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Author(s)	TAMASIGE, Mituo
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# Nerve-Supplied Single Muscle Fibre

By

**Mituo Tamasige**

(Zoological Institute, Hokkaido University)

(With 2 Text-figures)

In recent studies of neuromuscular transmission considerable efforts have been devoted to investigations on the electrical sign of activity of the junction, i.e., the endplate potentials (Eccles *et al.*, 1941; Fatt & Katz, 1951; Nastuk, 1953; and del Castillo & Katz, 1954). It is especially interesting as having dealt with a single element that Kuffler (1942) recorded electric potential changes from an isolated single neuromuscular junction. On the other hand, Tamasige (1953) recorded mechanical responses of an isolated muscle fibre to electrical stimuli; he undertook to clarify the factors determining the type of mechanical responses.

The present work was done in order to make a preparation of single muscle fibre with its nerve supply following Kuffler's method and to record mechanical responses of the muscle fibre to electrical stimuli through the attached nerve. The writer's own method was employed.

Fresh preparations were successfully made from the semitendinosus or biceps muscle of the frog, *Rana japonica*; the experimental arrangement is shown in Fig. 1.

Morphological aspects of the living nerve endings were found to be mainly similar to the photomicrographs of the rat muscle given by Carey (1941), but the arborization of a single axon was not so prolific in the frog muscle as in the rat muscle. In most cases the nerve ended with fine branches (nodular fronds of axonic projection, 1-5 micra in diameter) on the muscle fibre in the shape of a reindeer antler. Even by means of a phase-contrast microscope or a polarization microscope, no further distal branching could be observed from the axonic terminals noted above.

It is obviously demonstrated by recording the mechanical response of single muscle fibre to indirect stimuli through nerve attached that a preparation of an isolated neuromuscular junction possesses the normal transmitting function. In Fig. 2, the contraction of muscle fibre produced by indirect stimulation through nerve is compared with that by direct stimulation of the same fibre when it is curarized. It is seen in the figure that no essential differences exist between the

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Dedicated to Professor Tohru Uchida in memory of his 60th birthday.

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two responses in the case of single electrical shock. Moreover, the response of muscle fibre to indirect faradic stimulation at a frequency of 84 cycles/sec. through the nerve was the same as that to indirect single shock through nerve, so far as the preparation was fresh.

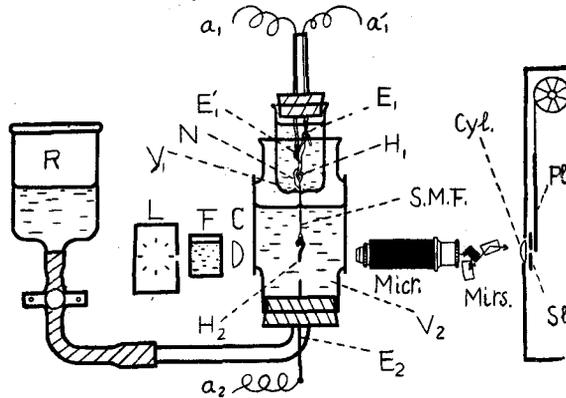


Fig. 1. Diagram of the experimental arrangement. R: Reservoir for Ringer's solution; L: Light source; F: Waterfilter; C: Condenser lens; N: Sciatic nerve; S.M.F.: Single muscle fibre;  $H_1$ : Glass hook to suspend the muscle fibre;  $H_2$ : Stained glass hook serving as a weight;  $E_1, E_1'$ : Ag-AgCl type non-polarizable electrode of upper vessel;  $E_2$ : the same type electrode of lower vessel;  $V_1, V_2$ : Upper and lower vessels containing Ringer's solution; Micr.: A horizontal microscope; Mirs.: Mirror combination for changing the image through the microscope at right angle; Cyl.: Cylindrical lens; Sl.: Slit; Pl.: Photographical plate which can be slid down at various speeds;  $a_1, a_1'$ : Lead wires for D.C. stimuli through the sciatic nerve in the upper vessel;  $a_2$ : Lead wire for D.C. stimuli of the muscle fibre directly between upper and lower vessel, coupling with  $a_1$  or  $a_1'$ , lead wire.

In the optical recording of the mechanical responses, the movement of the small stained glass hook,  $H_2$  (10 mg. in air), hung through the tendon left attached to the lower end of the muscle fibre is magnified by the horizontal microscope and registered on sliding photographic plates.

A muscle fibre was usually innervated by 2-4 axons or more as reported already by Kuffler (1942). When the innervated axons were cut off from the muscle fibre until only a single axon was left intact, indirect D.C. stimuli through the nerve produced either propagated or local contraction of the muscle fibre. The local responses seemed to be associated with the transmission through a small axon (within 5 micra) or through narrow-distributed axonic terminals.

In the next experiment a fresh preparation of a single muscle fibre with its nerve supply, covered by a thin layer of Ringer's solution, was held horizontally

in the air by glass hooks attached at the tendons. The nerve was cut off, and then the fresh-cut end was brought into contact again with the muscle fibre at a place apart from the originally innervated spot. The muscle fibre in this case could also be contracted in response to electrical stimuli applied indirectly through

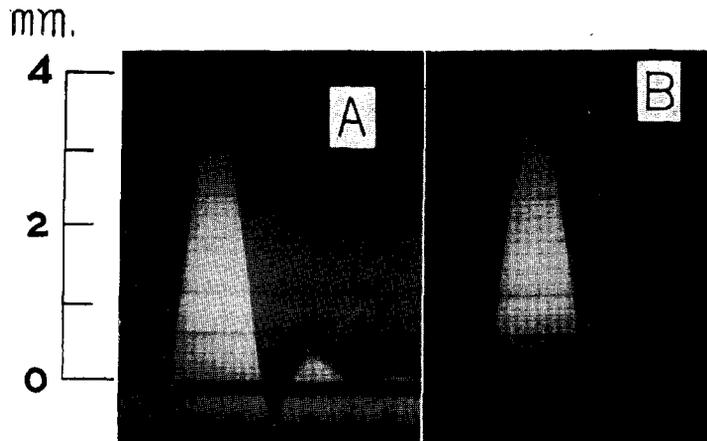


Fig. 2: Optical mechanograms recorded from an isolated muscle fibre with its nerve supply. All on same fibre from biceps muscle; diameter:  $72\mu$ ; length: 14 mm., at  $28^{\circ}\text{C}$ . Time signals are 30 cycles/sec. A: Contraction of the fibre produced by indirect single D.C. stimulus through the sciatic nerve attached. Stimulation: 1.5 v, 18 msec. B: Contraction of the fibre produced by direct single D.C. stimulus after curarization. Stimulation: 60 mV, 18 msec.

the nerve. Thus an artificial synapse ("ephapse") was successfully made from nerve to muscle fibre in which the endplate structure had no function. When the nerve was cut off from a definite distal part (the prongs or the dichotomous division of the axonic projection), the axonic terminals left on the muscle fibre were soon (within 10 min. or so) seen under microscope to have disappeared at room temperature ( $26^{\circ}$ – $30^{\circ}\text{C}$ ).

The results of the above observations seem to offer some substantiation to the electrical theory (Eccles, 1946) of the mechanism of neuromuscular junction rather than to the theory of chemical transmission on the same junction (Nachmansohn, 1946).

The greater part of this work was done from 1951 to 1953 while the author was in the Zoological Institute, Faculty of Science, Tokyo University. He is grateful to Professor Haruo Kinoshita for kind advice.

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