Responses of an Abdominal Ganglion of the Crayfish to Electrical Stimulation with a Sinusoidal Frequency Change[1)]

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(With 7 Text-figures)

In the transmission of information in the nervous system, all messages are conveyed in a code which is a sort of frequency modulation system of signaling, in which intensity of the nervous sensation is translated into frequency of the nerve impulses. Nervous sensation set up in a receptor organ and transmitted through a nervous system are usually related to a function of the stimulus intensity. But the output signals of some biological transducers are not always a simple function of the input one. In the eye of the cat (FitzHugh, 1957) and the infra-red receptor of the rattlesnake (Bullock & Diecke, 1956; Bullock, 1957) the nervous sensation was accompanied with a fluctuation of impulse frequency, i.e. a noise. In the central nervous system of the crayfish it was difficult to reproduce a particular response to the stimulation of the same region in a given preparation because previously mentioned fluctuation (Hughes & Wiersma, 1960).

In order to know the true response under a given condition, it is convenient to represent the relationship between the input and output signal of the biological transducer by the transfer function (Pringle & Wilson, 1952), which is determined by the statistical method for a noisy response (FitzHugh, 1957). In addition, the transfer function of a transducer which connects of several elemental transducers can be theoretically derived from the elemental transfer function; this situation must be applicable to the relationship between the nervous system and the neurones in it. In the present experiments, it was attempted to determine the transfer functions of the monosynaptic reflex pathway and the polysynaptic one and to find out the corresponding relation between the signal transfer characteristics and the structures of the two nervous pathways.

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Material and Methods

Material used was the abdominal ganglion of the crayfish, *Procambarus clarkii*. Dissection and mounting of the material and application of the electrodes have already been described in detail by Watanabe (1958). Some reflex pathways in *N. dorsolaterales*, the second root of the abdominal ganglion, were principally studied, for relatively simple responses were observed in this root.

Representation of the transfer function: The transfer function, which is ideally defined with the aid of a differential equation derived from the behavior of the transducer for any type of input, is usually expressed as the ratio of output signal to input one of the transducer using frequency as a parameter. The function can be expressed either in a mathematical formulation or its graphical representation, the former is not suitable for biological study because of the lack of accuracy of the measurements. Among several procedures for the derivation of the transfer function from the experimental results, the method using the sinusoidal input is much less cumbersome (Lippold, Redfearm & Vučo, 1958; Lowenstein & Finlayson, 1960). In the present experiments, the results are represented by the "Bode diagram" (Bode, 1945), in which "ratio" of amplitude of the sinusoidal input signal to that of output one and "phase shift" of the two signals were plotted on a logarithmic scale of frequency (Fig. 6).

Stimulation: The input signal, i.e., the electrical stimuli applied to the afferent nerve of the ganglion, consisted of repetitive square pulses the frequency of which was changed sinusoidally (the reciprocal of time interval value between pulses). It was obtained by the following procedure; when the sinusoidal current was supplied to the grid of the thyratron circuit as is shown in Fig. 1, the successive discharges of the thyratron formed

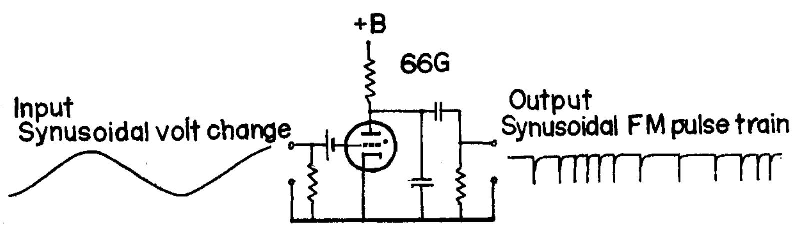

Fig. 1. Pulse-frequency-modulator with thyratron 66G-GT.

up the so-called frequency modulated pulse-train, because the discharge interval depends upon the grid voltage. The square pulse stimulator was triggered by such pulse-train. The whole device of stimulation and recording is shown schematically in Fig. 2. The frequencies of the sinusoidal current for frequency modulation were 20, 10 and 5 cycles/sec. The stimuli can be expressed in pulse frequency as follows:

$$S_1 = 100 + 50 \sin 7.2t$$
$$S_2 = 100 + 75 \sin 3.6t$$
$$S_3 = 100 + 75 \sin 1.8t$$

S_i: pulses/sec. ($i = 1, 2, 3$)
t : msec.

where in the right sides of the formulae the first terms are the pulse frequency of the carrier and the second ones represented the parts of the sinusoidal change (refer to Fig. 5).

Although the highest pulse frequency in the sinusoidal stimulation was about 200 pulses/sec., the afferent nerve impulses was always evoked by the individual pulse of stimulation, and there appeared no influence from the refractoriness of the nerve. Accordingly, the impulse-train on the afferent nerve had the same temporal pattern as that of the stimuli.

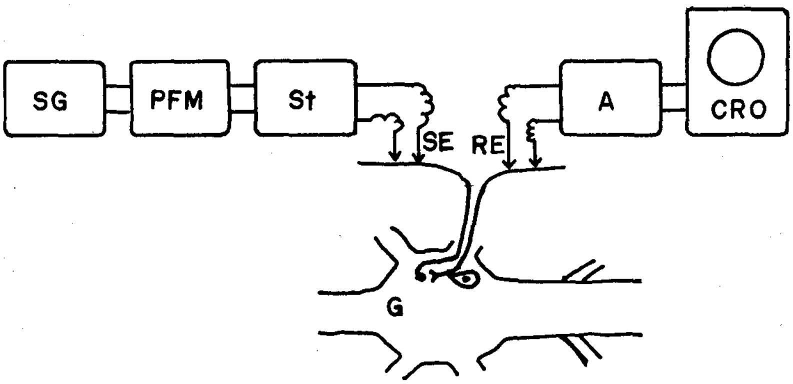

Fig. 2. Whole device of stimulation and recording. SG: Sine wave generator. PFM: Pulse-frequency-modulator. St: Square pulse stimulator. SE: Stimulating electrodes. G: Abdominal ganglion of crayfish. RE: Recording electrodes. A: Amplifier. CRO: Cathode-ray oscilloscope.

Results

Several types of ganglionic response: The typical simplest response of the ganglion to the stimulus of the constant frequency of pulses was formed of two parts, viz. the initial steep decay and the later slow decay in frequency of transmitted impulses (Watanabe, 1958), and was very noisy (the word "noise" does not refer to the purely electrical noise which arises in the recording and amplifying apparatus and obscures the action potential, but the random fluctuation of the discharge interval).

When the sinusoidal stimulation (strictly speaking, sinusoidal frequency-modulated pulse stimulation) was applied to the above mentioned pathways, several types of ganglionic responses occurred (Fig. 3). In a typical response the efferent discharge frequency of the ganglion usually changed without the phase difference from the stimulus (Fig. 3I); on the other hand there were other types in which the frequency of the ganglionic response did not clearly depend upon that of the sinusoidal stimulation (Fig. 3II) and in some cases the frequencies of the response occurred in inverse phase of the input (Fig. 3III). It is expected that the structural differences of the synaptic connection are among such transmission pathways where these types of the ganglionic responses occur.

The transfer function of the ganglionic transmission: Two examples of the above mentioned typical response were analyzed in detail. Their experimental records are shown in Fig. 4; A–type is not accompanied with the after-bursts but B-type is accompanied with remarkable after-bursts. In the records of each type, I is the response to the stimulation of the constant frequency of pulses, II, III and

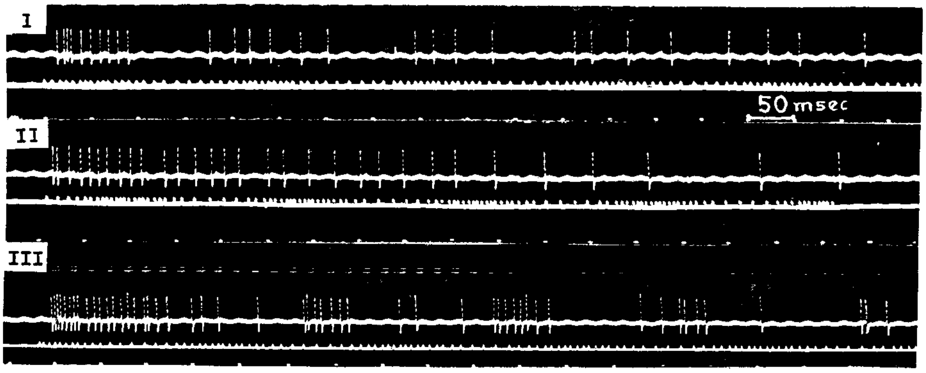

Fig. 3. Three types of ganglionic responses to the repetitive pulse stimulation changing frequency sinusoidally. Upper signal is the transmitted impulses through the ganglion and the lower one is the pulse-train for stimulation, in each record.

IV are the responses to the sinusoidal stimulations in modulation frequencies of 20, 10 and 5 cycles/sec. respectively. In order to see easily the relation between the input signal and output one of the ganglion, graphs representing the temporal patterns of the stimulation and the response in terms of pulse-frequency which could be derived from the records in Fig. 4 are shown simultaneously in Fig. 5. The time change in the output signals is more irregular than the temporal pattern of the input ones. This irregularity is due to the noise and, in part, probably to the non-linearity of the transmission (i.e. some frequency components which are not contained in the input signal appear in the output).

The sinusoidal component of the same frequency as the stimuli was detected from such irregular response by means of the so-called "correlation method", i.e., only a frequency component which had strong correlation with the input signal was taken out of the output one (Goldman, 1953). Consequently the sinusoidal components of the ganglionic response to the sinusoidal stimulation of 20, 10 and 5 cycles/sec. were as follows;

$$\text{A-type} \quad \begin{cases} R_1 = 14 \sin 7.2t \\ R_2 = 20 \sin (3.6t+54) \\ R_3 = 11 \sin (1.8t+90) \end{cases}$$

$$\text{B-type} \quad \begin{cases} R_4 = 13 \sin (7.2t+270) \\ R_5 = 11 \sin (3.5t+120) \\ R_6 = 19 \sin (1.8t+35) \end{cases}$$

Ri: impulses/sec. ($i = 1, 2, \ldots, 6$)
t : msec.

It is to be noted that, in each formula, the amplitude of the sinusoidal response means the range of the frequency change of the efferent impulses as shown in Fig. 5, and the phase shift (the 2nd term in the bracket) is in the similar situation. Thus the Bode diagrams representing the transfer functions of the two pathways of the

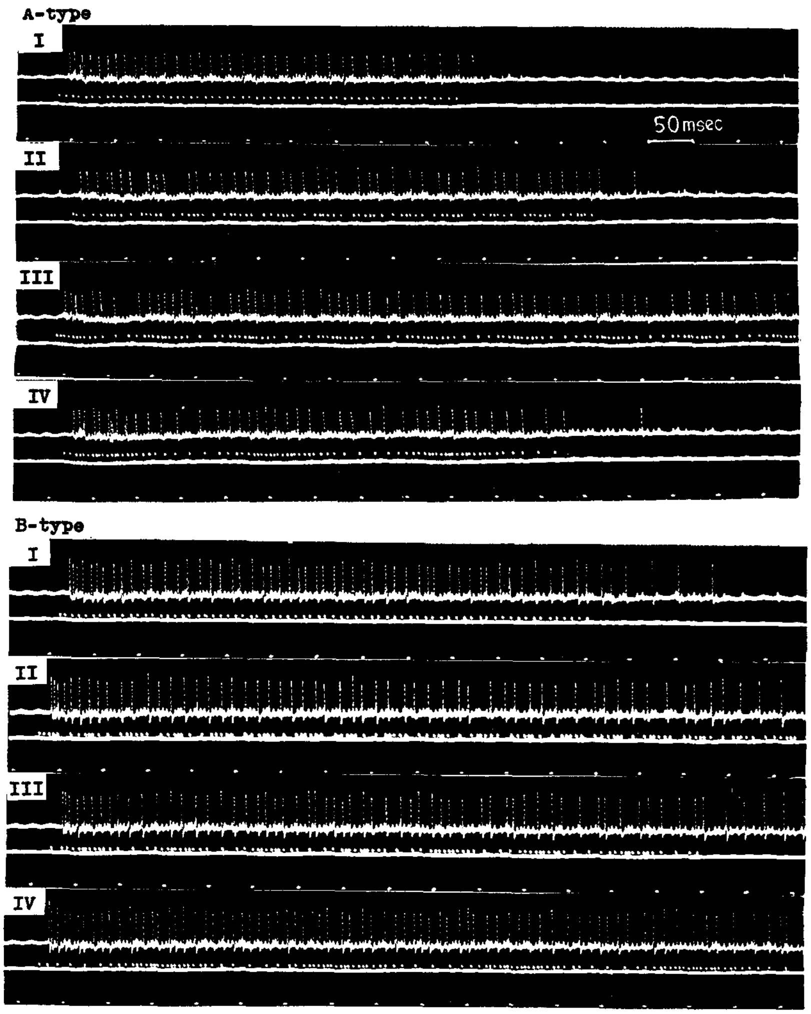

Fig. 4. Ganglionic responses to the repetitive pulse stimulation with the sinusoidal frequency change. A-type transmission is not accompanied with after-bursts and while B-type is accompanied with remarkable after-bursts. In each type, I is the response to the stimulation of a constant frequency, while II, III and IV are the responses to the sinusoidal stimulations of 20, 10 and 5 cycles/sec. respectively.

A– and B-type ganglionic response were obtained from above mentioned result as shown in Fig. 6. The theoretical curves of the transfer function of two ideal transfer systems, i.e., the first-order PD-transfer system with the time constant of 20 msec. against the A-type and the PID-transfer system against the B-type, are given in order to compare with the biological results (where P, I and D mean

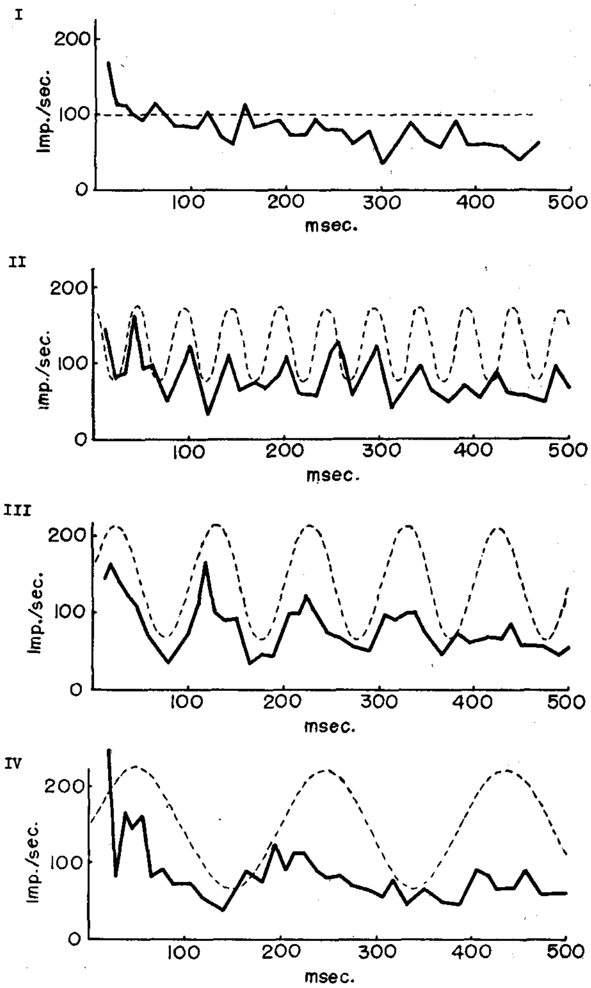

Fig. 5. Graphs representing the temporal patterns of the ganglionic responses in terms of the discharge frequency, reproduced from the records in Fig. 4A. *Abscissae*: Frequency of efferent impulses (pulses/sec.). In each graph the broken line and the solid line indicate the frequencies of the stimulation and the ganglionic response respectively.

"proportional, integral and derivative" respectively). A remarkable parallelism was seen between both curves of the ideal and experimental transfer function.

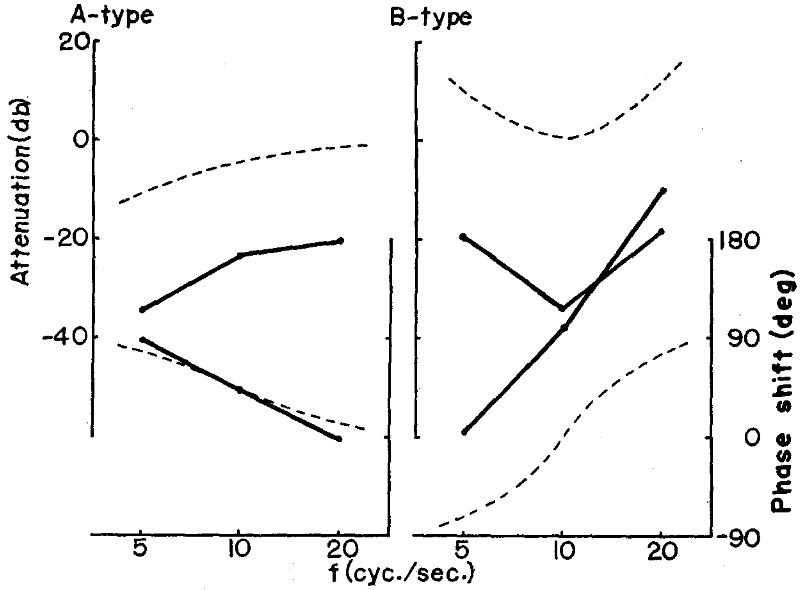

Fig. 6. Bode diagrams, graphical representations of the transfer functions, of the A- and B-type transmissions. *Abscissae*: The frequencies of the sinusoidal stimulation (cycles/sec.). *Ordinates*: The attenuation of the amplitude (decibells, in the upper left scales) and the shift of the phase angle (degrees, in the lower right scales) of the sinusoidal response from the stimulation. The solid lines are experimental results, while the broken lines indicate the theoretical curves of the transfer function of the 1st-order PD-transfer system against the A-type ganglionic response and PID-transfer system against the B-type ganglionic response.

Discussion

The ganglionic response to repetitive stimulation, even in monosynaptic transmission, is considerably complicated owing to accomodation and fatigue. When the prolonged constant current was directly applied to the spinal interneurone through the intracellular microelectrode, the initial frequency of the repetitive discharges evoked by the current rapidly decayed (Hunt & Kuno, 1959). In the abdominal ganglion of the crayfish, the exponential decay of the initial discharge frequency of the monosynaptic transmission may be regarded as the same phenomenon as that of the spinal interneurone, it is probably due to a nature of the postsynaptic membrane at which the phasic response arises even when the depolarization by the synaptic potentials remained constant.

At the same time the slow decay of the discharge frequency in the later part of the ganglionic response is supposed to be a result of the gradual decrease of the successive synaptic potentials. The two processes in a ganglionic response to repetitive stimuli are the fundamental characters of the monosynaptic transmission, in which the transfer function is similar to that of a first-order PD-transfer system. The ideal transducer which has a characteristic shown by the broken lines in Fig. 6A can be expressed by a rate network between input and output signals as is shown in Fig. 7A, although, strictly speaking, this transfer characteristic of such network corresponds only to the initial phasic response of the synapse.

On the other hand, in spite of resemblance of the initial phasic part of both A- and B-type responses, the former had a transfer function which differed from that of the latter; the B-type response was similar to that of PID-transfer system as is shown in Fig. 6B. The PID-transfer system corresponds to networks consisting of the feedback loops which contains the above mentioned rate network (Fig. 7B, C and D). Thus it is possible that the relationship between the A- and B-type transmission is analogous to that between the PD- and PID-system. The pathway of the B-type transmission is composed of several A-type transducers. In other words, it can be supposed that the B-type pathway corresponds to the polysynaptic neuronal connection which has the series, parallel and feedback network assumed from the circuits of the PID-system as is shown in Fig. 7.

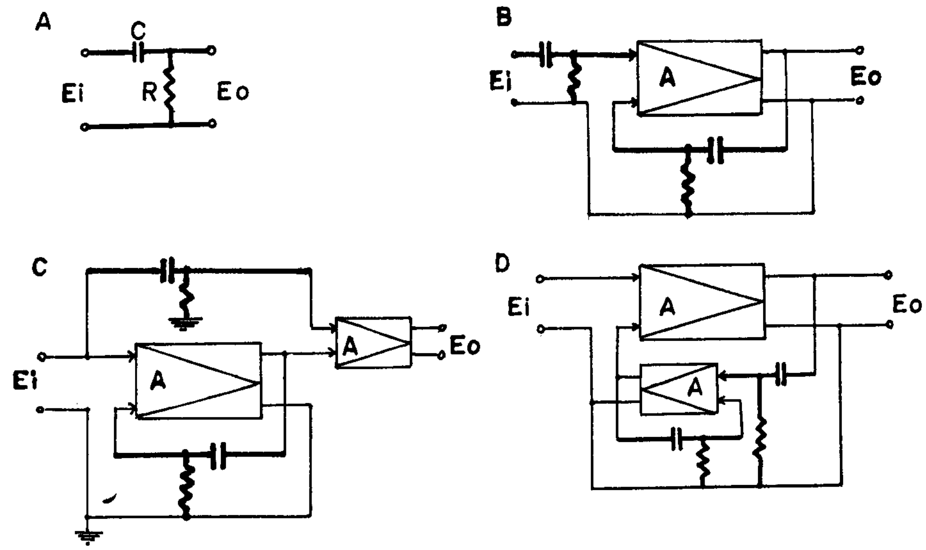

Fig. 7. Equivalent networks which express the theoretical curves of the transfer functions given by the broken line in Fig. 6. A: The rate network which has the 1st-order PD-transfer characteristics. B,C and D: The series, parallel and feedback networks which have the PID-transfer characteristics. Ei: Input signal. E_0: Output signal. C: Capacitor. R: Resistor. A: Amplifier.

A reason why the complete transfer characteristics of the signal transmission through the ganglion could not be obtained may be that the plotted points of the Bode diagram are only a few in number. As a matter of fact, it is impossible to estimate the complicated diagram of a high-order transfer function such as that of the B-type from the line connecting only three plotted points. In order to increase the number of plotting points, the number of stimulation pulses applied to a preparation must be large. In practice, the experimental results were obscured by the fatigue of the material owing to the large number of stimuli. In the study of ganglionic transmission, determination of the transfer function by means of the sinusoidal stimulation yields accurate information about the phase shift of the nerve signal, but is often inaccurate about the signal intensity dependent upon the method of application of the stimuli.

Summary

The transmission of nerve impulses through the abdominal ganglion of the crayfish was studied by means of pulse-train stimulation the frequency of which was changed sinusoidally.

When the sinusoidal stimulation was applied to some reflex pathways in the second root of the abdominal ganglion, three typical temporal patterns of the impulse-train through the ganglion were detected: I) The sinusoidal frequency change of the nerve impulses in the ganglionic response appeared in the same phase as that of the stimulation, II) no appreciable frequency change in the impulses was seen in the response and III) both the frequency changes in the stimuli and in the response were in the inverse phase to each other.

Two types can also be distinguished in the above mentioned I)-type responses; one of them was accompanied with remarkable after-bursts and the other without the after-bursts. Then the ganglionic responses of the two transmission types to the sinusoidal stimulations at frequencies of 20, 10 and 5 cycles/sec. were observed and the Bode diagrams of their transfer functions were obtained. The transfer function of the response without the after-bursts bore a close resemblance to the first-order PD-transfer system and the response with the after-bursts had a high-order transfer characteristic similar to that of the PID-transfer system (where P, I and D mean "proportional, integral and derivative", respectively).

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