SYNAPTIC ACTIVITY AND OVERSHOOTING SPIKES RECORDED FROM THE SOMA OF AN INSECT NEURON WITH BILATERALLY SYMMETRICAL BRANCHING

A THESIS

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Approved for Graduate Council

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INTRODUCTION

Insects, because of their wide range of behaviors and their small numbers of nerve cells, should provide ideal material for studying cellular mechanisms underlying behavior. But as Hoyle (1970) has pointed out in an important review, understanding of basic physiological mechanisms of insect nerve cells lags far behind that of other major animal groups. Hoyle suggests that basic technical problems of insect intracellular recording must be solved first, then fundamental questions must be answered, such as what electrical activity can be recorded from an insect cell body and are insect cell bodies excitable?

A review of the earlier literature indicates that relatively few publications have shown any significant activity in insect nerve cell bodies. Two exceptions are the studies of Hagiwara and Watanabe (1956) and Kerkut et al. (1969) who were able to penetrate cells and record overshooting spikes. Others (Bentley, 1969; Rowe et al., 1969) identified cell bodies by stimulation of the cell body.

A significant recent report by Hoyle and Burrows (1970) provided techniques which were applied directly or altered somewhat for use in this study. They showed that by improved procedures electrical activity could routinely be recorded from motorneuron cell bodies. However, the amplitude of the recorded spikes was small suggesting electrotonic invasions from a distant

part of the cell.

The present study presents information on improved recording conditions and on the anatomy, excitability, and function of a particular nerve cell found in the abdominal ganglia of the cockroach Blaberus craniifer. Four questions were used as guides for the investigation: (1) How can recording conditions be improved? (2) What electrical activity can be recorded from the cell body? (3) What is the anatomy of the cell? and (4) What is its function?

In attempting to answer the last two questions, the Procion Yellow dye injection technique (Stretton and Kravitz, 1968) was used. This technique utilizes a dye-filled microelectrode to record from and stimulate the individual cell. After this is completed the cell is filled with dye to determine its anatomy.

METHODS AND MATERIALS

I. Dissection

Most of the present experiments utilized isolated nerve cords taken from adult male cockroaches. After removing head, legs, wings, tergites, gut and excess fat body, the remaining tissues were washed with saline (Yamasaki and Narahashi, 1959). The nerve cord (from mesothoracic to sixth abdominal ganglia) plus longitudinal tracheae and peripheral nerves was freed from other tissues, transferred to a shallow pool of saline in a pinning dish made of transparent Dow-Corning Sylgard 184, and stapled to its normal length with short pieces of fine wire.

In early experiments the longitudinal tracheae were cut at points midway between ganglia and the cut ends were made into snorkels open to the surface of the saline. A ganglion was then desheathed with sharpened watchmakers forceps to allow penetration with microelectrodes. In later experiments the tracheae were removed and the sheath was softened for electrode penetration by exposure to a strong suspension of proteolytic enzyme (8% Calbiochem Pronase, 15 to 20 minutes, room temperature). This was followed by several rinses of saline.

II. Preparation of Microelectrodes

Microelectrodes were mechanically pulled from thin walled soft glass (Drummond 15 µl Microcaps). These were attached to glass slides, placed tip down in a beaker of 1 M potassium acetate, boiled for five minutes, and then transferred to a vacuum chamber

until bubbling ceased.

Electrodes to be filled with the fluorescent dye Procion Yellow were first filled with distilled water by the above technique. The water was then displaced by solution of 4% Procion Yellow injected by a 31 gauge syringe needle. The dye solution was filtered by means of a Millipore 0.22 μm filter on the syringe assembly. Procion-filled electrodes were used within three days of filling.

III. Physiology

Microelectrodes with resistances of 10 to 50 MΩ were connected to the input of an ELS-1 Bak Standard Wide Band Electrometer. Cell bodies were visualized under a dissecting microscope with oblique transmitted light. By means of a Pfeiffer PBL-2 micromanipulator the electrode tip was placed on the surface of a cell body. The manipulator was then gently tapped while the oscilloscope was observed for a resting potential. After penetrating a cell the bridge circuit on the Bak amplifier was used to stimulate the nerve cell through the recording microelectrode.

A single suction electrode allowed extracellular recording from a ventral nerve, dorsal nerve, or anterior or posterior connective of the ganglion of interest. A switch allowed the same suction electrode to be used for stimulation. A Tektronix Type 122 Preamplifier was used for recording and a Grass Model S5 Stimulator for stimulating.

The intracellular and extracellular recordings were displayed

on the two channels of a Tektronix Type 502 Oscilloscope and photographed by a Grass Model C4 Camera.

IV. Anatomy

Anatomical details of the cells were revealed by injecting dye from the Procion Yellow-filled electrodes. In most of the cases reported here both anatomical and physiological results were obtained from a single Procion Yellow-filled electrode. However, in a few cells the physiological experiments were carried out with potassium acetate-filled electrodes. Cells were filled with dye by hyperpolarizing pulses (10^{-8} to 10^{-7} amps x 0.5 sec at one per sec) delivered from a Grass S4 Stimulator with stimulus isolation unit. Filling was continued for about 30 minutes by which time the cell body was usually bright orange.

After injection the preparation was transferred to a moist chamber for six to twelve hours to allow diffusion of the dye into finer neuron branches. The tissue was fixed in Bouin's solution and 10 μm paraffin sections were prepared. Sections were mounted in Entellan, a low fluorescence embedding agent, and examined with a Leitz fluorescence microscope using a high pressure mercury lamp (Osram HBO 200) as the source with excitation filters BG 12 and BG 38, and blue-absorbing barrier filter (Lietz OG1). Both bright field optics and dark field optics were used. With the help of an ocular grid the profiles observed were drawn on graph paper. Photographs were made with Kodak Ektachrome X Film (ASA 64). A few of the preparations were inspected and photo-

graphed as temporary whole mounts in methyl benzoate before being embedded in paraffin.

RESULTS

I. Anatomy

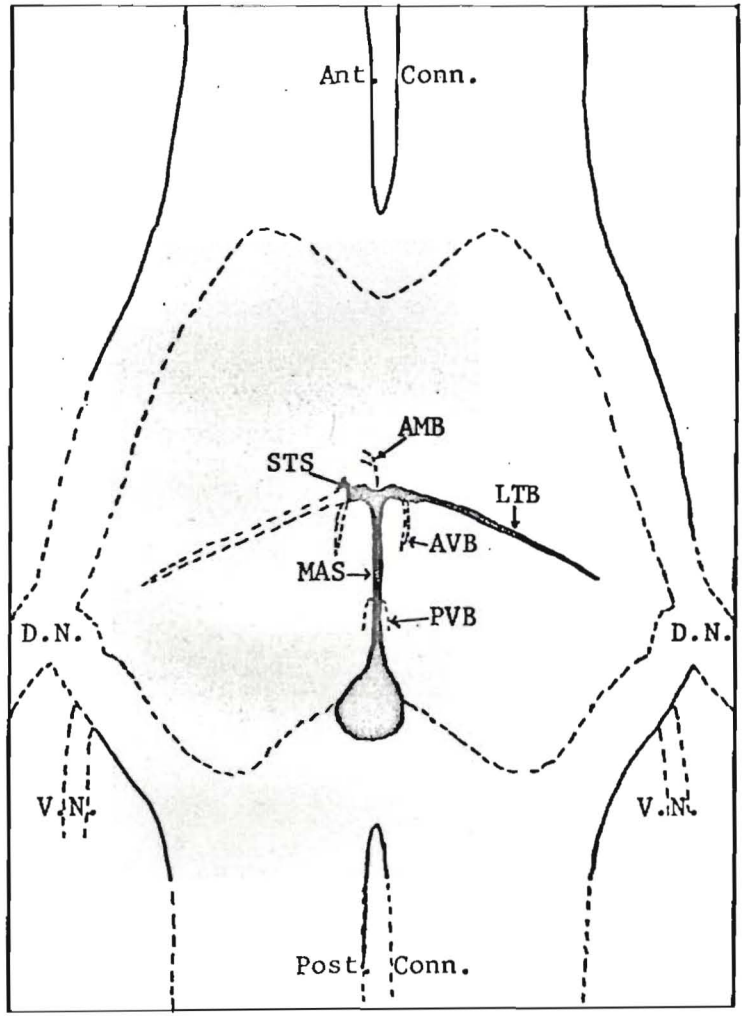
A. Location of Cell Body. Procion dye injection in abdominal ganglia one to five show the unpaired dorsal posterior midline (DPM) cell to be in approximately the same position in each ganglion. The cell body lies posterior to the cleft in the neuropile outline (Fig. 1), and has a diameter of approximately 50 µm so it is by a small margin the largest cell body in the ganglion.

B. Anatomy of DPM Cell Branches. The cell has a wide, unpaired midline axon segment (MAS, Fig. 1 and 2) approximately 7.5 µm in diameter by 150 µm in length. It apparently does not narrow at its junction with the cell body. As the midline axon segment passes anteriorly, it dips ventrally to depth of 100 µm before it gradually returns to within 50 µm of the dorsal surface (Fig. 2). At its lowest point several knob-shaped branches occur (KSB, Fig. 2 and 3). Anterior to the knobs, about 90 µm from the cell body, a pair of small diameter posterior ventrally directed branches (PVB, Fig. 2) were traced into a pair of longitudinal tracts which correspond to the "dorsal median tracts" described by Pipa et al. (1959) in Periplaneta thoracic ganglia. These branches were approximately 0.75 µm in diameter and the dye could be traced for a distance of about 55 µm.

About 150 µm from the cell body the midline axon segment splits into two short transverse segments (STS, Fig. 1 and 2), each

Figure 1A. Photomicrograph of wholemount preparation. Dark field fluorescence microscopy. B. Line drawing of ganglion and DPM cell in same orientation. Parts visible in A shown in darkened portion. Additional parts shown in dashed lines. MAS, midline axon segment; PVB, posterior ventral branches; AVB, anterior ventral branches; LTB, long transverse branches; AMB, anterior midline branch; STS, short transverse segment; DN, dorsal nerve; VN, ventral nerve; Post. Conn., posterior connective; Ant. Conn., anterior connective. Magnification x125.

A



B

Figure 2. Perspective sketch of DPM cell and branches as seen from a point to the right of and anterior to the ganglion. KSB, knob-shaped branches; amb-1 and amb-2, first and second branches of anterior midline branch; other abbreviations as in Fig. 1.

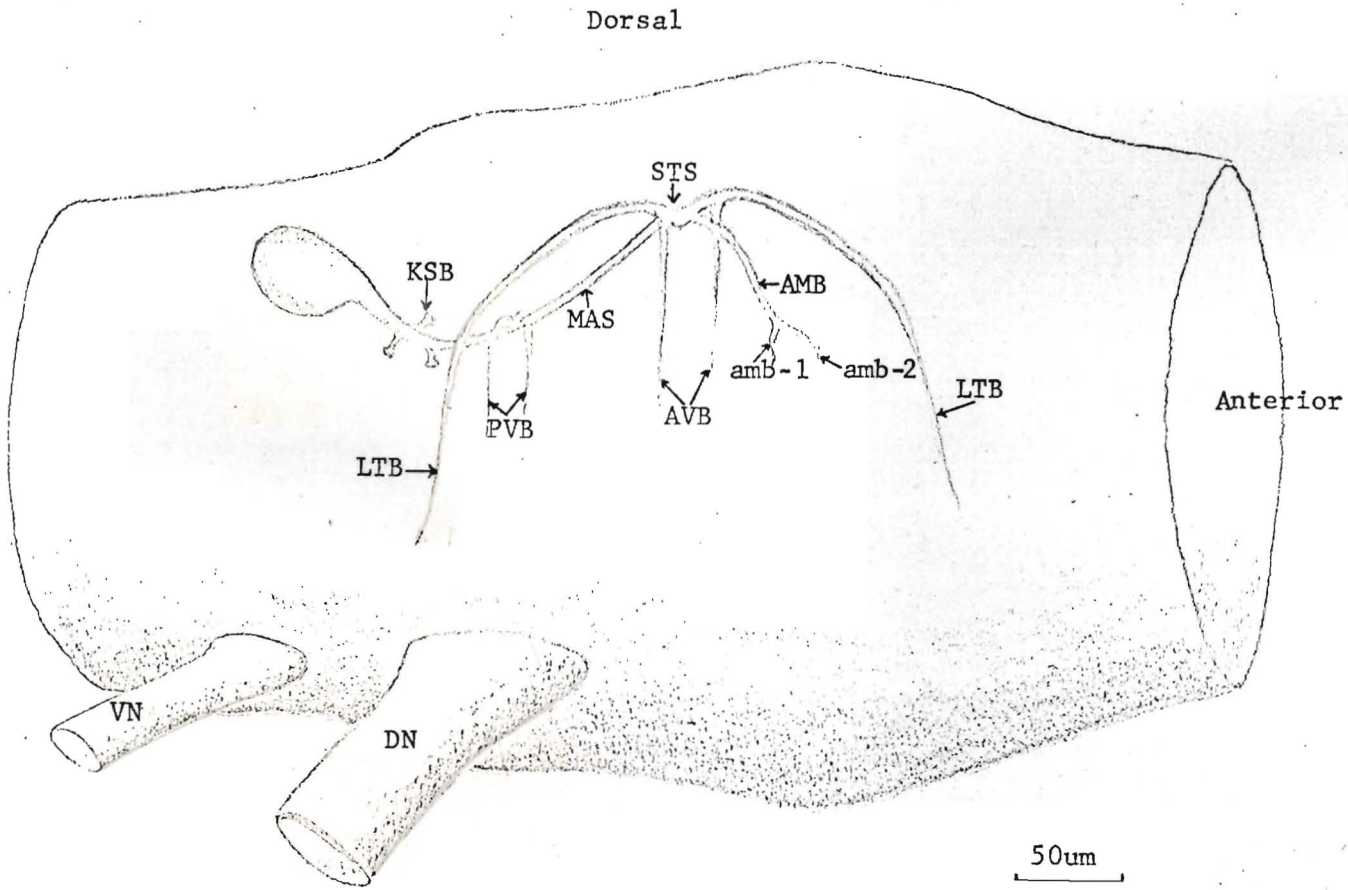
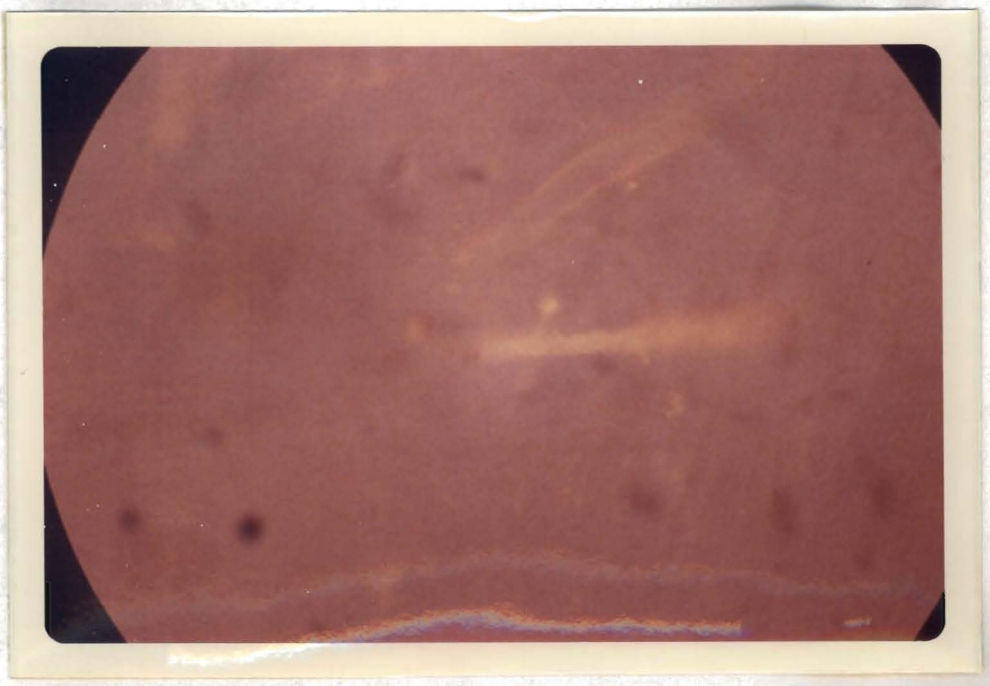
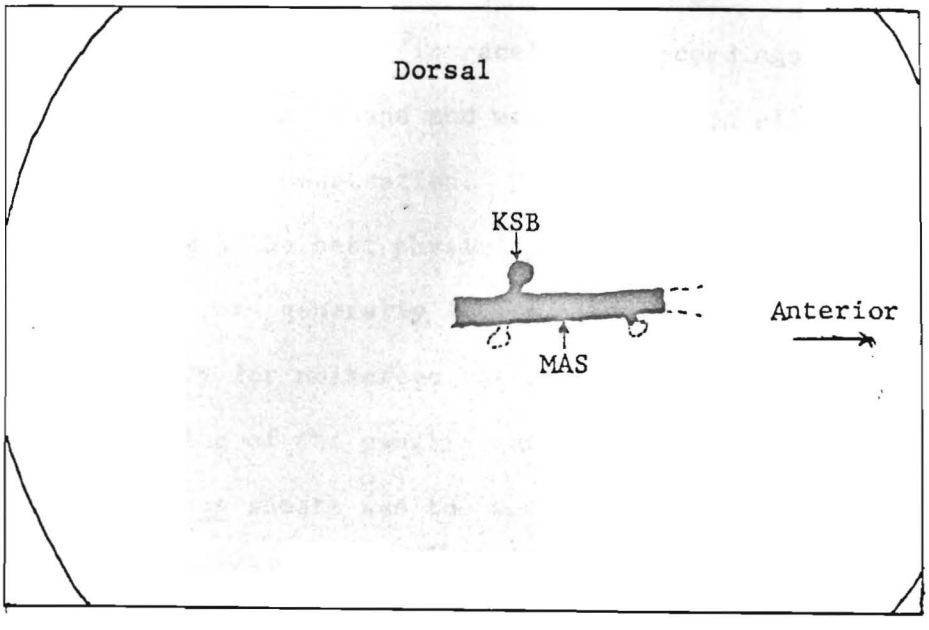

Dorsal
STS
KSB
MAS
AMB
amb-1
amb-2
LTB
Anterior
PVB
AVB
LTB
VN
DN
50um

Figure 3. Fluorescence photomicrograph of short length of midline axon segment (MAS) showing knob-shaped branches (KSB). Sagittal section. Dashed regions of drawing represent structures slightly out of focus. Magnification 1250x.




Dorsal
KSB
MAS
Anterior

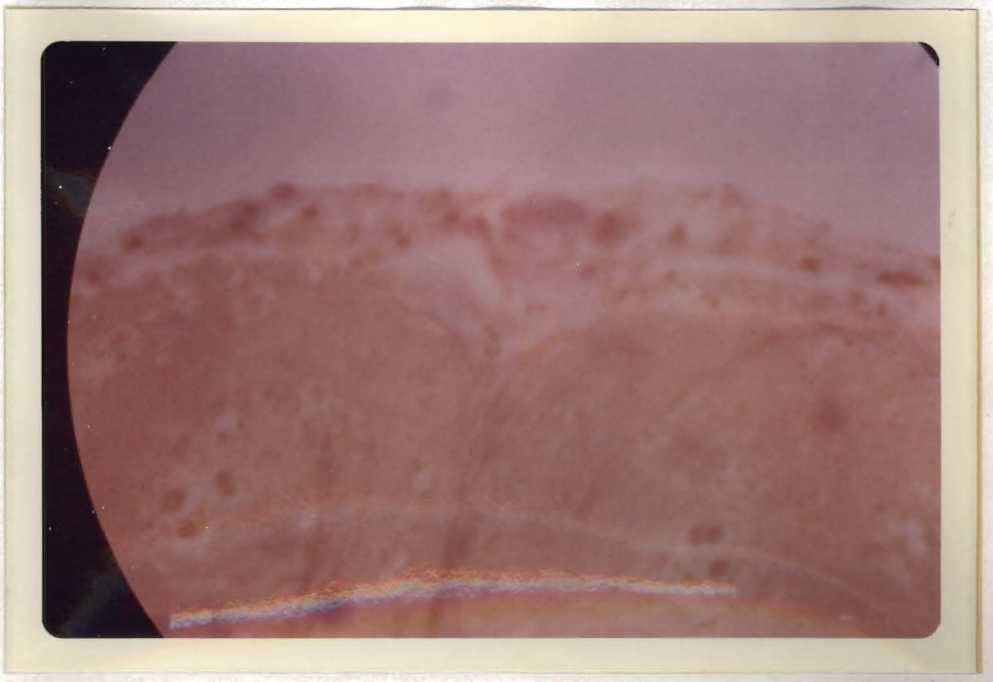
of which passes transversely 10 to 20 µm before branching again (Fig. 4) into a 1.5 µm diameter, long transverse branch (LTB, Fig. 1 and 2) and a smaller diameter, ventrally directed branch (AVB, Fig. 2). The two long transverse branches could be traced laterally and slightly posteriorly for 200 µm to near the exit of the ventral nerve. The two smaller anterior ventrally directed branches have a length of 100 µm and a diameter of approximately 1 µm. Like the posterior ventrally directed branches they also pass into the dorsal median tracts (Fig. 5).

In a few preparations a small diameter, rebranching anterior midline branch (AMB, Fig. 2 and 6) could be traced anteriorly about 75 µm from the main split in the midline axon segment.

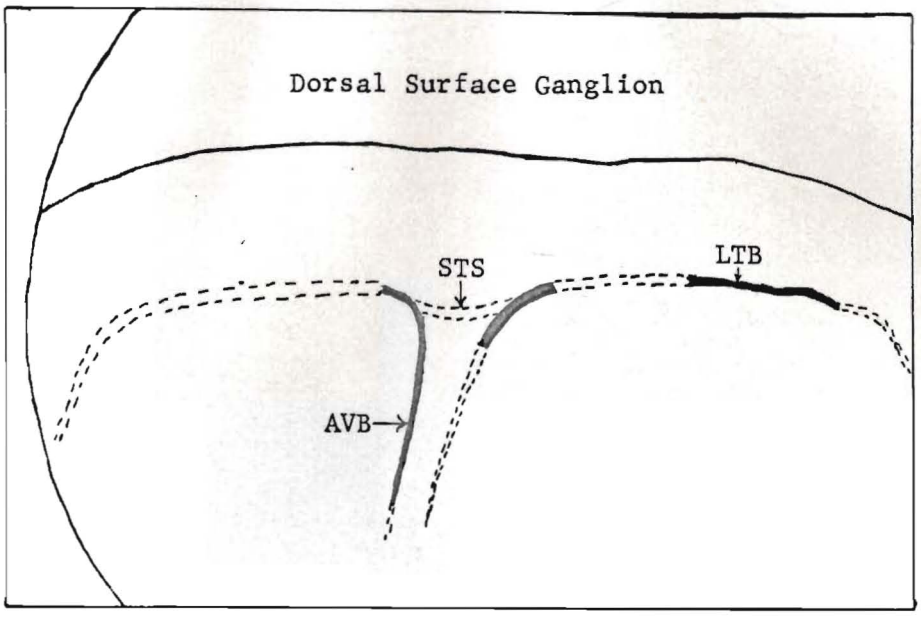
II. Physiology

A. Spontaneous Intracellular Activity. The quality (low noise level, few injury effects, large amplitude spikes and long lasting spontaneous activity) of intracellular recordings varied considerably with electrode shape and with the method of preparing the sheath for electrode penetration. Electrodes filled with potassium acetate gave the best physiological data. Electrodes filled with Procion were generally less satisfactory due to the larger tips necessary for noisefree recording and for filling. Mechanical desheathing of the ganglia gave very poor results and the untreated Blaberus sheath was too tough to allow punching through of stiff electrodes (technique of Hoyle and Burrows, 1970). Digesting the sheath with Pronase furnished the best physiological data provided overdigestion was avoided.

Figure 4A. Fluorescence photomicrograph of cross section showing anterior ventrally directed branches and long transverse branch as they split from the short transverse segment. B. Line drawing of A with parts visible in A shaded and additional parts shown in dashed lines. Abbreviations as in Fig. 1. Magnification 550x.

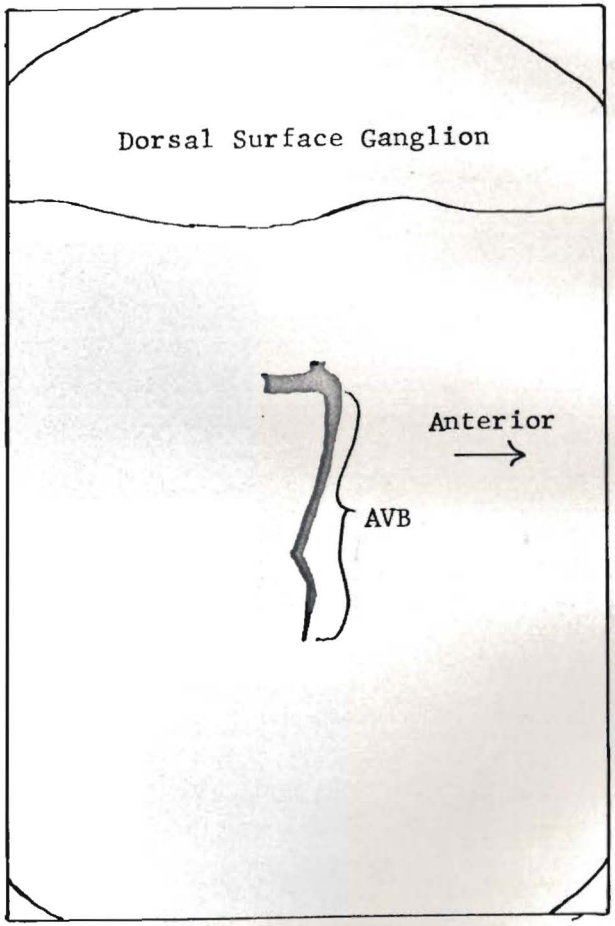


A



B

Figure 5. Fluorescence photomicrograph of 10 µm sagittal section of anterior ventrally directed branch. Magnification 550x.


Dorsal Surface Ganglion
Anterior
AVB

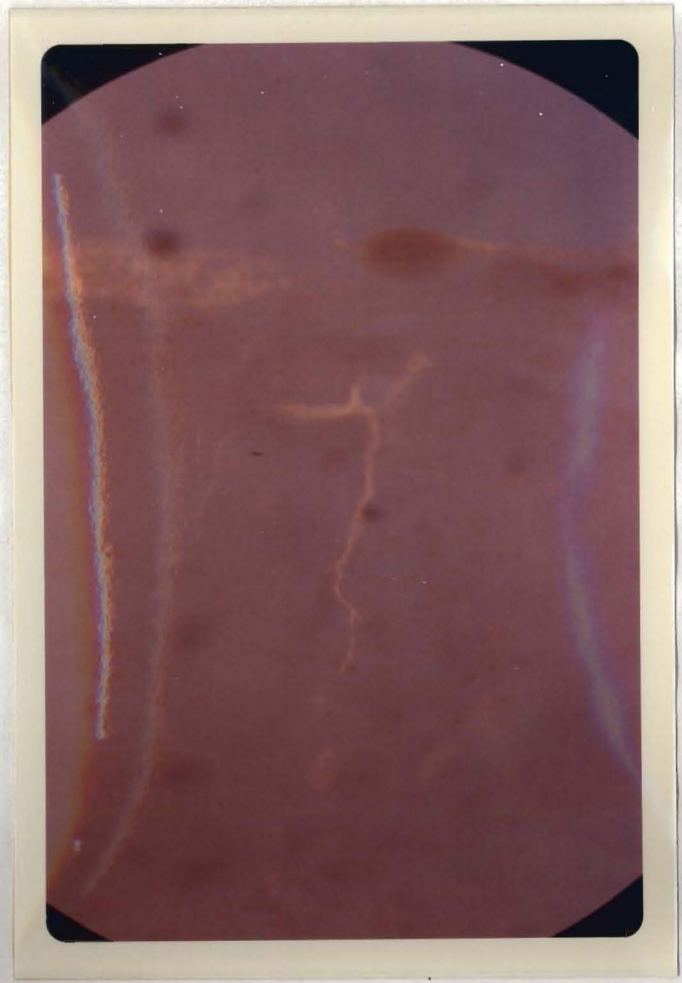
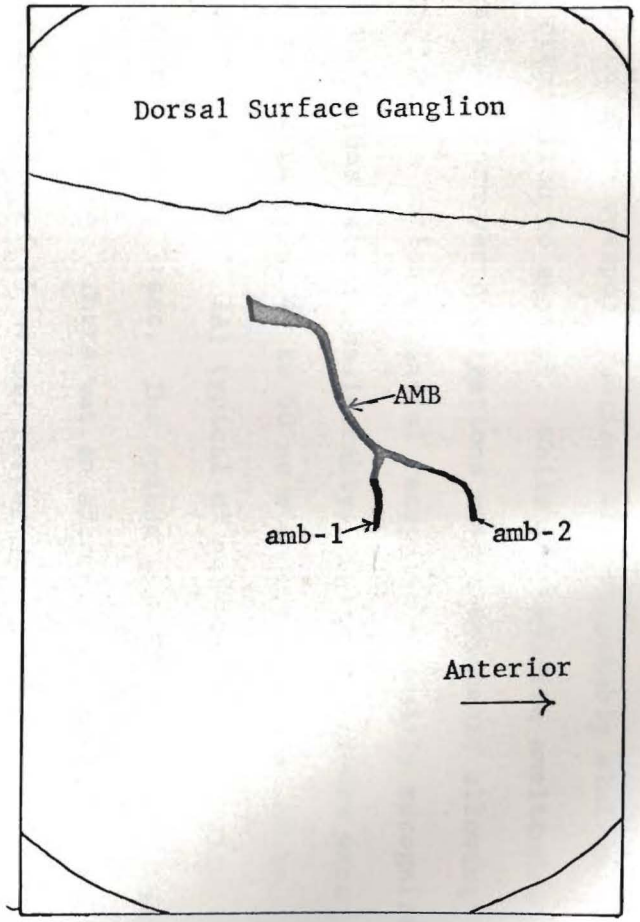
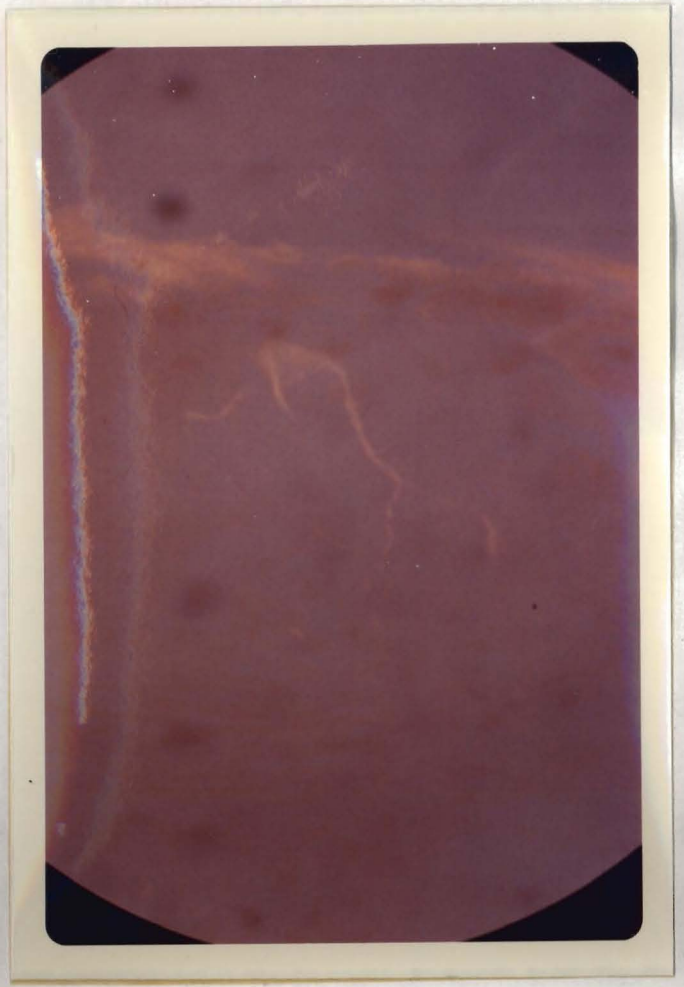


Figure 6. Fluorescence photomicrograph of anterior midline branch and its first and second branches. Sagittal section. Magnification 550x.


Dorsal Surface Ganglion
AMB
amb-1
amb-2
Anterior



When entering a DPM cell body a resting potential was generally observed. In desheathed preparations this was quite variable, ranging from 10 to 50 mv. In preparations digested with Pronase the potential most frequently fell in the range of 35 to 40 mv.

Spontaneous spike activity was infrequently observed in desheathed preparations. In contrast, one of the five available DPM cells of digested preparation nearly always produced some spontaneous spike activity.

Immediately after entering a cell a burst of spike activity usually occurred, then the spike activity settled down to a low firing frequency. Out of several hundred penetrations, the 30 or 40 best (in terms of large amplitude action potentials, 30 to 45 min. recording times, and responsiveness to stimulation) provide the basis for this paper. The rate of firing, the duration of spikes and afterhyperpolarizations were remarkably similar from one preparation to another. While the relative amplitudes of spikes and afterhyperpolarizations were consistent, allowing the "shape" of the action potential record to be easily recognized, the absolute values varied considerably. Spike amplitudes were generally in the range of 45 to 60 mv with overshoots of 10 to 20 mv. In a record (Fig. 7A) typical of the sample, the cell fired at approximately 2/sec. The spikes were 50 mv in amplitude and 4 msec in duration. There was an afterhyperpolarization which had an initial value of 15 mv and lasted approximately 12 msec.

Figure 7A. Spontaneous activity recorded from DPM cell body. B. Responses of a second cell body to ventral nerve stimulation at increasing frequencies. Large spikes in the cell body fail to follow every stimulus above 8/sec. EPSPs 9 to 15 mv by 40 msec can be seen leading into the spikes and in the responses in which spikes did not occur. Stimulus of 1 volt. Stimulus duration 0.5 msec. C. Activity in a third cell body during posterior connective stimulation from 2 to 20 stimuli/sec. Followed 1:1 up to frequency of 3/sec (line 2) but not at 10/sec (line 3). Stimulus of 8 volts. Stimulus duration 0.3 msec. EPSPs of 6 mv by 16 msec can be seen following many of the stimuli and leading into some of the spikes. Voltage calibration, 50 mv. Time calibration, 200 msec.

A
B
C

Both firing rate and spike amplitude usually decreased gradually with time. However, in one penetration the action potentials gradually increased from 10 to 100 mv after several experiments had been completed. The electrode probably formed an initial loose seal and later a tight seal with the cell membrane. This particular cell was still quite active after 90 minutes when the electrode was removed.

B. Effects of Stimulating Peripheral Nerves. After penetration of a DPM cell body with a microelectrode, the ventral nerve was stimulated in 5 different preparations. In all cases spikes followed each stimulus up to a frequency of about 7.5/sec (Fig. 7B). Above this frequency smaller potentials, possibly abortive spikes or excitatory postsynaptic potentials (EPSPs) were seen following each stimulus. These smaller potentials had a duration of 40 msec and an amplitude of 9 to 15 mv.

Responses were obtained in six of ten similar trials conducted with dorsal nerve stimulation, but the cell body failed to follow stimulation above frequency ranges of 12 to 20/ sec. Again small potentials, similar to those following ventral nerve stimulation, were observed at the higher stimulus frequencies.

In four out of five attempts to stimulate via a posterior connective, spikes were recorded from a cell body. In all four cases the cell failed to follow frequencies above 4/sec, but EPSPs tended to follow up to higher stimulus frequencies. In one of these experiments (Fig. 7C) some of the EPSPs did not directly follow the stimuli. These EPSPs were probably produced spontane-

ously or via more complex pathways. EPSPs elicited by connective stimulation were generally shorter in duration than those elicited by nerve stimulation (Fig. 7B and C).

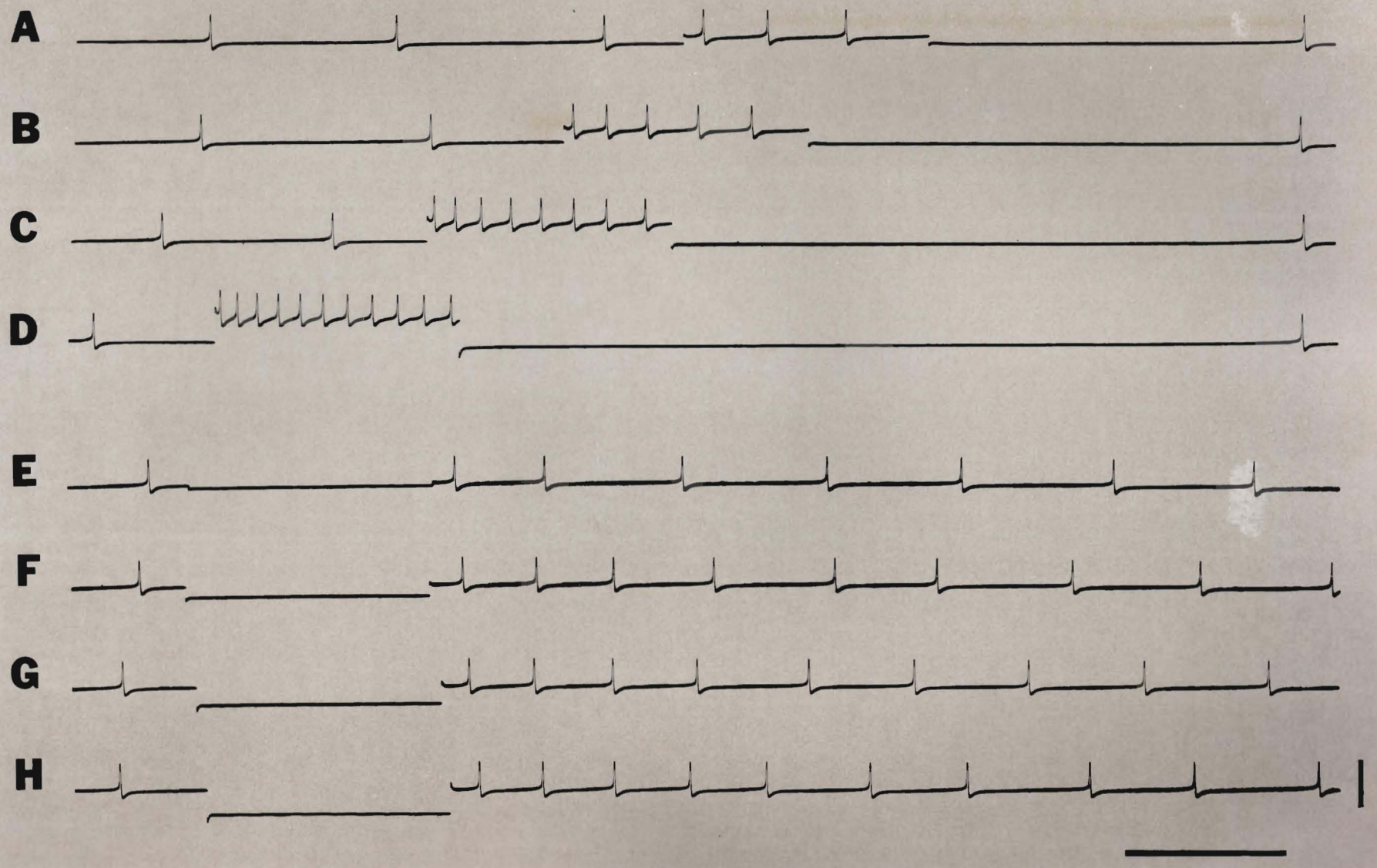
In four out of five trials with anterior connective, records similar to those obtained with posterior connective were observed.

C. Effects of Stimulating Cell Body. In isolated Pronase-treated preparations responses of the cell body to depolarizing and hyperpolarizing currents of 10^{-9} amps or less were recorded by a bridge circuit. A typical experiment (Fig. 8A-D) shows that with greater depolarization there was an increase in firing rate during stimulation. A rebound effect can be seen in this record; the greater the number of spikes during stimulation, the longer the recovery time to the spontaneous firing rate. In the same cell body (Fig. 8E-H), no activity was observed during hyperpolarization but a rebound firing occurred after each hyperpolarization, the rate of firing increasing with increasing hyperpolarization. Similar results were observed in approximately 25 other cells.

Physiological evidence that branches of this cell might extend into peripheral nerves was sought in the isolated Pronase treated cords. While stimulating the cell body, peripheral nerve activity was recorded from a connective or peripheral nerve. No response was observed in five trials with the ventral nerve, 10 trials with the dorsal nerve, 5 trials in the posterior connective, and 5 trials on the anterior connective.

In order to test the idea that activity of the DPM cell might

Figure 8. Response of cell body to stimulation through bridge circuit. A, B, C, and D: depolarizations of 0.4×10^{-9}, 0.7×10^{-9}, 1.1×10^{-9}, 1.5×10^{-9} amps respectively. With increasing current levels a longer silent period follows the depolarizations. E, F, G, and H: hyperpolarizations of 0.2×10^{-9}, 0.6×10^{-9}, 1.0×10^{-9}, and 1.4×10^{-9} amps respectively. With increasing current levels a higher firing rate follows hyperpolarization. Voltage calibration, 100 mv. Time calibration, 200 msec.


A
B
C
D
E
F
G
H

cause contraction of specific muscles in the abdomen (i.e., that the DPM cell is a motorneuron or an interneuron controlling motorneurons), about 20 cells were impaled and stimulated in semi-intact preparations. In these preparations the nerve cords were not isolated; instead a cautious dissection exposed the ganglia while leaving intact most of the peripheral nerve connections to abdominal muscles. The ganglia were desheathed with forceps and the impaled cell bodies were depolarized while observing the peripheral abdominal muscles for reactions. No muscle responses were observed.

DISCUSSION

I. Recording Conditions

Intracellular recording techniques have been generally unseccessful in insects, for a reason which now seems clear. Until recently mechanical desheathing was the standard procedure for getting a glass microelectrode through the tough connective tissue sheath which surrounds insect ganglia. Following the lead of Willows (1968), who found that brain cells of Tritonia deteriorated when the sheath was removed, Hoyle and Burrows (1970) avoided desheathing and used glass micropipette electrodes stiff enough to puncture the sheath of locust ganglia. They obtained dramatically improved recordings.

The present study confirmed Hoyle and Burrows' experience that it is bad practice to mechanically desheath insect ganglia. Preparations which were mechanically desheathed provided only a few cells with spontaneous spikes and none which responded to bridge stimulation. Attempts were made in this study to drive electrodes through the sheath of Blaberus but all ended in failure due either to breakage of weak electrode tips or to excessive compression of the ganglion by overly stiff tips. Therefore Pronase digestion was used in this study and appears to provide a practical alternative to Hoyle and Burrows' sheath-penetrating stiff electrodes, particularly for those species which have an unusually tough sheath.

The amount of current required to stimulate cells provides additional evidence that mechanical desheathing is bad practice. In this study and that of Hoyle and Burrows (1970) electrical activity of cells could be elicited by direct stimulation of the cell body through a bridge circuit using current levels of approximately 10^{-9} amps. In earlier studies using mechanical desheathing, cell bodies had to be stimulated with 5×10^{-8} to 10^{-6} amp pulses to produce muscle movements (Bentley, 1969; Rowe et al., 1969; Rowe, unpublished data).

Two explanations for the deleterious effects of mechanical desheathing are destruction of the ion-regulating function of the sheath (Hoyle, 1952, 1953; Treherne, 1965) and excessive mechanical disruption of the nerve cells. The present study provides some support for the latter explanation. While enzyme digestion presumably avoids mechanical disruption, it probably interferes with ion regulation. On the other hand punching through the sheath probably overcomes both problems.

The ideal microelectrode is an elusive compromise between conflicting requirements. The electrodes must be mechanically strong with fairly large diameter tips, since smaller (higher resistance) tips have higher "noise" levels, make it hard to balance the bridge circuit, and tend to block during dye injection. However, electrodes with smaller tips do less damage to the cell and permit more sustained intracellular recording.

Choice of electrolyte for the microelectrodes also presented

conflicting requirements. In preliminary experiments potassium chloride-filled electrodes produced very poor results. Potassium acetate-filled electrodes (suggested by Kerkut et al., 1969) were used in some of the later experiments and gave the best physiological records. However, since it was important to mark cell body positions and to demonstrate anatomical details of the DPM cell, Procion Yellow was used as the electrolyte in most of these experiments. Physiological recordings and stimulation through the bridge circuit using Procion Yellow electrodes was hampered to a certain extent since dye-filled electrodes had much higher resistances than potassium acetate-filled electrodes of the same shape.

II. Do Cell Bodies Participate in the Electrical Activity of Insect Cells?

The first report of spontaneous activity in insect neurons was by Hagiwara and Watanabe (1956). They recorded 60 mv resting potentials, large spontaneous EPSPs and 70 mv overshooting action potentials from cicada tymbal muscle motorneurons. Hagiwara and Watanabe presumed they were recording from cell bodies, but later experimenters had great difficulties in recording from insect neurons. It was suggested (Hoyle, 1970) that the cell bodies of insect neurons were silent and the few available insect intracellular records were from smaller parts of the cell in the neuropile. However, studies by Kerkut et al. (1968, 1969) reported overshooting action potentials from specific cell bodies whose penetration by microelectrodes was visually observed. It is clear

that the overshooting spikes recorded in this study were also from cell bodies since the electrode position was repeatedly verified both visually during penetration and by Procion dye injection after recording. Since Kerkut et al. (1969) describe the Periplaneta cells from which they recorded overshooting spikes as "dorsal midline cells" of the metathoracic and caudal ganglia, it is quite possible that they recorded from homologs of the DMP cells reported here.

Previous anatomical studies suggested the generalization that the attachment of the cell body to its axon was very narrow in insects (Zawarzin, 1924; Wigglesworth, 1959). Hoyle (1970) pointed out that the high resistance of the axon-soma junction could greatly attenuate spikes conducted electrotonically into the soma and account for the small amplitude spike activity recorded from most insect neurons (Callec and Boistel, 1965, 1966; Rowe, 1969). The large spikes found in the DPM cell body of this study could be due to the atypically large diameter of the axon-cell body junction, which could allow axon spikes to be passed electrotonically to the cell body with little attenuation.

Stimulation of the DPM cell bodies with a bridge circuit provided additional evidence that these may be atypical insect neurons. Hoyle and Burrows (1970) and Hoyle (1970) were able to identify most motorneurons of the metathoracic leg muscles of locust. Because they made simultaneous intracellular records from muscle cells and from cell bodies and were able to record identical spike patterns from the two sources it is quite certain that they

recorded from motorneurons. Compared with the results presented here the spike amplitudes were much smaller and were triggered by much larger depolarizations. They concluded that the motorneuron soma membrane is electrically inexcitable although invaded by the action potentials electrotonically. In contrast the large overshooting spikes from the DPM cell seem to indicate that the cell body or some very nearby point of the axon is electrically excitable.

III. Functional Inferences From Anatomical Details of the Cell

Unfortunately the Procion Yellow technique does not reveal the position of either pre- or postsynaptic sites, and it has the additional shortcoming that one can never be sure that all branches of a cell have been revealed. However, the following speculations seem justified. The responses in the cell body to anterior and posterior connective stimulation could be following a pathway suggested by the two pairs of vertical branches (AVB, PVB, Fig. 2) that pass into the abdominal homologs of the "dorsal median tracts". According to Pipa et al. (1959) this tract in the thoracic ganglia is made up of long extensions of the connective fibers. The vertical branches thus could possibly receive synaptic input from fibers in the longitudinal tracts. Also records of responses to stimulation of the ventral and dorsal nerves have possible, anatomical explanations. The long transverse branches (LTB, Fig. 2) appear to be heading toward the roots of the ventral and dorsal nerves. It is possible that there could be synaptic connections to both nerves.

Other possible sites of synaptic activation are the knob-shaped branches (KSB, Fig. 2) extending from the initial dipping portion of the axon. These are similar to clubshape, possibly synaptic structures in Aplysia (Lewis et al., 1969) and to postsynaptic structures on the vertebrate Mauthner neuron (Furshpan and Furukawa, 1962). Postsynaptic surfaces as large as these knobs and as close to the cell body could well account for the large EPSPs recorded in response to peripheral nerve and connective stimulation. Further work using electron microscopy could determine whether these are pre- or postsynaptic structures.

The most original revelation from an anatomical view is the bilateral symmetry of the dorsal posterior midline cell. To this investigator's knowledge no previous work in insect neural anatomy has shown cells with this type of symmetry.

IV. Possible Overall Function of DPM Cell

Thus far the physiological data seem to rule out a motorneuron or motor interneuron function for the DPM cell, since stimulation of the cell body produced no detectable motor response in either the abdominal musculature or the peripheral nerves leaving the ganglion. Since all studied motorneurons (Hoyle, 1970) have small spikes, the large amplitude spikes recorded from the DPM cells also tend to rule out a motorneuron function. Previous generalizations that the ganglion is arranged with motor cell bodies ventral or lateral and all sensory cell bodies outside the ganglion (Zawarzin, 1924; Snodgrass, 1935; Cohen and Jacklet, 1967) suggest

that this dorsal posterior midline cell would have an interneuron function. Although synaptic input from collaterals of antidromically activated motorneurons cannot be ruled out it seems more likely that the EPSPs in response to peripheral nerve stimulation are from sensory cells. Overall, it seems reasonable to suggest that this cell serves a sensory interneuron function and that the bilateral symmetry of the cell may allow it to integrate sensory input from both sides of the ganglion.

SUMMARY

1. Anatomical and physiological studies have been carried out on a large dorsal posterior midline neuron found in cockroach abdominal ganglia 1 through 5, using microelectrodes filled with Procion Yellow.

2. Large overshooting spontaneous spikes were recorded from the cell body, indicating that the soma membrane or a portion of the axon close to it is electrically excitable.

3. Action potentials and EPSPs were recorded from the cell body in response to stimulation of dorsal and ventral nerves and anterior and posterior connectives.

4. On passing currents of the order of 10^{-9} amps through the cell body, overshooting spikes could be recorded in the cell body but no responses were observed in peripheral nerves, connectives or abdominal musculature.

5. The Procion Yellow dye injection technique demonstrated details of the branching pattern and showed that the cell was bilaterally symmetrical.

6. It is suggested that the cell has an integrating sensory interneuron function.

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LITERATURE CITED

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