Location of Synaptic Action in an Abdominal Ganglion of the Crayfish by Aid of Histological Methods[1)]

By

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(With 6 Text-figures)

In previous papers the present writer (1958, 1961) described some physiological characters of the synaptic transmission of nerve impulses in the abdominal ganglion of the crayfish. They were similar to the transmission characters of the cat spinal cord (Eccles, 1953, 1957) but differed from those of the giant synapse of the crayfish, which have been investigated in detail (Furshpan & Potter, 1959; Watanabe, A. & Grundfest, 1962). The physiological investigations of the giant synapse are on reliable histological basis, because there have been hitherto a number of investigations on the structure of it (Wiersma, 1961).

Kennedy & Preston (1960), who described observations on the synaptic transmission in the caudal ganglion of the crayfish similar to the present writer's concluded that their synaptic responses were obtained in the region of the neuropil from the measurement of the microelectrode displacement beneath the surface of the ganglion. However, no information in detail is available on the structure of the neuropil unlike the giant fibre system. It is required to locate the position of the microelectrode which records the synaptic potential by a more convincing method than only by the measurement of the microelectrode displacement. For such purpose, many authors have introduced some staining substance into the preparation from the electrode tip and recovered the staining spots in histological sections (Burns, Grafestein & Olszewski, 1957; Tomita, Murakami, Sato & Hashimoto, 1959; Mitarai, 1960; Brown & Tasaki, 1961).

In the present experiments, on the basis of the location of the position of the microelectrode tip attempts were made to find the correspondence between the synaptic response and the ganglionic structure.

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Material and Methods

Preparation: The abdominal cord was dissected out of the crayfish, *Procambarus clarkii*, and was mounted in a specimen chamber filled with Harreveld's solution[1]. The sheath of the ventral side of the ganglion was removed in order to facilitate the insertion of the microelectrode.

Stimulation and recording: Stimulation was applied by a pair of Ag-AgCl electrodes to the first root of the abdominal ganglion, *N. pedis spruii*, lifted out of the saline in the specimen chamber. The electrical arrangement was the same as that described in the previous paper (Watanabe, 1958). The synaptic responses were recorded by a glass capillary microelectrode filled with 20% potassium ferrocyanide solution.

Marking: The position of the microelectrode tip in the ganglion was marked by electrophoretic injection of a small quantity of ferrocyanide ions by means of application of anodic current of 2–10μA through the electrode for 5–10 sec. after recording of the synaptic response. Next the deposit of the ferrocyandie was stained by immersion of the marked ganglion into 10% ferrous chloride solution mixed with an equal amount of 1N-HCl solution. Then the marked ganglion was fixed by means of Bouin's solution or 10% neutralized formaline, stained with azo-carmine, imbedded in paraffin and sections were made in series. The position of the microelectrode tip was marked as a blue spot (Fig. 6A and B). Besides, Nissl staining and Mallory-azan staining were employed for the histological study of the structure of the ganglion.

Results

Histology: The detailed histology of the crustacean nervous system has been repeatedly investigated, but the connections among the sensory axons and inter- and effernt neurones have been not particularly resolved in relation to its functions, except for the giant fibre system.

First an outline of the neuronal configuration of the abdominal ganglion was investigated by Nissl staining (Fig. 1). Cell bodies of several sizes, 10–100μ in diameter, are found typically in a layer of the dorsal side of the ganglion. The cell layer holds a mass of neuropils, under the ventral side, which can be distinguished in structure from surrounding axon-bundles. The bundles of the root, axons enter the neuropil, although the fine structure is not brought out by this staining which failure is partly due to thickness 20μ of the sectioned specimen. Then the schematic structure of the ganglion can be made up from the serial sections (Fig. 2), consequently the position of the cell bodies and neuropil can be roughly pointed out in the figure.

In order to study the structure of the neuropil in detail, thin sections, 3–6μ in thickness, were made after Mallory-azan staining (Figs. 3 and 6). Although the structure of the neuropil was seen to be homogeneous in Fig. 1, it is shown by this staining to have a considerable variety. Numerous axons entered into the neuropil from the segmental roots, cell bodies and interganglionic connectives, and they

1) NaCl: 1.2%, KCl: 0.04%, $CaCl_2$: 0.15%, $MgCl_2$: 0.02%, H_2O: 98.6%; pH to 7.4 by $NaHCO_3$.

dispersed into it; on the other hand some axons passed by it. In the vertical sections, a dense meshwork structure is found just underneath the layer of the cell bodies. i.e., under the dorsal side of the neuropil. The rough meshwork found

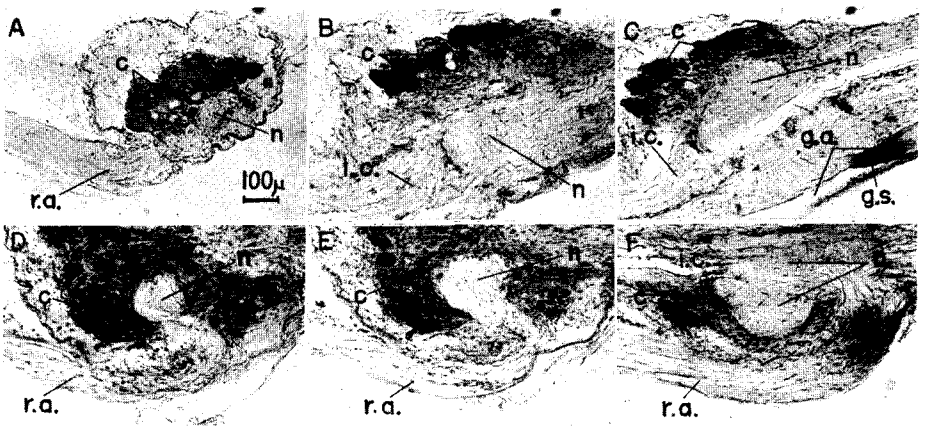

Fig. 1. Microphotographs of the serial sections of the abdominal ganglion of the crayfish. A, B and C; ventral sections along the antero-posterior axis of the abdominal ganglion. D, E and F; horizontal sections along the antero-posterior axis of the abdominal ganglion. c.: cell body. n.: neuropil. i.c.: interganglionic connective axon-bundle. r.a.: root axon-bundle. g.a.: giant axon. g.s.: giant synapse. Formalin fixation. Nissl staining. 20μ thick sections.

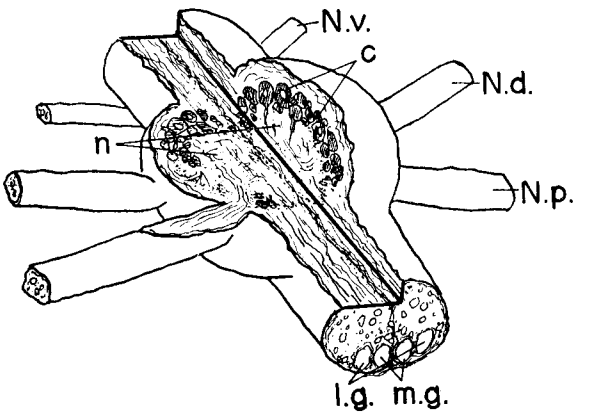

Fig. 2. Semischematic sketch of the construction of neuronal elements in an abodminal ganglion of the crayfish. c.: cell body. n.: neuropil. N.p.: the first root. N.d.: the second root. M.v.: the third root. m.g.: medial giant axon. l.g. lateral giant axon.

in the ventral side is not exactly the neuropil but a cross section of the contralateral axons, which extend between the right and left sides of the ganglion and can be clearly recognized in the horizontal section of the ventral region of the ganglion (Fig. 3C and D). It is assumed that the dense meshwork is the very neuropil (Fig. 4 and Fig. 6C, 6D), in which several arborizations of the tangled fine dendrites are found, although the precise structure of synaptic junctions can not be recognized in it by the present method even under high magnification (Fig. 4C and D).

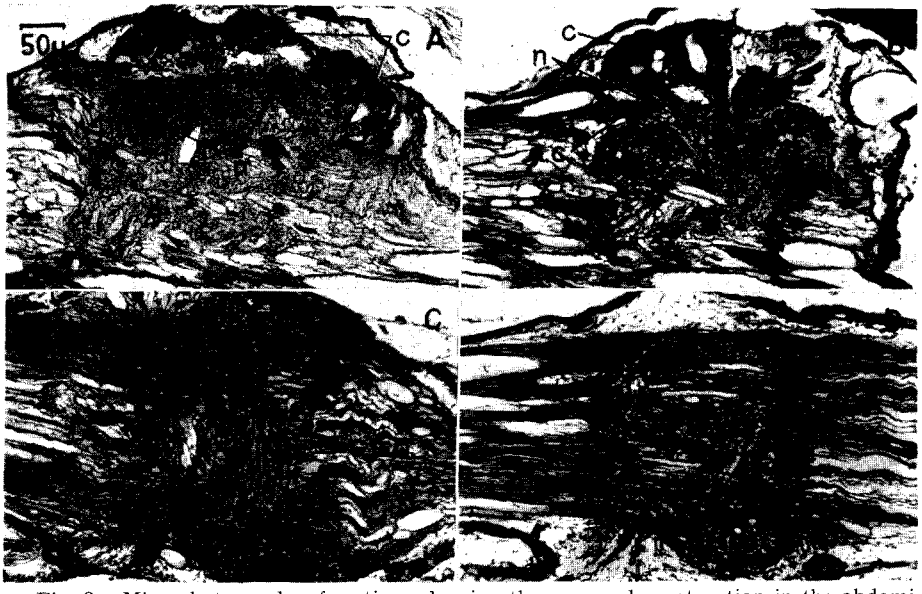

Fig. 3. Microphotographs of sections showing the neuronal construction in the abdominal ganglion. c.: cell body. n.; neuropil. a.c; axon which enters into the neuropil from the cell body. c.c.a.; contralateral connective axon. Formalin fixation. Malloryazan staining. 6μ thick sections.

Marking: Repetitive stimulation by rectangular pulses was applied to the first root of the abdominal ganglion and then the synaptic responses were recorded intracellularly by the above mentioned microelectrode. Fig. 5A1 shows the typical synaptic responses which consist of remarkable synaptic potentials and synaptic spikes set up by them. The position of the microelectrode tip corresponding with the responses of Fig. 5A1 is shown in Fig. 5A2, which is a horizontal section of the dorsal region of the ganglion; the latter section is magnified in Fig. 6A. In this case the staining spot is found in the center of the neuropil placed between two axon-bundles through the ganglion.

The synaptic potentials of the responses shown in Fig. 5B1 were smaller than those in Fig. 5A1. Then the staining spot was found in the boundary area between the neuropil and surrounding axon-bundles (Fig. 5B2). In this case the

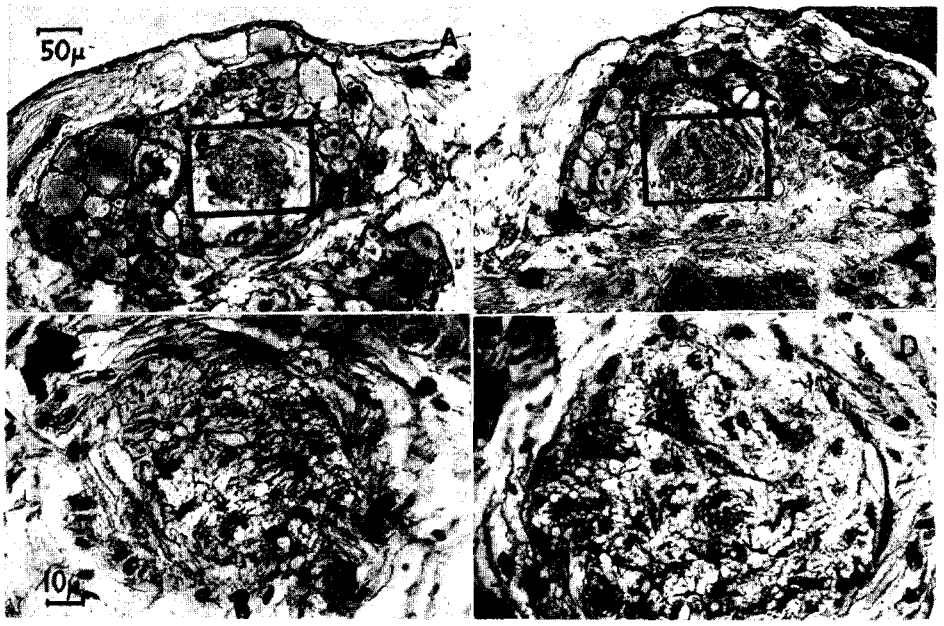

Fig. 4. Microphotographs of sections showing the typical dendric structure of the neuropil. A and B: location of the neuropil in low magnification. C and D; fine dendric structure of the neuropil under high magnification.

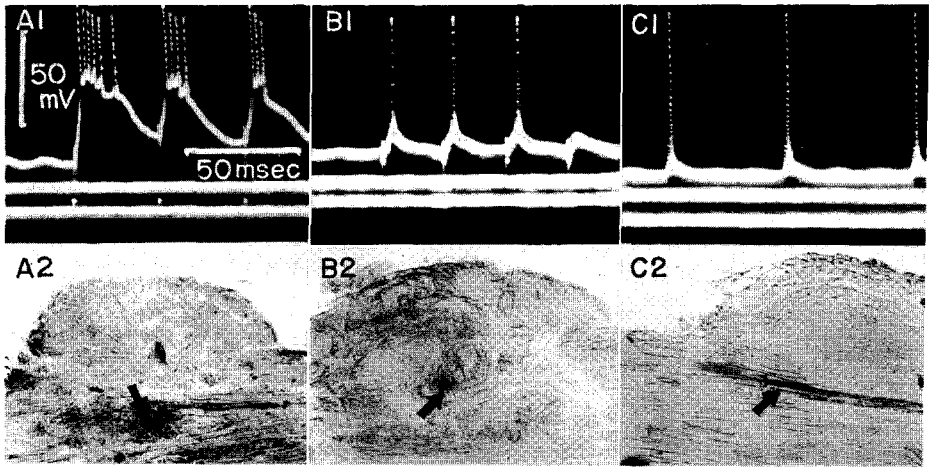

Fig. 5. Synaptic responses recorded by the microelectrode and corresponding marked spots at the position of the electrode tip in the section of the ganglion. Arrows indicate the marked spots. Synaptic response of A1 was recorded at the marked position of A2, response B1 at the mark in B2 and response 1C at the mark in C2.

electrode tip was distant from the dense dendric meshwork in the neuropil.

When the recorded impulses were not accompanied by the synaptic potential (Fig. 5C1), the staining spot elongated along the axon as is shown in Fig. 5C2. It can be considered that the electrode was inserted into the axon, not into the synaptic region, consequently the staining substance spread along the inside of the axon.

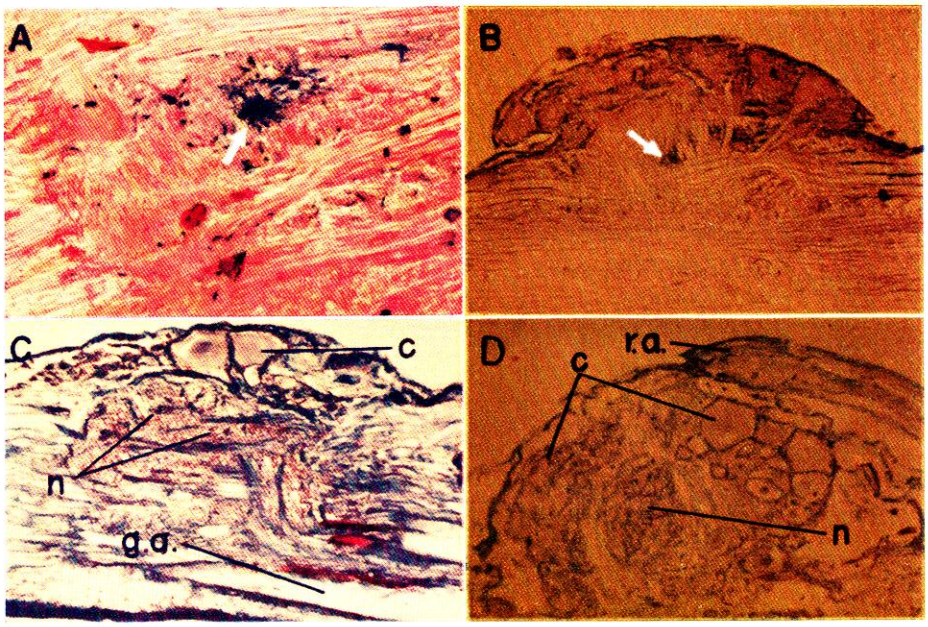

Fig. 6. Microphotographs of the sections showing the colour relation of the stained tissue and the marked spot (A and B), and Mallory-azan stained section (C and D). Arrows indicate the marking spots. c.: cell body. n.: neuropil. g.a.: giant axon. r.a.; root axon-bundle.

In every trial, except for such instances as the last case, the staining spot was measured within a range of a few tens of micra in diameter. Therefore it can be concluded that the recording position by the microelectrode was nearly indicated. However the thickess of the sections prevented successful marking because of wash ing out of the staining substance.

Discussion

From the previous experiments, in which the insertion of the microelectrode into the synapse was found to be more unstable than the insertion into the axon, it was assumed that the synaptic region of the abdominal ganglion of the crayfish

had very fine structure. Undoubtedly this fine structure was neither the cells body nor axons, but thin dendrites in the neuropil (Fig. 4). In general an axon from the nerve cell divided repeatedly, forming dendric structures, and enters the neuropil formed by the endings of the sensory axons and branching of central and efferent neurones (Wiersma, 1961). However it was impossible to discriminate among the dendrites of them and recognize the detailed structure of junctions in the present histological investigation. Nevertheless, when the typical synaptic responses were picked up, the microelectrode tip was certainly found to be placed in the dense dendric structure of the neuropil. On the other hand, when the electrode tip was placed near the circumference of the neuropil, the synaptic potential decreased in height, in spite of the constancy of the spike height. In this instance the meaning must be that the position recorded by the microelectrode is not in or near the region where the synaptic junctions exist densely. Consequently it can be concluded that most of the synaptic junctions in the abdominal ganglion of the crayfish are in the dendric structure of the neuropil and that agrees with Preston & Kennedy's opinion (1960). The synaptic region of the crayfish ganglion may have the most delicate structure among the synapses of several animals hitherto investigated physiologically.

Summary

The site of origin of the synaptic response was studied on the abdominal ganglion of the crayfish by aid of histological methods.

1. In Nissl-stained serial sections, the cell bodies of the neurones are found typically in a layer of the dorsal side of the ganglion. The cell layer held a mass of neuropils under its ventral side. A schematic model is presented for the neuronal configuration of the abdominal ganglion.

2. The fine structure of the dendritic arborizations was rendered visible in the neuropil by means of Mallory-azan staining. However it was difficult to recognize the further detailed structure of the synapses among the dendrites of the sensory axon and inter- and efferent neurones.

3. The position of origin of the synaptic response was determined by the electrophoretic injection of a staining substance through a microelectrode into the ganglion. The remarkable synaptic response was found in the dense dendric structure of the neuropil.

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