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On the Conduction Rate of Successive Impulses in Muscle and Nerve Fibres

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Introduction

Many authors have studied the conduction rate of muscle and nerve fibres when successive electrical stimuli were applied. These authors include Lucas on the nerve, skeletal and heart muscle of the frog; Samojloff on the muscle; Gotch on the nerve; Gasser and Erlanger[5] on the nerve; and Forbes and others on the nerve and muscle. Recent studies on this subject have been done by Bullock on the frog's nerve and on the giant axon of the earthworm, by Wakabayashi[17] on the conduction rate in a single muscle fibre of a toad by applying double stimuli with pore electrode, and by Ichioka on the conduction rate of the nerve of a toad. The present experiment by this author was conducted for the purpose in confirming these results and extending the research from double stimuli to three or more ones. Finally experiments were done on a nerve-muscle preparation.

Methods and materials

The experiment was conducted in the period from October to June on the excised muscle of a Japanese toad (*Bufo vulgaris*), at temperatures ranging from 10.5° to 26.0°C. The muscle preparation was mounted on a strip of filter paper soaked with Ringer's solution. The zinc-zinc-sulphate-gelatine was used as stimulating non-polarizable electrodes. In the case of stimulating a single muscle fibre Pratt's pore electrode was placed on the nerve free pelvic end of the sartorius muscle of the toad, and the indifferent electrode was placed on a strip of filter paper which was laid underneath the muscle preparation (Fig. 1 a.).

For stimulating a single nerve fibre, the sheath was stripped off beforehand at the proximal region of sciatic nerve and Patt's electrode was placed on it. In some of the experiments on the nerve-muscle preparation, a fluid electrode as shown in Fig. 1 b. was adopted. Stimulation was done at St. and recording at L.

Brief electrical shock of rectangular pulse was used for stimulation by utilizing the "Stosskontakt"[10] of Helmholtz' pendulum. In addition, break induction shock of a inductorium was often used. The shock interval was always regulated by the Helmholtz' pendulum interruptor.

Since the diametre of the pore of Pratt's electrode was much less than that of a muscle fibre, a single muscle fibre was stimulated easily. But the pore was not so sufficiently small for the nerve fibre that more

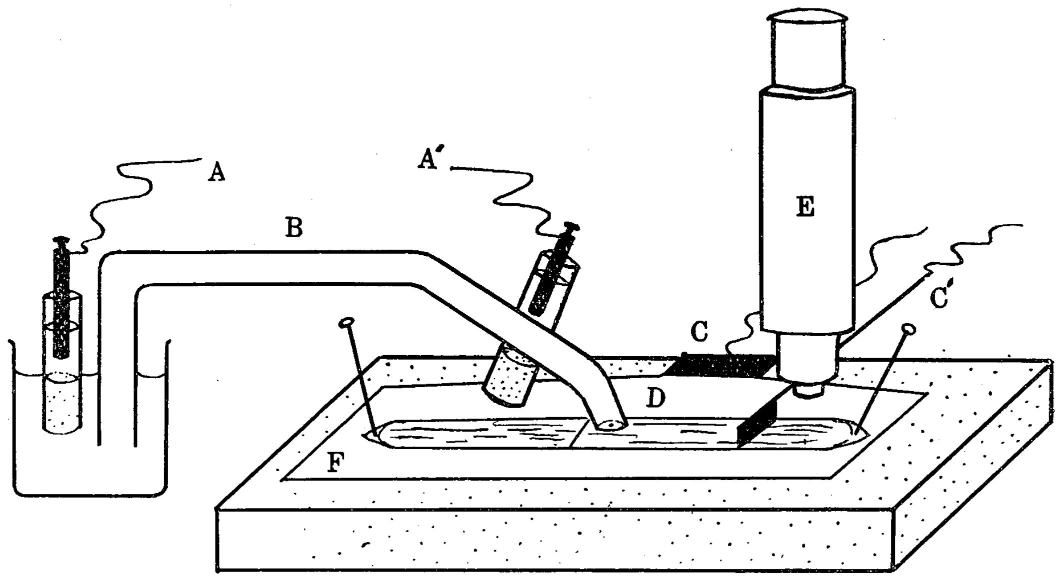

Fig. 1. a. Shematic illustration of the apparatus for a muscle fibre. A & A': Stimulating electrodes with Pratt's electrode (B). C & C': Recording electrodes of silver-edge and -plate. D: Bufo sartorius on a strip of filter paper (F) soaked with Ringer's solution. E: Microscope to observe the contraction of a single muscle fibre.

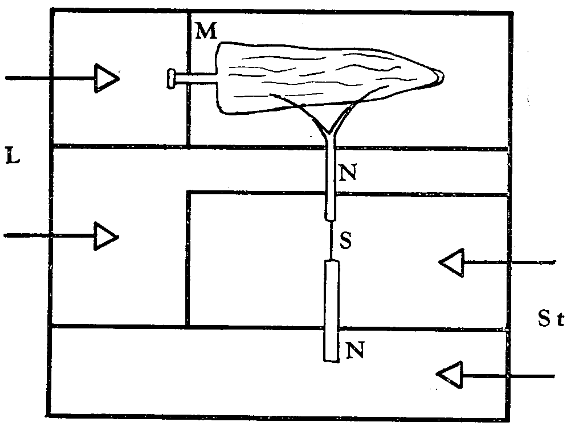

Fig. 1. b. Shematic representation of the fluid electrode for a nerve-muscle preparation to stimulate a nerve fibre and to record an action potential from a minimum number of muscle fibres. Indirect stimuli are applied by vacuum tube stimulator. St: electrodes from the stimulator, L: recording electrode, N: nerve trvnk, S: a single fibre of a frog sciatic nerve, M: sartorius muscle whose pelvic end are cut off except a few fibres. Each compartment are filled with Ringer's solution.

than a single fibre were unavoidably stimulated simultaneously. Neverthless it was ascertained by observing the action potential of the same form and size that fibres which were stimulated were always the same.

Multiple stimuli with irregular intervals were applied by using two tetanizing induction coils, whose secondary coils were connected in parallel. And the current was rectified with a 30 MC vacuum tube to exclude the current of the opposite direction. In every case the stimulus was done with cathodic current.

For leading the action potential, an indifferent electrode of silver plate was attached on the verge of the piece of filter paper (Fig. 1. a.), and an edge of a silver plate as an exploring electrode was attached perpendicular to the muscle surface at various distances from the stimulating electrode. The action potential led off in this way was amplified by C-R coupled four-stage amplifier, and recorded with an electromagnetic oscillograph or by using Braun tube oscilloscope.

The conduction velocity was obtained by measuring the conduction distance and the time interval from the moment of stimulation to the initiation of action potential. After Wakabayashi[19] the moment of slight downward deflection at the beginning of the action potential in the record was adopted as an initiation point of the potential. By this procedure he obtained best results in his experiment with pore electrode. And author followed his advice. In the most case of this report, only the shock-response intervals were measured, ignoring the exact value of the velocity, because it was enough to have relative values for the purpose of this experiment.

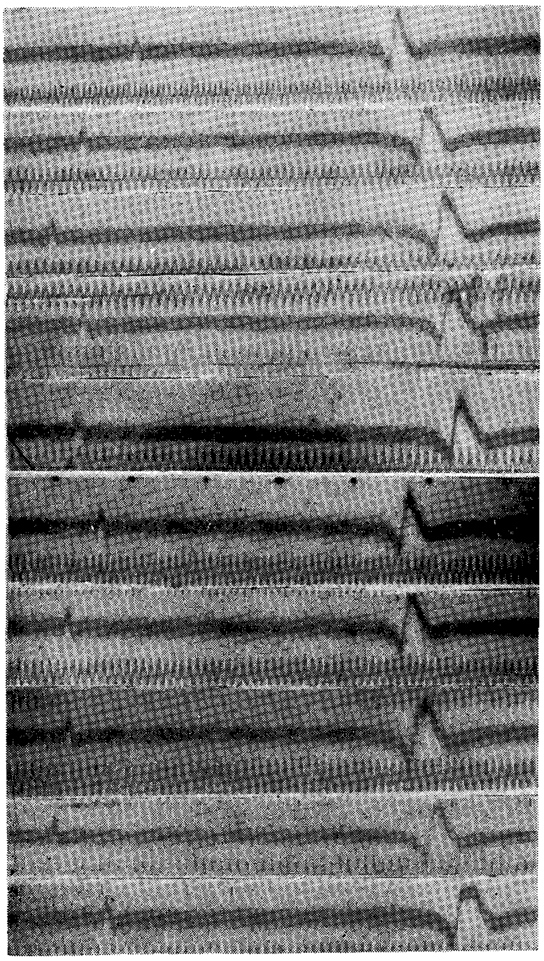

Fig. 2. A preliminary experiment. Single muscle fibre of a toad. Response interval was tested with different strength of stimulus by use of Pratt's electrode. From above to below the strength of stimulus was increased. The speed of the recording paper was not constant in each case. The results of measurement are represented in Table I. Time mark; 1.000 c.p.s. Temperature; 10.2° C. Dec. 26th, '52.

Results

A) *Preliminary experiment*

These experiments were carried out to examine whether the stimulus-response interval was really constant or varied with the strength of stimulus as was used in this experiment. The results as shown in Fig. 2. and Tab. I, that the rate of conduction did not depend upon the strength of the stimulus applied to a single muscle fibre. Thus a current that is slightly stronger than the threshold value was always adopted as a safety stimulus in this experiment.

B) *Muscle fibre*

1) *Stimulus interval, spike height, and its speed:*—When the interval

of two stimuli was so small as was close to the least interval, the conduction rate of the second action potential in the muscle fibre was observed to be retarded. This was also reported with a whole muscle and a nerve trunk by various authors (Forbes and others, Gasser and

Table I. The test of independence of the shock-response interval on the strength of stimulus. The same data as in Fig. 2.

Strength of stimulus (Ω)	Interval between shock and action potential (msec.)
2800	44.3
2900	44.1
3000	44.1
3100	44.0
3200	44.0
3300	44.0
3500	44.0
3800	44.1
4100	44.0
4500	43.8

The strength of stimulus increases from above to below.

Erlanger, Gotch, Ichioka, Lucas, and Samojloff). But when the interval became slightly longer than this until the stadium which was considered to be a supernormal phase, the conduction rate of the second impulse increased above the normal rate and this period of increased velocity

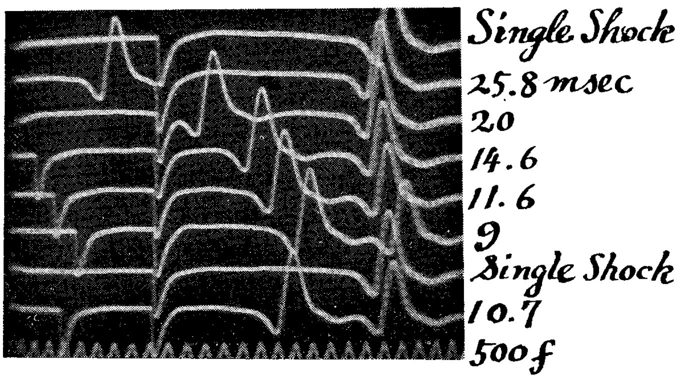

Fig. 3. a. Shock-response interval of double shocks. Sartorius muscle of a toad. Stimulation of a single fibre with Pratt's electrode. The distance from the stimulating electrode to the recording one was 37 mm. At the right of the record the stimuli interval are indicated. Each stimulus artefact is recorded as a simple downward deflection. Artefacts arranged vertically in a straight line in the record are that of the second impulses, when not indicated especially. Numbers at right side are interval of conditioning and test stimuli.
Time mark; 500 c.p.s., temperature; 14.7°C, Dec. 5th, '53.
The same data is represented in Fig. 3. b.

was considerably long. An example of the result is shown in Fig. 3. a. and b. It is worth noticing that the second impulse traveled faster than the first, although the height of the former was smaller than the latter. The height of the second impulse became larger, when the stimulus interval became longer, but it did not exceed the first one. This observation was also reported by Gasser and Erlanger[5] on the nerve fibre in supernormal phase. Inversely the height of the second impulse became smaller as the interval became shorter, but no impulse was found to be so minute that scarcely perceptible i.e. the second impulse disappeared abruptly when the interval was shortened beyond

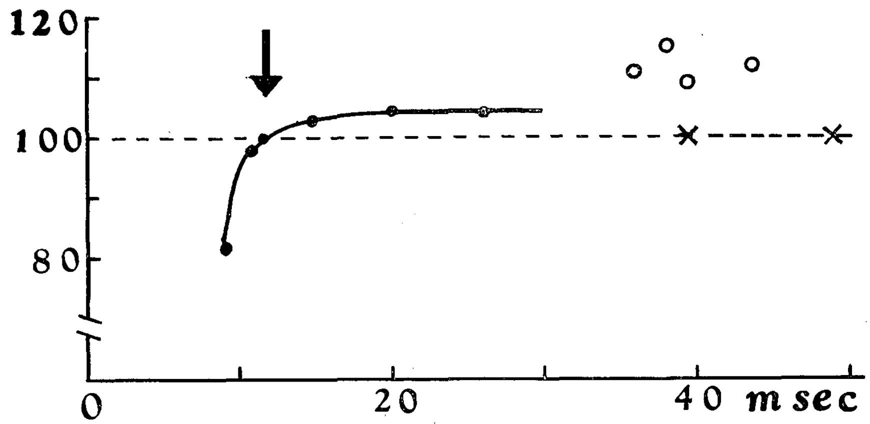

Fig. 3. b. The change of the conduction velocity of the second action potential. Single muscle fibre of a toad. Ordinates are velocity of impulses represented by unit of normal value. Abscissae are time in msec. elapsed after the first stimulus. Dots represents the same data as un Fig. 3. a. Crosses; that of the data of Nov. 1st, '50. Temperature; 13.0°C. Open circles; the data of March 19th, '53. Temperature; 14.7°C. Arrow; see in discussion.

the least interval. This may possibly be happened as a special result that is caused by stimulation with a pore electrode. Uchizono used an intracellular electrode for muscle fibre of a frog and a giant fibre of a crayfish and reported that the interval of the stimuli became shorter, the smaller the height of the second impulse until scarcely perceptible. This discrepancy may possibly be explained as follows. By using a pore electrode, the fibre could not be stimulated by strong current for fear of spreading the current to surrounding fibres. As with an intracellular electrode as was used by Uchizono, a strong current can be used for stimulation of a single fibre without any current spread. Such an extremely short interval of stimuli was not adopted in the following experiment.

2) *Three successive stimuli*:—Three stimuli were applied to a single muscle fibre in succession and the action potential was led to record from the different point of the muscle fibre along its length. The record

is shown in Fig. 5. Its velocity diagram relating the time and the distance in conduction is represented in Fig. 4. a. & b. This shows that the conduction rates of the second and the third impulse were above normal. In other words, the slope of the velocity diagram of

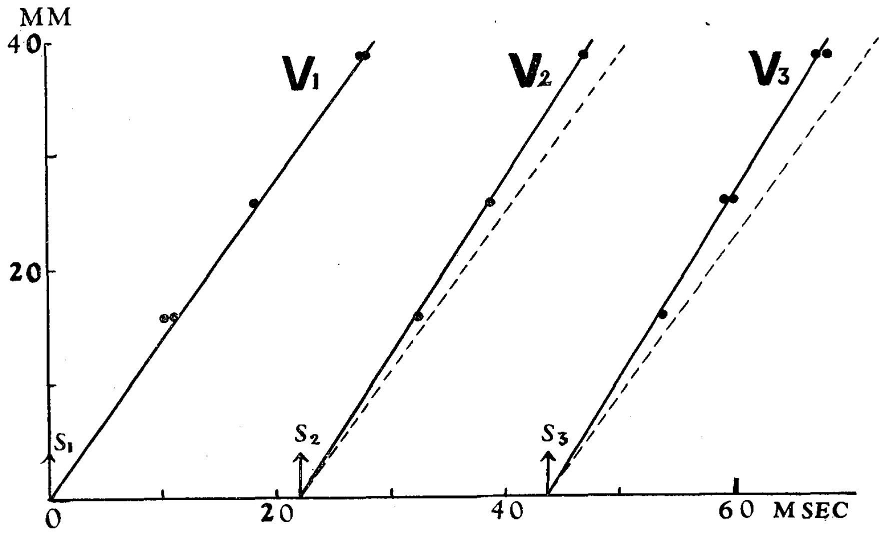

Fig. 4. a. Velocity diagram of the successive impulses in a single muscle fibre. Ordinates: distance of the muscle fibre from the stimulated point. Abscissae; time elapsed after the first stimulus. S_1, S_2, S_3; moment of the stimulus. Time interval S_1—S_2 is 21.9 msec., S_2-S_3; 21.7 msec. V_1, V_2, V_3; solid line indicates velocity of each impulse. Two broken lines indicate lines drawn parallel with the line V_1 (normal velocity). Lines V_2 and V_3 represent the increase of conduction rate. The line V_2 approaches the line V_1 as longer distance the impulse is conducted.

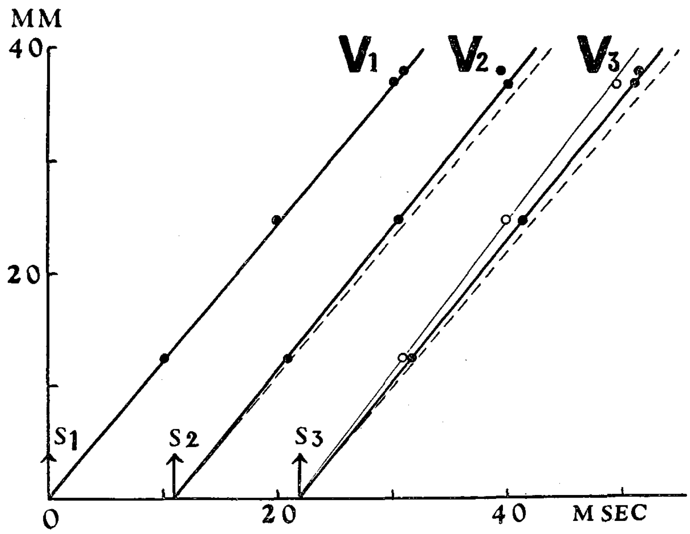

Fig. 4. b. The same as Figure 4. a. S_1—S_2; 10.9 msec., S_2—S_3; 10.9 msec.; Both intervals are very near the least interval. The thin solid line V_3 indicates the increase of the velocity of the last impulse when the intermediate one was abortive or omitted. Temperature; 14.7°C, March 18th, '53.

the second and the third impulse were steeper than that of the first one. This diagram shows that the shortening of shock-response intervals will became rather remarkable when the impulse has traveled through a longer pathway.

The Fig. 4. b. shows the case in which each interval is near the least interval. The thin line represents the speed of the last impulse which became faster than before when the second stimulus was omitted or abortive. This abortion is probably due to the fluctuation of excitability. This may be expected from the data of Fig. 3. b. in which the last impulse became the second one by omitting the intermediate stimulus.

To examine whether the first impulse influenced the speed of the third one, a conditional stimulus was applied preceding two stimuli

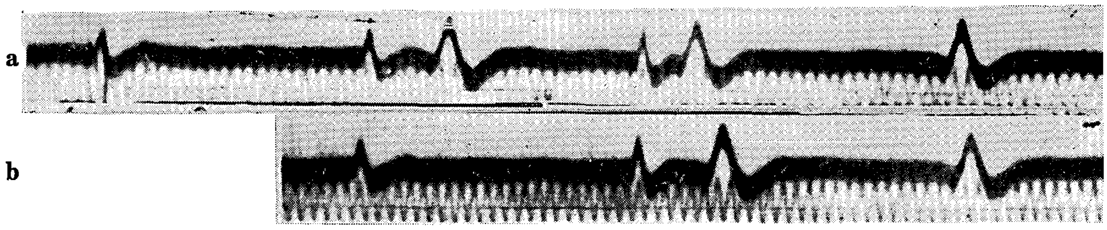

Fig. 5. Action potentials of a single muscle fibre of a toad stimulated with pore electrode. Temperature; 14.7°C. March 19th, '53. a; three stimuli, whose intervals are 21.8, 21.7 msec. b; the first of the three was omitted. The distance of the lead off electrode from the stimulating point is 39 mm in a and b. time mark; 1.000 c.p.s. Speed of the recording paper was not always the same. The figure was not retouched. The same data is represented in Table II.

Table II. Response time of a single muscle fibre.

Interval of stimuli (msec.)		Response time of action potentials (msec.)		
St_1-St_2	St_2-St_3	St_1-Ap_1	St_2-Ap_2	St_3-Ap_3
21.8	21.7	27.4	25.5	24.8
	21.0		27.2	25.0

The original record is shown in Fig. 4.

properly spaced. The result is shown in Tab. II. and Fig. 5 which indicates that the conduction velocity of the last impulse was not found to be affected by the conditioning stimulus.

3) *Irregular successive stimuli:*—The results in Fig. 6 and Table III. The rate of conduction was compared by examining shock-response intervals of each impulses. The result shows that only the first impulse traveled with a normal speed but all subsequent traveled faster. Speed of the latter is almost similar with each other and indicates that the length of the interval did not show such a significant influence on the velocity of impulses in this degree of fibre length. This is shown in Table III.

Bullock stated that the speed of the conduction velocity of impulses of a giant axon of an earthworm and of a sciatic nerve of a frog stimulated with multiple stimuli of regular interval may reach maximum

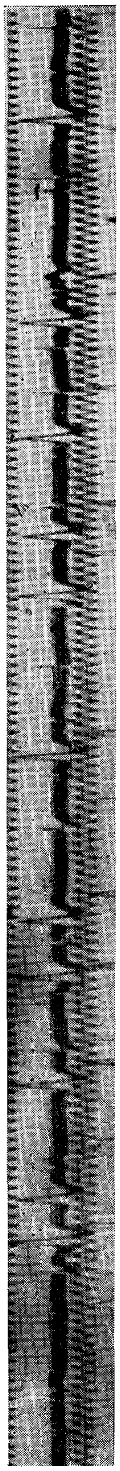

Fig. 6. Multiple stimulation with irregular interval. Single muscle fibre of a toad. Stimulation; with Pratt's electrode and two induction coils. Temperature; 18.0°C., April 1st, '54. The result of measurement is indicated in Table III. Time mark; 500 c.p.s.

Table III. Multiple stimulation of a single muscle fibre with successive irregular intervals.

Interval between stimulus and action potential (msec.)	Interval between stimuli (msec.)	Interval between action potentials (msec.)
16.0	10.8	9.2
14.4	24.4	24.4
14.4	22.6	23.2
15.0	12.4	12.2
14.8	35.6	35.0
14.2	35.4	ca. 35.2
ca. 14.0	12.8	ca. 13.2
14.8	22.0	21.8
14.6	25.6	25.4
14.2	9.8	11.0
15.6		

after several impulses. This author's experiment did not confirm Bullock's findings.

C) *Double stimuli of nerve fibre*

The next step is to do the same on the nerve fibre by stimulating with a pore electrode. It was appropriated to adopt the Braun tube oscilloscope to record the event of the nerve, because its velocity is higher than that of the muscle.

Adrian observed that the supernormal phase in the nerve became large when treated with acidified Ringer's solution but with alkalinity the refractory period was prolonged and its excitation was depressed. In the supernormal phase, an increase may be expected in the conduction velocity of nerve fibre. For this reason, an experiment was conducted on the nerve fibre treated with Ringer's solution of different acidity.

The result showed that it is nearly the same in nerve and muscle. The increase of the speed of the nerve fibre was ascertained in the record, as shown Fig. 7. but it was difficult to measure it numerically as done in the case of the muscle fibre. To see whether the facilitation of conduction was really directly connected with supernormal phase, an attempt was made to measure the conduction in different alkalinity. But this was not successful because the fluctuation of the threshold of the nerve increased too much to make the measurement difficult.

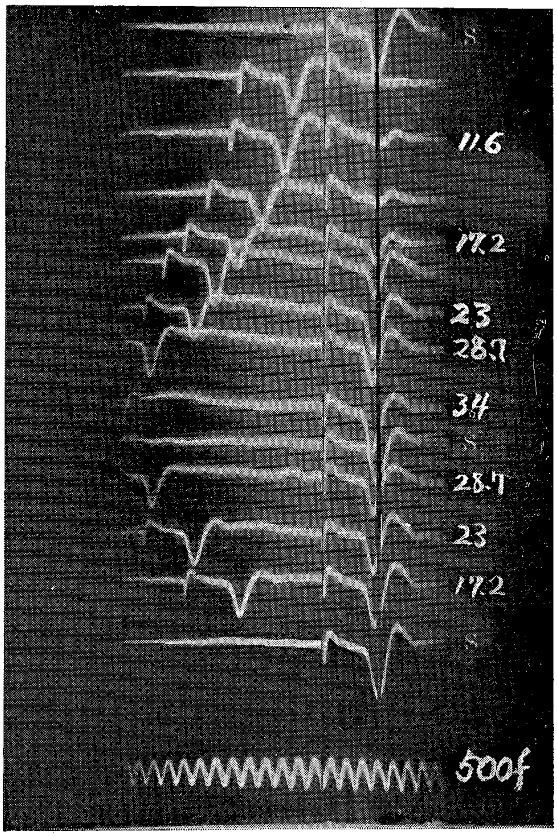

Fig. 7. Double shocks of a few nerve fibres with pore electrode. Toad's sciatic, Temperature, 14.0°C. Jan. 18th, '54. Due to the position of the recording electrode at extremely peripheral portion of the sciatic nerve, the form of the action potential was somewhat abnormal. The other explanation of the record is the same as Fig. 3. a. S;only a single shock was applied. With the aid of a vertical line the change of velocity of the second impulse shown. Time mark, 500 c.p.s.

D) *Indirect stimulation of nerve-muscle preparation*

In this case, (i) a few nerve fibres were stimulated by using a pore electrode placed on the stripped sciatic nerve trunk. Action potential was led from the sartorius muscle. (ii) All fibres in the sciatic nerve of a frog was cut except for one fibre, and the pelvic end of muscle fibre was also cut off but several muscle fibres remaining intact. A vacuum tube stimulator combined with a Helmholtz' pendulum was used for stimulation. Then by stimulating the proximal portion of the nerve, the action potential was led from the pelvic end of the muscle whose fibres were reduced to a minimum as shown in Fig. 1. b.

In (i), successive growth of action potential due to the facilitation of junctional transmission, and the increase of conduction rate in the second and the third impulses was observed as in Fig. 8. The phe-

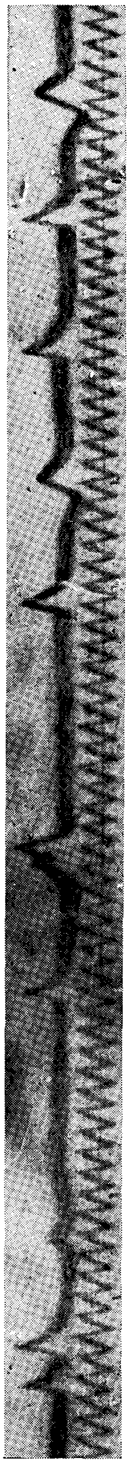

Fig. 8. Multiple stimulation of a few nerve fibres with irregular interval. Toad muscle preparation. Temperature; 16.0°C, Feb. 25th, '55. Time mark; 500 c.p.s. Retouched. Stimulus artefacts are indicated by numbers. The second and the third stimulus are given in absolutely refractory period. The result of measurement is shown in Table IV.

Table IV. Multiple stimulation with irregular interval.

Ieterval between stimulus and action potential (msec.)	Interval between stimuli (msec.)	Interval between action potentials (msec.)
13.4	38.6	36.8
11.6	38.4	37.8
11.0	38.0	38.4
11.4	35.8	36.4
12.0	41.6	40.6
11.2		

The same data as shown in Fig. 8.

nomenon of facilitation was not remarkable in (ii) but the general feature of the conduction rate appeared rather striking in the case of the nerve-muscle preparation. This is shown in Fig. 9. In this figure, in which the action potential of the nerve and muscle are simultaneously indicated, the advance of conduction is clearly recognized in the second and in the third impulse in muscle as compared with the same in the nerve fibre which shows neither advance nor retard in conduction. In this case the length of the nerve was too short to make any increase of speed noticeable. Then this advance of action potential which shows the increase of conduction rate can be caused possibly not only by the neuromuscular junction but also by the property of the muscle fibre. No matter which of the tissues may be responsible for the phenomenon, it is noticeable that the increase of conduction rate is clearly observed by indirect stimulation.

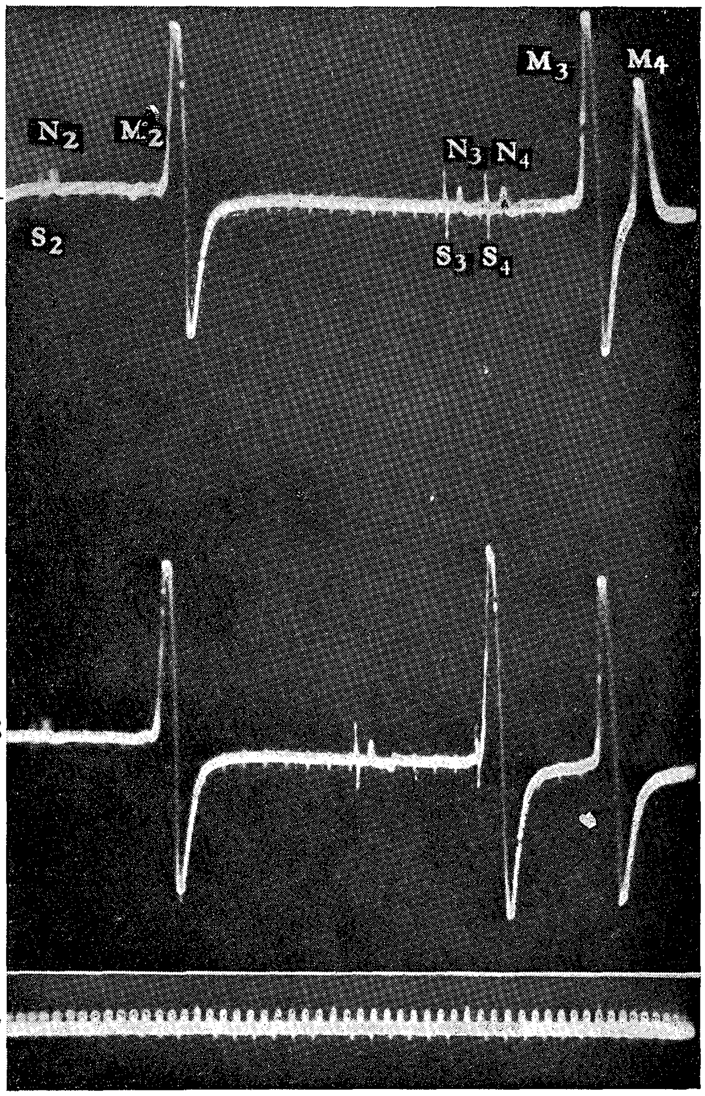

Fig. 9. a. Multiple stimulation of a frog's nerve-muscle preparation. A single nerve fibre was stimulated. The action potential was recorded from a few muscle fibre. Temperature; 24.5°C. July 5th, '56. S; stimulus artefacts, N: action potential of a single nerve fibre, M: the same of a few muscle fibres. A: Interval of stimuli; S_1 (the first)—S_2 (the second), ca. 20. (S_1 is not represented here), S_2—S_3, 30., S_3—S_4, 2.9 msec. Response time; I_2 and I_3 both are 8.3 msec., and that of I_4 is 10.0 msec. B: Interval of stimuli; S_1—S_2, Ca. 20., S_2—S_3, 24.3, S_3—S_4, 8.6 msec. Response time; I_2, I_3, and I_4 are 8.4, 7.9, and 8.6 msec. Time; 10/7 msec.

Discussion

The results obtained here did not coincide with Lucas, Samojloff, Gotch, Forbes and others, Gasser and Erlanger, and Ichioka's. They observed that with two stimuli in succession the conduction rate of the second action potential became slower as it propagated further. And when the interval of two stimuli was too short, the second action potential was delayed, which Lucas called "irresponsive period". Cooper reported that in the nerve of the frog the conduction velocity of action potential appeared to become faster in supernormal phase, and thought it to be probably an error. Although Graham observed also the increase of conduction rate of the nerve, she and Lorente de Nó reported in 1938 that the increased rate of conduction velocity did not occur at least in perfused mammalian nerve fibre and that the increased rate, if observed, may be due to the result of "poor condition" of the preparation.

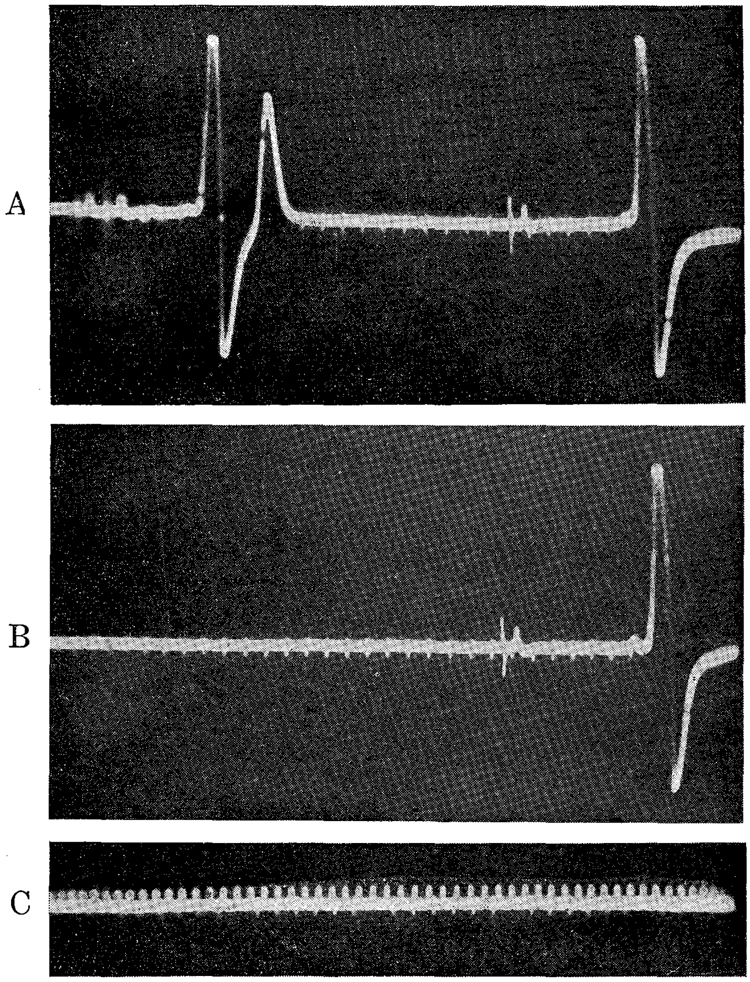

Fig. 9. b. The same as Fig. 9. a. A: Interval of stimuli; S_1—S_2, 2.3, S_2—S_3, 29.4 msec., response time of I_1, I_2, and I_3 are 9.3, 10.2, and 8.3 msec. respectively. B: Single shock. Response time is 9.4 msec. (normal value). The change of velocity of impulse is known by comparing this normal value with each response time.

With the use of the Pratt's pore electrode, Wakabayashi observed that in the case of double stimuli, the second action potential of muscle fibre of a frog traveled faster; his results are substantiated by (B) in this experiment. As for the nerve, Bullock used a giant axon of the earthworm and frog's sciatic nerve trunk and achieved almost the same results as (C) of the present report. He got also the relationship between recovery curve and conduction velocity, but doubted whether the phenomenon was caused by the same process as the supernormal phase.

In double stimuli the second impulse ran after the first with supernormal speed when two stimuli properly approached close each other (See Fig. 3. b.). However, its speed became subnormal when the two stimuli approached too closely. And then, in the former case, the spatial distance between two impulses in the fibre should become narrower as they propagate further, and in the latter case, it should become wider. If the fibre were unusually long, a certain irregular series of impulses at the start could become regular temporarily and spatially in the long run. Logically the final interval of this hypothetical regular series will be given by the point where the curve of the conduction rate of the second impulse and the line of normal one cross, as shown by an arrow in Fig. 3. b. It is easily expected that the speed of impulses should be normal one in this final state. These results were very similar to Ichioka and Wakabayashi, who obtained the curve of speed with the nerve trunk of the toad, although their curve of conduction rate was somewhat different from this experiment. They obtained the decrease in speed and no increase in the refractory and supernormal phase. This discrepancy might be caused by different methods of stimulation. A precise explanation of this has not been obtained, concerning a tendency for self-regulation in the impulse interval, but from their results also a similar or the same event may be expected, namely that irregular intervals of impulses tends to regular as the impulse is travelling long way.

The experiment with pore electrode indicated the existence of some intimate relationship between recovery of amplitude of the action potential with its velocity. It is worth noticing that the height of the second action potential could be small even when the conduction rate was faster than the first.

It seems paradoxical that the conduction rate is large in the phase in which the recovery of the action potential is still incomplete. But this could be understood by the hypothesis that during the process of restitution after excitation in nerve and muscle, the membrane of the fibre can be easily depolarized to a critical level by conducted impulse for initiation of an impulse and is able to produce only small action potential than normal. Similar fact of speeding up conduction is also observed in cathelectrotonus of a nerve in which a small action potential is conducted rather faster. It may be suggested from this, that the second impulse is conducted in the cathelectronic region of fibre which is left just behind by preceding impulse.

Further investigation is required to find out whether such hypothesis will substantiate all the facts in this connection.

Acknowledgment

The author is indebted to Professor Wakabayashi of the Physiological Institute, Tokyo University, for his kind encouragement during this experiment and also wish to thank Dr. Nakanishi in Wakabayashi's laboratory for valuable assistance in permitting the use of the stimulator which he constructed.

Summary

1) The conduction velocity of a single fibre of a muscle, nerve, and

nerve-muscle preparation of a toad was observed when two or more stimuli were applied.

2) Conduction rate did not depend upon the strength of the stimulus applied.

3) At a point in close proximity to the least interval, the conduction rate of the second action potential became slower. When the interval became slightly longer, the second impulse traveled faster than the first one even when the height of the former was smaller than the latter.

4) If a stimulus could not evoke an impulse, the speed of the impulses that followed was not affected by the first stimulus.

5) When the muscle was stimulated directly or through nerve fibre with irregular interval, the response time of the first action potential was somewhat longer than the second. And there after they were almost the same.

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