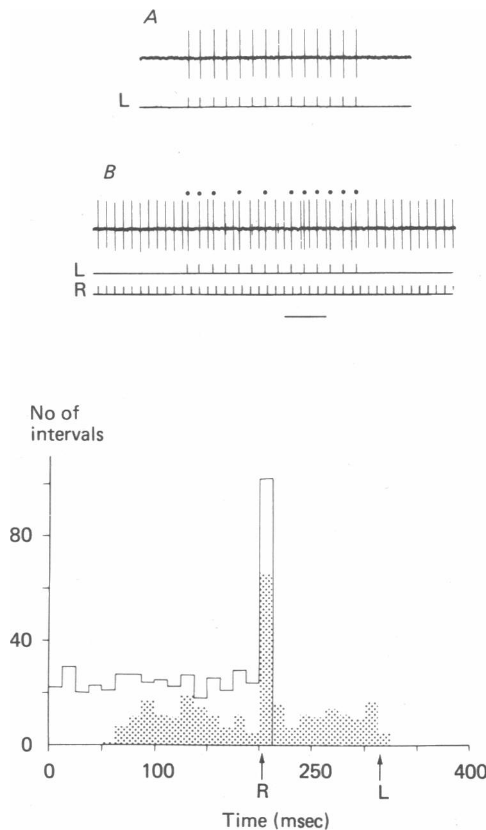

Fig. 6. Mixing of impulse discharge originating from individual tendon organs. Top record (*A*), impulse discharge from L in response to short mechanical pulse stimuli (lower trace) applied at a 312·5 msec interval. Record *B*, a single-trace record of response to combined stimulation of both tendon organs. While the partner organ (R) was stimulated repetitively at a 203 msec interval (bottom trace R) L was stimulated once every 10 sec in the same was as in record *A* (second trace from the bottom, L). In *B*, impulses originating from L are marked by dots. Three impulses from L were blocked during combined stimulation, leaving small stimulus artifacts behind. Stimulus intensity for both L and R was 1·2 × threshold. Stimulus pulse duration, 1 msec. Time bar, 1 sec. From single-trace records similar to record *B* frequency histograms were constructed for both interimpulse intervals (dotted histogram) and interstimulus intervals (continuous line histogram) during combined stimulation. Arrows with letter R and L indicate interstimulus intervals applied to R and L, respectively. Bin width, 12·5 msec. Total number of interstimulus and interimpulse intervals are 489 and 299, respectively. Input to output ratio = 0·611 (299/489). Tendon organ pair no. 9.

single trace records as shown in the lower part of Fig. 6. It is clear that the two frequency histograms are quite different. The frequency of occurrence of interimpulse intervals (dotted histogram) is generally low compared with that of the interstimulus histogram (continuous line histogram), the distribution extending to the area where no interstimulus interval exists. In addition, no interimpulse intervals occur corresponding to interstimulus intervals of less than 50 msec. These features of interimpulse intervals may be accounted for by the rather weak stimulus intensity

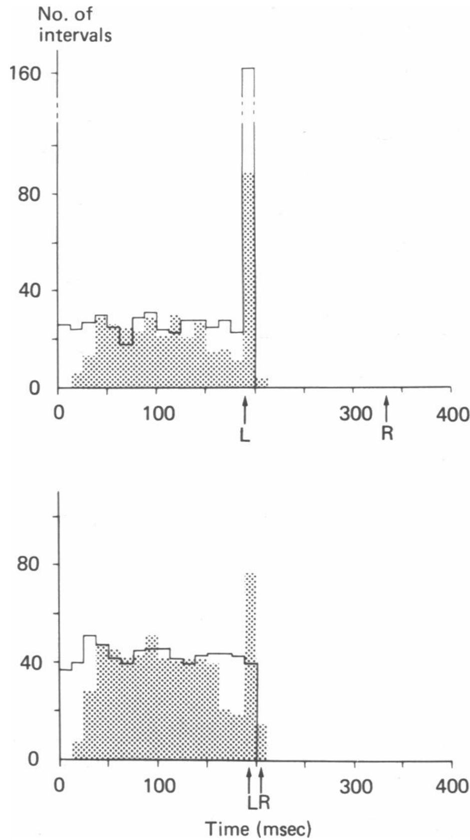

Fig. 7. Frequency histograms of interimpulse intervals (dotted) as well as interstimulus intervals (continuous line) during combined stimulation of both tendon organs constructed from records similar to those shown in Fig. 6. Mechanical stimulus intensity was 2 × threshold for each organ. The interval at which short (1 msec duration) mechanical pulses were applied to each organ, 191·3 msec for L and 333·8 msec for R in the upper histograms and 191·3 msec for L and 205 msec for R in the lower histograms. These values are indicated by arrows. Bin width, 12·5 msec. Total number of interstimulus intervals, 533 for the upper and 695 for the lower histogram, respectively. Total number of interimpulse intervals, 459 for the upper and 602 for the lower histogram, respectively. Input to output ratio, 0·861 (459/533) for the upper and 0·866 (602/695) for the lower histogram, respectively. Tendon organ pair no. 10.

(1·2 × threshold) employed in this experiment which makes those stimuli occurring at short intervals fail to initiate an impulse, resulting in spreading of the distribution. In contrast, the distribution of interstimulus intervals is clearly limited to and rather uniform in the area below the shortest interval of stimuli applied to individual tendon organs (arrow R). A peak frequency of distribution may occur at this shortest interval as may be seen in Fig. 6. The ratio of the number of interstimulus intervals to the number of interimpulse intervals (input to output ratio) was found to be 0·611.

By increasing the stimulus intensity the frequency histogram of interimpulse intervals may be expected to approach to that of interstimulus intervals, together

with the increase in the input–output ratio. Results obtained with increased stimulus strength ($2 \times$ threshold) are illustrated in Fig. 7. As expected, the frequency histogram of interimpulse intervals fits closer to that of interstimulus intervals and the input–output ratio increased to 0·861 and 0·866 for the upper and the lower histogram, respectively. The shortest interimpulse intervals now extend down to the second block of the histogram. When the interstimulus interval of a set of stimuli applied to one of the pair of tendon organs (R) was made shorter, approaching the interval of stimuli applied to the partner (lower histogram), the peak frequency of interstimulus intervals disappeared (see below), whereas the peak frequency of interimpulse intervals remained. Another feature common to all interimpulse interval histograms is the relatively low frequency of occurrence for a few blocks on the left of the peak frequency, making a dip in this region. The occurrence of this dip may be explained as follows. Suppose the shortest interstimulus interval ($T = s_{n+1} - s_n$) applied to one of a pair of tendon organs (e.g. L in Fig. 7) were divided into two segments during combined stimulation by s, one of another set of periodic stimuli applied to the partner organ (e.g. R in Fig. 7). A pair of unequal length of segments can be obtained by dividing T closer to either s_n or s_{n+1}, and when $s - s_n$ in the former case is equal to $s_{n+1} - s$ in the latter case these two pairs of segments are identical in terms of interstimulus intervals. The situation where s is too close to s_n to initiate an impulse (due to the reduced excitability caused by the preceding impulse by s_n) may account for the lack of interimpulse intervals for the first few blocks of the histogram as well as the peak frequency of interimpulse intervals exceeding that of interstimulus intervals (lower histogram in Fig. 7) because the interimpulse interval corresponding to T lacks the interstimulus interval. In the second situation where s is close to s_{n+1}, s_{n+1} does not initiate an impulse for the same reason as above, but s does. Again the short segment of interstimulus interval ($s_{n+1} - s$) lacks the corresponding response, whereas the longer segment ($s - s_n$) does. If the above two situations occurred with the same frequency the frequency of interimpulse intervals for the longer segment of T would be a half of that of the corresponding interstimulus intervals. An essentially similar argument may be applied to several blocks of intervals where the dip occurs.

For the superposition of several periodic sequences of events a theory has been developed by Cox & Smith (1953) which can be applied to the frequency histogram of interstimulus intervals shown in Figs. 6 and 7. The frequency of uniform distribution (q) predicted for intervals shorter than the shortest periodic interval and the frequency of a point concentration (Q) occurring at the shortest periodic interval were calculated using the following equations derived from the general equations developed by Cox & Smith (1953). $q = 2 \times n \times d/(\theta_1 + \theta_2)$ and $Q = n \times (\theta_1 - \theta_2)/(\theta_1 + \theta_2)$ where θ_1 and θ_2 ($\theta_1 > \theta_2$) are intervals of periodic stimuli applied to individual tendon organs, n the total number of intervals and d the bin width of histogram. Values of q and Q predicted for each histogram are listed below together with the observed value (mean $\pm$ S.E.) in parenthesis. $q = 23{\cdot}7$ ($24{\cdot}2 \pm 3{\cdot}4$), $Q = 103{\cdot}9$ (102) for the histogram in Fig. 6, $q = 25{\cdot}4$ ($25{\cdot}9 \pm 3{\cdot}3$), $Q = 144{\cdot}6$ (162) and $q = 43{\cdot}8$ ($43{\cdot}3 \pm 3{\cdot}5$), $Q = 24{\cdot}0$ (40) for the upper and the lower histogram in Fig. 7, respectively.

DISCUSSION

The terminal ramification is a common feature among most sensory axons subserving various sensory modalities. The basic question is to ask how information

carried by individual branches is processed before it is sent to the central nervous system. Pringle (1938) has discussed several modes of interaction (resetting, pacemaker switching and impulse collision) among impulse activities carried by individual branches. Resetting of the excitability cycle of a pace-maker (or impulse initiation site) in a branch by impulse activities in other branches has been demonstrated for cutaneous mechanoreceptors (Lindblom, 1958; Lindblom & Tapper, 1966; Horch *et al.* 1974), cold receptors (Duclaux & Kenshalo, 1973) and corneal tactile receptors (Tower, 1940). The collision of an antidromic and an orthodromic impulse has been reported to occur in terminal branches of splanchnic slowly adapting mechanoreceptors (Floyd & Morrison, 1974) and intradental receptors (Matthews, 1977). Another mode of interaction, the probabilistic mixing of impulse trains generated in two branches of a sensory axon, is discussed by Clifford & Sudbury (1972) as a consequence of impulse collision in a branch. For taste receptors the summation of a generator currents along the unmyelinated terminals of a single afferent neurone has been suggested (Miller, 1971).

Single sensory axons supplying muscle spindles and tendon organs in vertebrates also show terminal ramification (Barker, 1974). Based on the effect of fusimotor stimulation upon the response to stretch of primary endings, pacemaker switching has been suggested to occur between impulse initiation sites, one situated in branches innervating nuclear bag fibres and the other in those terminating on nuclear chain fibres (Crowe & Matthews, 1964; Emonet-Denand, Hulliger, Matthews & Petit, 1977). Multiple sites of impulse initiation have been suggested for myelinated terminal branches of an axon innervating a frog spindle (Katz, 1950; Ito & Vernon, 1975) and for those supplying a lizard tendon organ (Proske & Gregory, 1976). Brokensha & Westbury (1974) have suggested probabilistic mixing of impulse discharge originating from individual impulse initiation sites in terminal branches of a frog spindle to account for the two distinct phases of discharge adaptation observed during a maintained stretch.

For the single-impulse injection experiments like those shown in Fig. 1 the following three possibilities could have occurred. (1) The injected impulse has no effect on the subsequent impulses in the train, (2) the injected impulse eliminates only the succeeding impulse in the train, leaving the rest impulses unaffected and (3) the injected impulse resets the timing of occurrence of the subsequent impulse train. The first possibility may occur when there is no interaction between receptors: each receptor behaves independently and the output seen in the parent axon is simply a sum of impulse activities of individual branches. The second case may be accounted for by collision of an antidromic and an orthodromic impulse in the branch carrying a train of impulses. The last possibility, 'resetting', may occur when the injected impulse causes a decrease in excitability of the partner receptor, and delays the subsequent impulse discharge in the train. The present results clearly demonstrate 'resetting' as the mode of interaction observed. The possible site of this interaction may be either the branching point of an innervating axon or the impulse initiation site in each Golgi tendon organ. If the first possibility were the case, then the reduced excitability curves obtained by switching the conditioning tendon organ from one to its partner should be identical. As shown in Fig. 4 this is usually not the case. The possibility of the point of bifurcation being the impulse

initiation site seems to be less likely because just subthreshold stimulation of both tendon organs failed to initiate an impulse in the parent axon.

For the situation where simultaneous resetting occurs among multiple encoding sites·Eagles & Purple (1974), based on probability theory, predicted that, when the distribution of intervals of successive impulses from individual encoding sites is overlapping each other, then their combined activities would result in a faster and more regular discharge in the parent axon than the discharge from any single encoding site alone. This prediction seems to be supported by the result shown in Fig. 3.

A significantly longer recovery cycle of a single sensory receptor than that of the innervating sensory axon itself, the finding first noted by Adrian & Zotterman (1926) for frog muscle spindles, has been demonstrated also for cutaneous mechanoreceptors (Lindblom, 1958; Lindblom & Tapper, 1966, 1967; Horch *et al.* 1974) and corneal tactile units (Tower, 1940). The refractory period of a single Ib axon measured by its electrical stimulation near a tendon organ and recording the propagated action potential in the parent axon was found to be about 5 msec, suggesting that whenever an impulse is initiated in one of a pair of tendon organs it propagates along the branch into the parent axon and mixes with impulses originating from the partner. Since tendon organs have significant dynamic sensitivity (Houk & Henneman, 1967; Houk, 1967) this suggests that impulse mixing may occur *in vivo* when asynchronous twitch contractions of individual motor units activate tendon organs supplied by branches of a single axon.

It is evident that when 'resetting' occurs among tendon organs innervated by branches of a single axon only the information carried by the most strongly activated organ is transmitted to the central nervous system, the information carried by others being neglected. In contrast, 'impulse mixing' may provide information on individual organ's activities to the central nervous system because the probability of mixing appears to be a function of the magnitude of twitch contractions developed by a number of motor units whose muscle fibres directly insert into the tendon organ.

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