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Axon-axon Transmission of Nerve Impulses, as Tested by Motor Axons of the Cheliped of the Crayfish*)

With 2 Text-figures and Plate I

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(*Communicated by* T. YAMAMOTO)

1. A considerable number of investigations has been accumulated, in which action potentials on nerve fibers accompanying the conduction of impulses are proved to exert some influence on adjacent fibers running in sufficient proximity, and, in some experiments, direct transmission of impulses between the juxtaposed axons has been demonstrated. Jasper and Monnier ('38) first succeeded in obtaining transmission of excitation across a contact region of two nerve bundles, which were dissected out of the nerve trunk of the crustacean limb and placed in contact in the form of a T. Such a mutually excitatory effect between adjacent fibers is probably not the case in the nerve trunk *in vivo*, because of the relatively large insulating effect of their sheath. (In fact, such a transmission of impulses from axon to axon would fail to account for the definite pathways shown to be independently activated.) Thus, Rosenblueth ('41) worked out the fact that impulses travelling in a group of fibers in cat phrenic nerve set up new impulses in neighboring fibers at the point where their excitability had been enhanced locally by means of electrotonus. On the other hand, Arvanitaki ('40, '42) achieved an experimental axon-axon junction between two giant axons of *Sepia* arranged in contact with one another for a short distance at their terminations. In such a preparation, if the contact region had

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been previously treated with Na-citrate, it was found that action currents of one axon acted as liminal stimuli to the other.

Conditions for analysis may be much more favorable with an axon to axon preparation such as Arvanitaki's, and, if possible, with the transmission surfaces of natural contact, so as to exclude many unknown conditions such as number of active fibers, elements influenced, position and condition of the contact surfaces, amount of shunting, etc. The present investigation is concerned with this sort of axon-axon transmission of nerve impulses.

2. Motor axons innervating the claw-adductor of the cheliped of the crayfish, *Cambarus clarkii*, were used. The adductor muscle is innervated by only three efferent axons, one of which is the inhibitory axon common with the extensor of the propodite and the other two are the motor axons of its own, respectively referred to as the 'slow' and 'fast' axon (Harreveld and Wiersma, '36, '37; Wiersma and Harreveld, '38, '39). As was done by the author for the Japanese giant crab, *Macrocheira kaempferi* (Nagahama, '41), their topographical relation in real appearance may be indicated by a 'nerve-map' as shown in the accompanying plate figure. They are easily recognizable by their larger size (more than 20μ in diameter in the meropodite region) in comparison with the other sorts of nerve fibers (less than 10μ), and by their definite course in the nerve trunk. Moreover, they can be distinguished from each other by their relative sizes; viz. the fast axon of greatest diameter (50~90μ) running close together with the slow axon of medium size (35~50μ) in a nerve bnndle B_2-*1*-*b*-*Ad* (cf. nerve-map), and the inhibitory axon of least diameter (20~30μ) which comes out at the middle region of the carpopodite from the bundle B_1-*a*-*Ab* to join the motor axons at the entrance of the propodite.

The two motor axons are distinguished physiologically from one another as follows. To a single impulse of the fast axon the muscle responds with a single twitch, though in not a completely fresh preparation at least two impulses suitably spaced are required to evoke contraction and in this case the characteristics of contraction depend on the number and frequency of the impulses, while no visible contraction is obtained on a single impulse in the slow axon, a train of nerve impulses at suitable intervals being required for a visible contraction.

The cheliped was cut off at the natural point of fracture. After exposing the nerve trunk running in the meropodite and carpopodite by removing the halves of the shell of those segments together with the underlying extensor muscles, the shell on the opposite sides was removed, together with the flexor muscles, under a binocular microscope.

Care was taken to leave the nerve trunk intact, thus providing a nerve preparation extending over the full length (2~3 cm) of the meropodite and carpopodite and connected with the tissues in the propodite. Further preparation with the aid of the nerve-map will be referred to in the following paragraphs. During and after preparation the nerve was prevented from excessive stretching and kept immersed in Harreveld's solution except for the interval during which stimulation was applied to it.

The tendon of the abductor was cut and the tip of the dactylopodite was connected with an isotonic lever, by means of which the mechanogram of the adductor was registered as usual. Induction shocks with the make shocks cancelled, or repetitive shocks of direct current of short duration were applied to the nerve through Ag–AgCl electrodes covered with cotton thread moistened with Harreveld's solution. The stimulation intensity was regulated by a potentiometric device (unless otherwise stated, kept low so as to obtain a single impulse on each stimulus and yet to avoid repetitive discharges).

Experiments were done at room temperatures between 15 and 20°C.

3. Except for the nerve bundle containing two motor axons in question (B_2–*1–b* in the nerve-map), all bundles were cut at the entrance of the propodite and were carefully seperated from each other over the full length, leaving only a small portion proximal to the cut point (2~5 mm) in natural contact. After cutting all bundles at the proximal ends of the meropodite region, the bundle reaching the adductor and one or more of the other bundles were seperately hung on glass hooks with threads ligating the proximal ends. When repetitive shocks were applied near the proximal ends of bundles thus severed from the muscle, stimulation thus applied never gave rise to response of the muscle, irrespective of its intensity and frequency, no matter which conditions shown in the following paragraph were taken. Therefore it may be said that Jasper and Monnier's artificial synapse as previously cited is not realizable in the present case.

4. Retaining the bundle containing two motor axons for the adductor, all the bundles of the nerve preparation were removed and then the fast and slow axons were completely separated over the full length, leaving only a small portion (2~5 mm) near the entrance of the propodite. After cutting the fast axon at the entrance of the prododite, the two axons were separately hung on glass hooks with threads ligating the proximal ends. Repetitive shocks were then applied to the fast axon near the proximal end for suitable periods (Fig. 1).

The results obtained are shown diagramatically in Fig. 1, *a*, *b*, *c* and *d*.

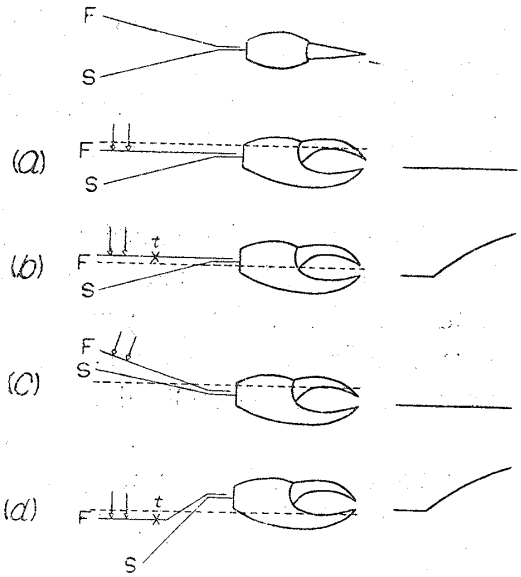

Fig. 1. Diagrams showing axon-axon transmission across the contact region. F: fast axon; S: slow axon; t: ligated or narcotized part. In the right column, the corresponding contraction curves are shown semidiagramatically.

a. In the first place, when the whole axons are submerged just beneath the surface of the physiological solution, stimulation given to the fast axon elicits no contraction in the adductor, irrespective of its intensity and frequency. (Control stimulation given to the slow axon with suitable intensity and frequency evokes a slow contraction.)

b. On the other hand, when the whole axons are temporarily lifted into the air away from the surface of the physiological solution, stimulation to the fast axon does elicit response of the muscle and the resulting contraction is in all its essential features the same as the slow contraction.

c. However, when the contact region of the two axons is immersed in the physiological solution, stimulation to the fast axon evokes no contraction of the muscle, even if the stimulated region remains in the air.

d. Conversely, in the situation that the contact region is lifted out of the solution and the stimulated region is immersed just beneath the surface of the physiological solution, stimulation applied to the fast axon again gives rise to a response of the muscle which is of the slow contraction type.

In the case of *b* and *d*, no contraction appears in the muscle following the stimulation of the fast axon in those preparations in which the fast axon is ligated with a thin thread or placed at a point between the stimulated and contact regions (*t* in Fig. 1) on a strip of filter paper soaked with a mixture of ether and Harreveld's solution. On the other hand, stimulation of the slow axon instead of the fast axon gives rise to contraction of the muscle. The narcotizing effect is reversible unless it is applied too long. Hence it appears that the excitatory effect on the muscle following stimulation of the fast

axon is not due to a spread of the electric current to the slow axon at the contact region.

The evidence of the transmission of nerve impulses across the contact region from the fast axon to the slow one will be brought out more convincingly in the experiments with variation in intensity and frequency of stimulation applied to the fast axon. The rate and height of the evoked contraction depend exclusively on frequency, and the stimulation intensity has no marked influence on them. As can be seen in Fig. 2,

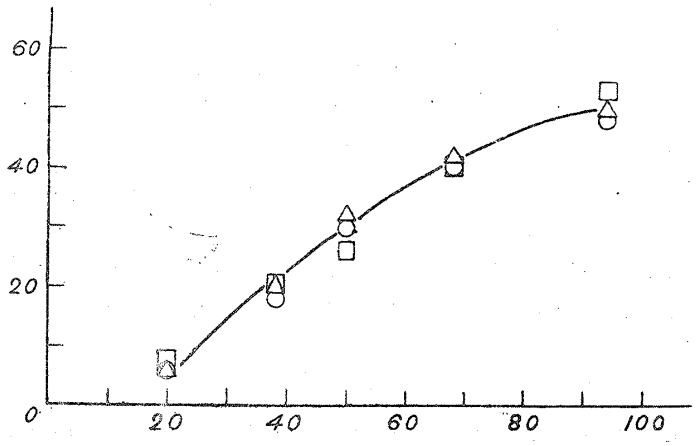

Fig. 2. Relation between frequency of stimulation applied to the fast axon and that of impulses evoked in the slow axon. The latter may be estimated from the curve of elicited contraction in comparison with that obtained in the case of stimulation of the slow axon itself. Relative stimulation intensities are 30 (□, just above the threshold), 60 (△) and 90 (○). Each point on the curve represents the mean value obtained from five different preparations. Tem., 18~20°C.

the frequency of the impulses evoked in the slow axon does not coincide with that of the stimulation given to the fast axon (accordingly, that of the impulses travelling through the fast axon, since the fast axon has little tendency of repetitive discharges). Furthermore, so long as the fast axon is firing, the slow axon is activated even with threshold intensity and in spite of three-fold variation in relative stimulation intensity, there is no noticeable difference in the number of evoked impulses in the slow axon corresponding to the frequency of stimulation. Such constancies as occur make it unlikely that the slow axon is activated directly by spread of the stimulating current.

It should be remarked here that the curve in Fig. 2 can not be extrapolated in the direction of the origin because of the fact that the slow system shows a perceptible response only when the frequency of

impulses is higher than 5 per sec. Thus, the starting point of the curve may be taken to indicate a ratio of about 4 between the frequency of impulses in the fast axon and that in the slow axon activated by the fast impulses. This ratio is found not to be constant at different stimulation frequencies but to tend to decrease, the higher the frequency becomes, though, at the higher range investigated (up to about 100 per sec.), a slight increase occurs with an increase in the frequency, thus revealing that the activating effect on the slow axon increases, the more rapidly the impulses in the fast axon follow each other.

Thus it may well be concluded that, if the interstitial fluid in the contact region of two nerve axons is sufficiently ineffective in shunting the action current in the exterior conductive media, the stimulating effect of the action current of an axon elicits a propagated excitation in an adjacent axon. In addition it must be noticed here that, as is clearly shown in Table 1, the reaction time of the slow contraction induced by stimulation of the neighboring fast axon is noticeably longer than one giving rise to exactly the same contraction but induced by

Table 1.

Reaction time of the slow contraction evoked either by stimulation of the slow axon or by axon-axon transmission from the fast axon.

Number of preparation	Frequency of stimulation to the fast axon (per sec.)	Reaction time (msec.) by stimulation of		Difference of the two reaction times
		Fast axon[1)]	Slow axon[1)]	
1	38	406	382	24.4±4.5[2)]
2	38	386	364	22.0±2.5
3	38	433	405	28.0±3.9
4	38	417	391	26.4±2.2
Average				25.2±2.1
1	68	126	108	18.0±1.8
2	68	146	128	18.4±3.2
3	68	141	127	14.0±3.0
4	68	154	137	16.8±2.8
Average				16.8±1.0

Each value is a mean of five measurements. Length of contact region, 2 mm ; temp., 18~20°C.

1) The distance between the stimulating electrodes (the polar distance of 4~5 mm) and the end of the contact region distal to the muscle was adjusted always to be 15 mm on the axon.

2) With reliability of 95 %.

stimulation of the slow axon. Although it varies somewhat with the stimulation frequency, the difference between the two reaction times is found to be of the order of 15 to 27 msec. Supposing that the difference in conduction times along the fast and slow axons from the stimulated point to the contact region at which transmission across the two axons may occur is neglegible, the above-mentioned difference in reaction time may be mainly attributed to the time delay for transmission of impulses across the contanct region from the fast axon to the slow one. The value is comparable with that reported by Jasper and Monnier ('38) and explained by Arvanitaki ('42) as an indication of rhythmical and gradually increasing local electric activity preceding the propagative excitation.

The length of the contact region of the two axons, even if it is more than 5 mm in the usual preparation, has no appreciable influence on the results, although if it is less than 1 mm, transmission hardly takes place, probably because of unfavorable conditions of the contact surfaces induced during preparation.

It should be pointed out here that for the effectiveness of the action current as a stimulus, the electric field created in the exterior conductive medium and its variation with the propagation of the spike along the active axon play important parts. As has been demonstrated by Arvanitaki ('42), among others, when a spike along an active axon ceases to propagate further at a point such as a cut end, variation of the potential developed in the vicinity of that point subjects the contiguous axon to an action of the electric field most favorable to excite it. Such seems to be the case with the preparation used in the present investigation.

5. If the slow axon was cut off from the muscle instead of the fast axon, stimulation of the latter did not give any indication of the axon-axon transmission. It is supposed that in this case transmission may have failed because of some peculiarities as follows. a) It is very likely that action currents of the slow axon are less effective as stimuli, because their peak value is much smaller than that of the fast axon. b) The effectiveness of an action current as a stimulus, depends, on the other hand, on the excitability characteristics of the receptive axon. In this respect, the fast axon does not lend itself very well to this role of receptive axon, in contrast to the slow axon which is characterized by its facility of giving repetitive discharges. In not a completely fresh preparation, the fast axon usually shows repetitive discharges only with difficulty. c) As has already been mentioned, during preparation the muscle comes to respond only to two or more successive impulses of

the fast axon reaching it at an interval of less than about 40 msec. (On the contrary, in the slow system the liminal frequency of impulses for perceptible contraction is about 5 per sec., although the number of impulses required to evoke it is much more than in the case of the fast system.) Hence it is conceivable that, in the present investigation, the transmission of impulses from the slow axon to the fast one has apparently faild because the interval between activated impulses was too long to evoke the contraction, although the fast axon may have been activated in reality.

Summary. Axon-axon transmission of nerve impulses across the contact region of fast and slow motor axons innervating the adductor of the cheliped of the crayfish was demonstrated. When the contact region is lifted into the air, impulses are capable of being transmitted from the fast axon, which has previously been cut off from its connection with the muscle, to the slow axon to evoke the slow contraction; while, if the contact region is submerged in a physiological solution, this sort of phenomenon never takes place. The excitatory effect is not due to a spread of the stimulating current, since the shape of the contraction curve depends not on the intensity of the stimulation, but on its frequency. Delay in transmission of impulses across the contact of two axons is 15 to 27 msec. On the contrary, there is no indication of axon-axon transmission from the slow axon to the fast axon.

In connection with the physiological investigation, a nerve-map of the cheliped was constructed for the four distal muscles (plate figure).

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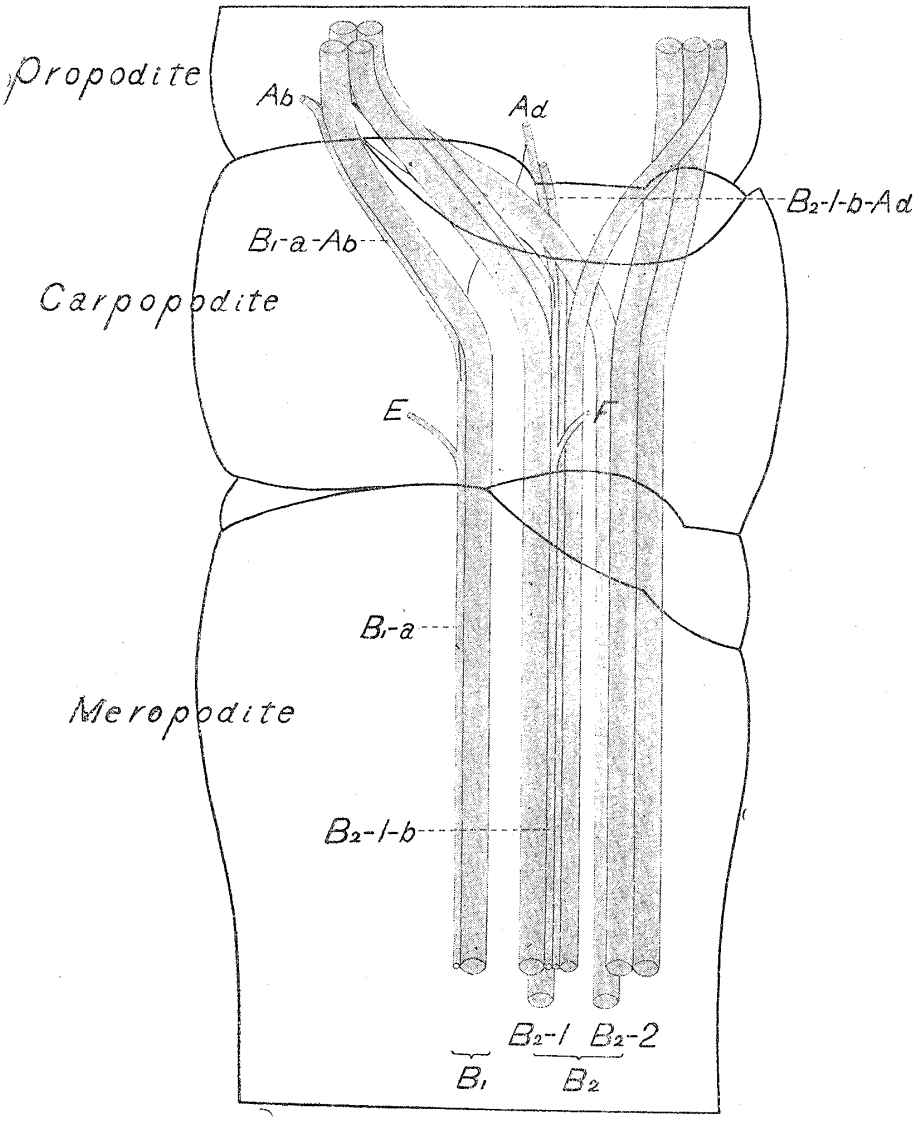

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Explanation of Plate

Nerve-map of left cheliped of *Cambarus clarkii*, viewed from inner side. The distribution of efferent axons for the four distal muscles is represented. In order to construct the map, principally the same method was applied as that described in detail for the Japanese giant crab (Nagahama, '41).

Though in the ischiopodite region the nerve appears to consist of only one trunk, it may be divided into two main trunks, a smaller (B_1) and larger (B_2) one, immediately after entering the meropodite. Each of these main trunks consists of independent nerve bundles connected to one another more or less firmly with thin connective tissues. The bundles forming the larger trunk may be divided into two groups (B_2—*1* and B_2—*2*), so that, although these two groups are also connected loosely to one another, they can easily be separated by slight teasing. While each bundle consists of a number of thin afferent axons, in a bundle (B_2—*1*—*b*) belonging to the group B_2—*1* of the large trunk there exist six efferent axons, together with a small number of afferent ones, three of which come out of the main mass after entering the carpopodite to innervate the flexor of the propodite (*F*) as the fast and the slow motor axons and the inhibitory one; two, the fast and the slow motor axons (contained in bundle B_2—*1*—*b*—*Ad* together with a few afferent axons), reach the adductor; and the remaining one branching out of the main near the entrance of the propodite is the inhibitory axon for the abductor (represented by the extra full line starting from B_2—*1*—*b*—*Ad*) and takes its way to meet another axon for the abductor (contained in B_1—*a*—*Ab*). The abductor is innervated by two axons, one of which is inhibitory as mentioned above and the other, running in a bundle (B_1—*a*) belonging to the small trunk, a motor axon (of the slow type) common with the extensor of the propodite. The remaining efferent axon is one common inhibitory axon for both the extensor of the propodite and the adductor, existing also in the bundle B_1—*a*. Thus, a branch (*E*) of B_1—*a* in the carpopodite contains ramifications of the two above-mentioned axons innervating the extensor, while the common inhibitor, after leaving the main mass, joins the two motor axons for the adductor at the entrance of the propodite so as to run to the muscle (represented by the extra full line starting from B_1—*a*—*Ab*).