J. comp. Physiol. 110, 233–250 (1976)

Journal of Comparative Physiology · A

Auditory Neurons in the Ventral Cord of the Locust: Morphological and Functional Properties*

Hansgeorg Rehbein

Lehrstuhl für Allgemeine Zoologie, Ruhr-Universität Bochum, Postfach 2148, D-4630 Bochum, Federal Republic of Germany

Received June 14, 1976

Summary. 1. An extracellular recording and staining technique has been used to study the structure of individual ventral-cord elements in the auditory pathway of *Locusta migratoria.*

2. Three groups of auditory ventral-cord neurons can be distinguished: (a) neurons ascending to the supraesophageal ganglion, (b) T-shaped neurons, and (c) neurons limited to the thoracic ventral cord.

3. The ventral-cord neurons ascending to the supraesophageal ganglion link the auditory centers of the thorax to those of the supraesophageal ganglion. These are, at least in part, richly arborized neurons of large diameter.

4. The ventral-cord neurons with T structure send equivalent signals along both arms of the T; they resemble the neurons of the first group in that they make synaptic connections in the supraesophageal ganglion, but they also conduct auditory information to caudal regions of the thorax via the descending trunk of the axon.

5. In the supraesophageal ganglion there are several extensive projection areas of the auditory ventral-cord neurons. No direct connections to the mushroom bodies, the central body or the protocerebral bridge could be demonstrated.

6. The thoracic ventral-cord neurons act as "short" segmental interneurons, providing a connection between the tympanal receptor fibers and the ascending and T-shaped ventral-cord neurons. They play a crucial role in auditory information processing.

7. The possible functional properties of the various morphological sections of the auditory ventral-cord neurons are discussed, with reference to their connections with motor and other neuronal systems.

* This work formed part of the program Sonderforschungsbereich 114 (Bionach) and was supported by the Deutsche Forschungsgemeinschaft

I. Introduction

A large number of papers have been published which treat the auditory systems of various insect species from a predominantly physiological or ethological point of view (for surveys of the literature see Schwartzkopff, 1974; Elsner and Huber, 1973). The morphology, however, has been examined closely only at the first station on the auditory pathway – the tympanal organ and the receptor neurons. On the other hand, little is known about the morphology of the higher-order auditory neurons in ventral cord and brain. Not until very recently were the structural details of certain auditory ventral-cord neurons of the migratory locust discovered, by means of an extracellular recording and staining method (Rehbein et al., 1974a, b).

The present paper extends that study of the ventral-cord neurons in the locust auditory pathway. Since the physiology of this system has been carefully examined, its morphological and functional properties are of particular interest. The aim of this research has been twofold: to clarify the essential principles of structure of auditory neurons in the ventral cord, and to infer, from these morphological findings and the available physiological data, what may be the general functional principles of the system and its connections to other neuronal systems.

II. Methods

A. Axonal Cobalt Iontophoresis

Axonal iontophoresis (Iles and Mulloney, 1971) is a method of "filling" nerve cells with dye via their transected axons, so that they are selectively revealed for microscopic study. The auditory ventral-cord neurons marked here with this method had previously been identified by the extracellular recording and staining technique described below. In order to limit the marking as nearly as possible to the neurons of interest, the connective was usually split so that a particular bundle of fibers could be isolated.

Iontophoresis was carried out either in a plexiglass apparatus or in the living animal. The end of the fiber bundle was placed in a solution containing cobalt at concentrations between 50 and 100 mM, and direct currents of 0.1–1 μA were passed through solution and bundle. In one series of experiments no current at all was applied, in order to avoid the movement of cobalt into neighboring axons which is occasionally observed. The staining times were between 2 and 48 h.

Immediately after the experiment, the cobalt that had migrated into the axons was precipitated with a 1% $(NH_4)_2S$ solution, leaving a black CoS deposit. The nerve tissue was fixed with formalin. After dehydration in an increasing series of alcohol concentrations, the ganglia could be rendered transparent by soaking in styrol or methyl benzoate and sealed into hollow-ground microscope slides with Canada balsam. Some of the preparations were imbedded in paraplast for later histological examination (see below).

B. Histological Methods

The fine structure in the areas to which the neurons marked with cobalt project was studied in some of the ventral-cord pieces following iontophoresis. These were prepared by standard histological techniques (imbedding in paraplast and cutting into 10-μm sections) and stained by Timm's method as described by Tyrer and Bell (1974). This procedure allowed detailed examination of

the morphological relationships between the branches of the auditory neurons stained with CoS and other neuronal systems. In addition, the silver staining method of Fink and Heimer (1967) was used, since it clearly shows the fiber tracts and the neuropile regions of the ganglion.

C. Extracellular Recording and Staining Technique

In this procedure (Rehbein et al., 1974a), an electrophysiological experiment is immediately followed by development of the stain and preparation of the tissue for histological study.

The electrophysiological methods have been extensively described by Kalmring et al. (1972b). Experiments in which recording was combined with staining departed from their method only in that the glass microelectrodes were filled with a 2M $CoCl_2$ solution rather than with KCl. The electrode resistance was 2-10 MΩ. As a rule, the electrodes were filled immediately before use by injection of the solution through a fine-tipped cannula. A silver wire was inserted into the abdomen to serve as an indifferent electrode.

When the electrophysiological part of the experiment had been completed a 1% $(NH_4)_2S$ solution was dripped into the ventral cord for about 5 min, and the tissue was then prepared by the histological procedure described above.

This combined recording and staining technique differs from the intracellular injection of $CoCl_2$ in the following ways:

(a) The activity of the axons (auditory neurons) was recorded extracellularly.

(b) The migration of cobalt into the axon occurred during the electrophysiological experiment. The cobalt was precipitated as CoS as soon as recording was completed.

(c) No artificial iontophoresis voltage was applied between the electrodes; however, the quality of the staining can presumably be distinctly improved by the application of, for example, pulses of direct current (such as are used for intrasomatic injection) even though the electrode is extracellular.

Staining during extracellular recording is successful only when the tip of the recording electrode is in the immediate vicinity of the neuron under study (giving a spike amplitude of several millivolts) and recording is continued for at least a few minutes. One can be confident that the nerve cell stained during the experiment is always identical with that from which the recordings were obtained, for the following reasons:

(a) When a response pattern of a given type was recorded in different animals, the morphological picture resulting from staining was always the same (even though the recordings were obtained from different parts of the nerve cell).

(b) When the activity of only one neuron is recorded in an experiment, only one cell is found to be stained. But if one records successively or, as rarely occurs, simultaneously from two or even three auditory neurons, a corresponding number of neurons are stained.

An important advantage of extracellular recording and staining is that it permits structural analysis of neurons with axons of relatively small diameter.

III. Results

The auditory neurons of the ventral cord can be divided into three groups on the basis of morphological criteria (Fig. 1). These are as follows:

(a) neurons ascending directly, without synapsing, to the supraesophageal ganglion,

(b) T-shaped neurons, with one branch making a direct connection with the supraesophageal ganglion and the other descending in the cord,

(c) thoracic neurons, the projection of which is restricted to the thorax.

All the auditory neurons described here are similar in that their cell bodies lie in the thoracic region of the ventral cord. This property clearly distinguishes

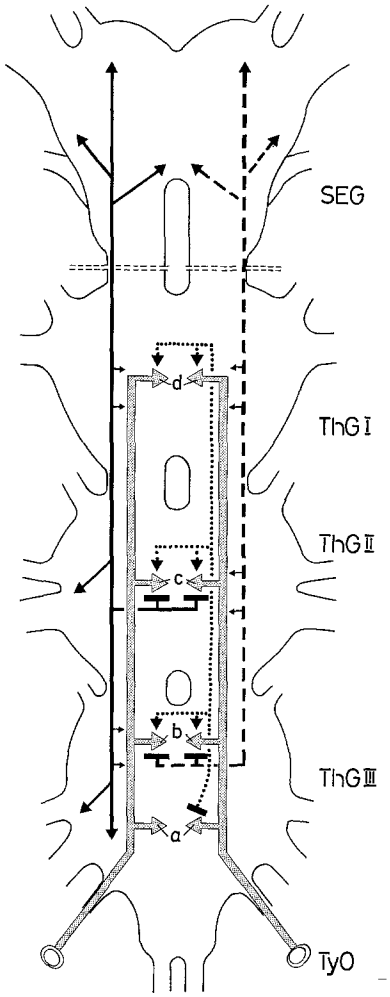

Fig. 1. Principles of the arrangement of units in the auditory pathway of *Locusta migratoria*. The grey underlaid double lines show the course and synaptic regions (auditory neuropiles) of the tympanal receptor fibers (the auditory neuropile areas are termed *a, b, c,* and *d*); heavy line, T-shaped ventral-cord neuron; dashed line, ascending ventral-cord neuron, terminating in the supraesophageal ganglion; dotted line, thoracic ventral-cord neuron; *SEG*, supraesophageal ganglion; *ThG I, II, III,* first, second and third thoracic ganglia (the subesophageal ganglion is absent); *TyO*, tympanal organ. Each type of ventral-cord unit is represented bilaterally in the animal but drawn here on only one side of the ventral cord (note the "loop" arrangement of the auditory elements in the thorax)

them from the receptor neurons [1], the cell bodies of which are located peripherally on the tympanum, and from the neurons of the supraesophageal ganglion, which have somata in the protocerebrum. The designation of auditory neurons

[1] The central projection of the receptor fibers is described in detail elsewhere (Rehbein, 1973; Rehbein et al., 1974a); those aspects necessary to understand the results that follow are presented in Figure 1

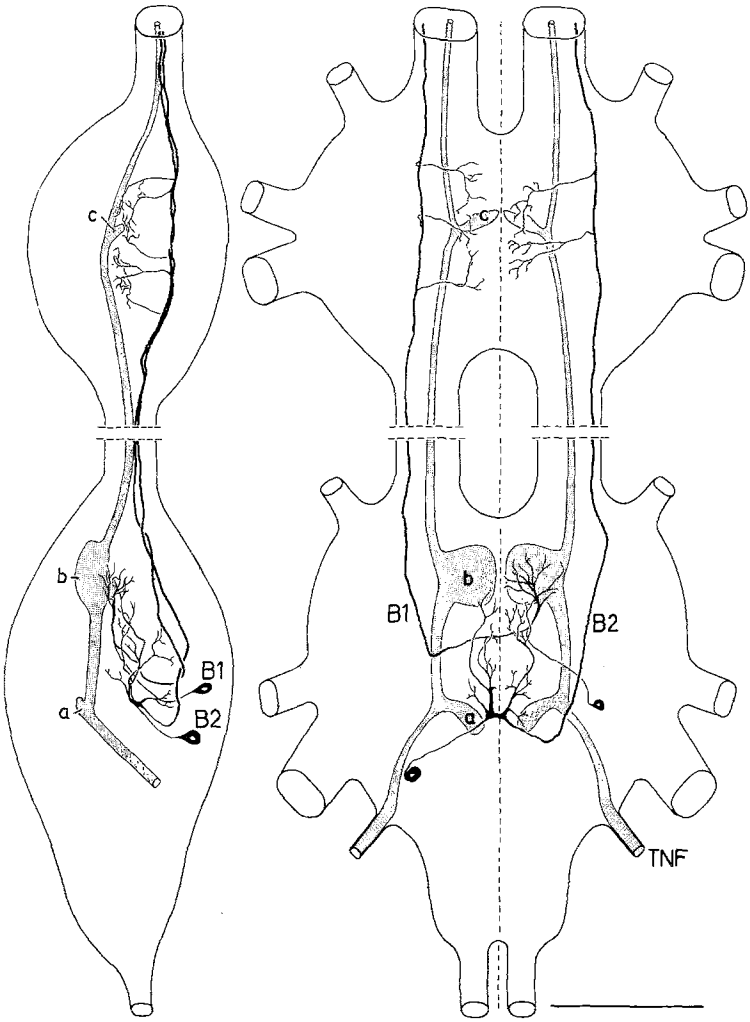

Fig. 2. Drawings of neurons B1 and B2 in the second and third thoracic ganglia; *right*, view from above; *left*, from the side. The underlaid double lines indicate the course of the tympanal nerve fibres (*TNF*) and location of the auditory neuropiles (*a*, *b* and *c*). The scale line represents 500 μm

in the ventral cord by capital letters (e.g., B and G) is derived from an older categorization by Popov (1967) and was developed by Kalmring (1971) and Kalmring et al. (1972a, b). In this paper, typical neurons belonging to each of the three groups will be presented as examples, and their morphological and functional peculiarities will be described.

A. Ventral-Cord Neurons Ascending to the Supraesophageal Ganglion

The ascending ventral-cord neurons constitute a direct connection between the auditory neuropiles of the thorax and those of the supraesophageal ganglion

(Fig. 1). All the neurons in this group known so far receive input only in the metathoracic ganglion, though this does not exclude the possibility that there are neurons ascending to the brain from the auditory centers of the second and first thoracic ganglia (neuropile areas c and d). Two neurons will be described (B1 and B2 in Fig. 2), which despite certain characteristic differences have a number of morphological and physiological traits in common. The axons of the two neurons run close together to the supraesophageal ganglion. Both neurons send out thin, medially directed side branches in all the ganglia through which they pass; these end near the midline but are entirely restricted to the ipsilateral half of the ganglion. In the metathoracic ganglion the axons cross to the opposite side, where their somata occupy a dorsolateral position. The decussation of the axon of Neuron B2 lies in a more caudal commissure than that of Neuron B1; here its diameter is several times that over the rest of its length. Near the midline Neuron B1 has one, and Neuron B2 two, clusters of branches which extend rostrally. These branches are probably the dendritic, information-receiving structures of the B neurons, since after transection of the meso-metathoracic connective the characteristic response patterns of the neurons in the metathoracic ganglion are unchanged. The response patterns of the B neurons, then, must be generated in the third thoracic ganglion.

Neuron B2, like Neuron B1, is particularly sensitive in the near-threshold region of intensity. The response patterns of both neurons are determined primarily by inputs from the ipsilateral (with respect to the axon) tympanal organ. The dendritic (input) structures of both neurons show a high degree of arborization; only a few of these branches enter the auditory neuropiles of the metathoracic ganglion. Most of the dendrites are distributed through a large region of metathoracic neuropile apart from the auditory synapse regions (Fig. 2).

In the following discussion, neurons of this group will be referred to simply as "ascending ventral-cord neurons".

B. Ventral-Cord Neurons with T Structure and Corresponding Function

T-shaped auditory ventral-cord neurons are a characteristic structural and functional type not limited to the auditory systems of Ensifera; they are also to be found in the acridid ventral cord. The "G neuron" of the migratory locust (Kalmring, 1971) is an example which has been thoroughly studied with respect to both morphology and physiology. The diameter of its fiber and the size of its soma make it, along with the B neurons, one of the largest ventral-cord neurons in the *Locusta* auditory system. The soma of the G neuron occupies an extremely caudolateral position in the metathoracic ganglion, near the entrance of the meso-metathoracic connective. As it crosses to the other side of the cord, the axon sends out two sturdy branches, one on either side of the midline, which run in a rostral direction and branch extensively (Figs. 3 and 4a, c). By alternately eliminating the input from one or the other tympanal organ (or meso-metathoracic connective) while recording from this neuron, it can be shown that these branches must be the dendritic structures of the G neuron (see below). Only a few end branches of the dendrites penetrate

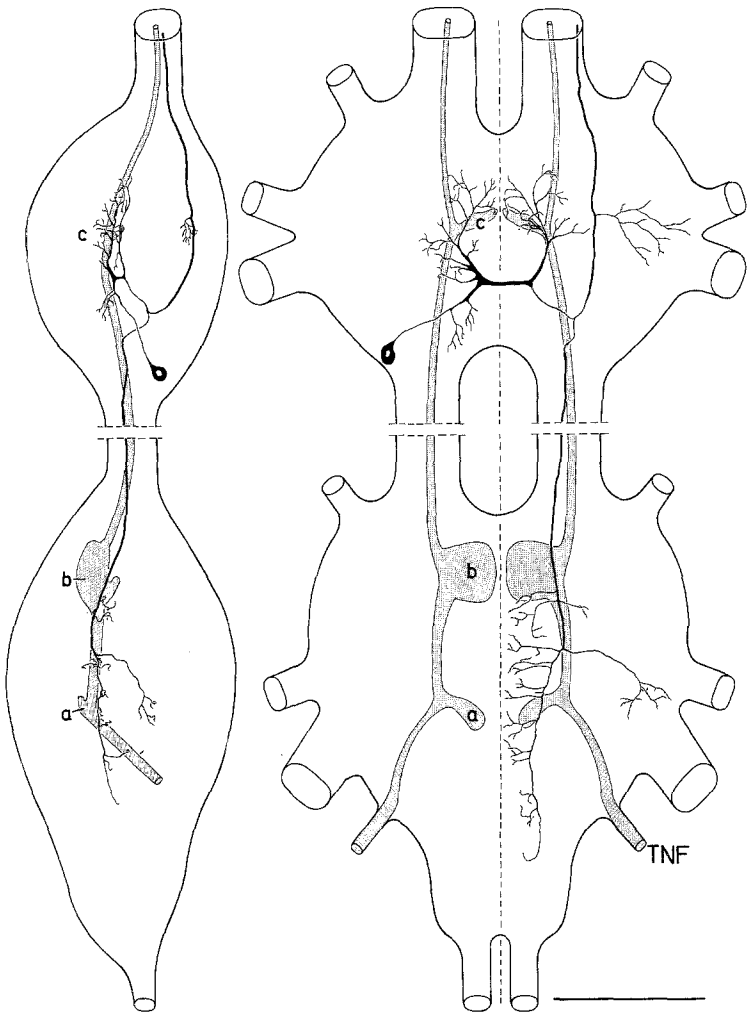

Fig. 3. Drawing of the G neuron at the level of the second and third thoracic ganglia; *right*, view from above; *left*, from the side; the underlaid double lines indicate the course of the tympanal nerve fibres (*TNF*) and location of the auditory neuropiles (*a*, *b* and *c*). The scale line represents 500 μm

the auditory neuropiles (formed by receptor fibers) in each half of the second thoracic ganglion (Neuropile c), where synaptic contact is made with the collateral branches of auditory receptor cells. Most of the dendritic mass spreads through large regions of the ventromedial neuropile apart from the auditory synapse regions. The possible function of these dendritic branches is discussed in Section IV.A. A striking property of the neuron is the unusually pronounced swelling of the two main branches of the dendrites (Figs. 3 and 4a) and of the axon in the commissure region. With high magnification one can see that near the point of largest swelling there are fine lateral processes, only a few μm long (Fig. 4b), which may also receive synaptic input.

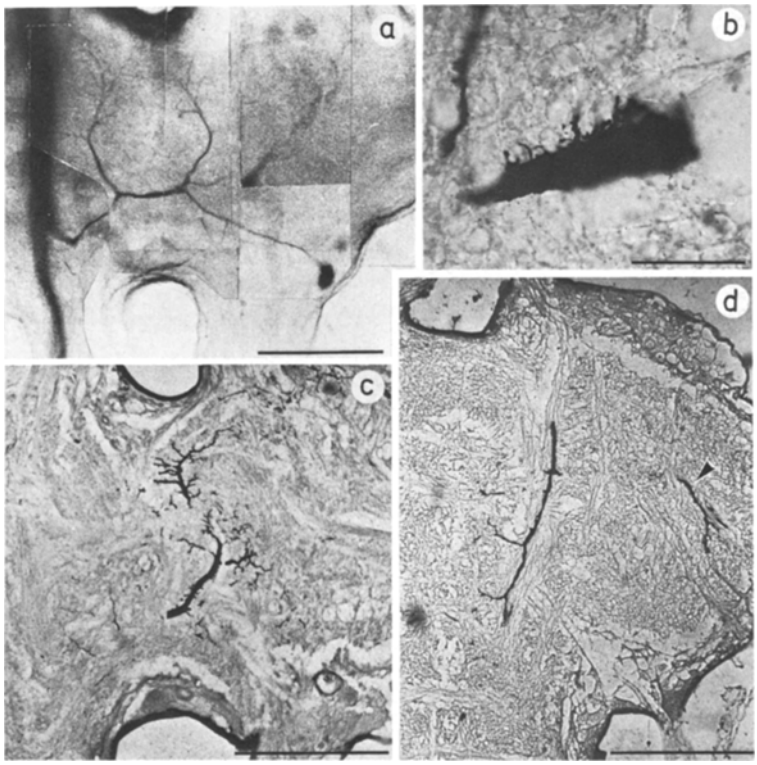

Fig. 4a–d. Photomicrographs showing details of the G neuron. **a** as revealed in the second thoracic ganglion by CoS staining (whole mount preparation, photomontage); **b** axon in the region of the commissure, with short side processes (the integration segment); **c** dendritic branch in the second thoracic ganglion; **d** the descending trunk of the axon in the third thoracic ganglion, showing medial and lateral (arrow) side branches. In **b**, **c** and **d** the cobalt stain was enhanced by silver, following the method of Timm. The scale lines represent 250 μm in **a**, **c** and **d**, and 25 μm in **b**

On the side of the ganglion contralateral to the soma the axon divides into two trunks, one ascending to the supraesophageal ganglion and one descending to the metathoracic ganglion; the same electrophysiological responses can be recorded from both branches. In this sense the T structure of the neuron is reflected in its function. At all levels of the ventral cord (metathoracic, prothoracic, and subesophageal ganglia) both axons send toward the midline thin branches with a string-of-beads appearance. These side branches make no direct connections with the auditory neuropiles of the third thoracic ganglion.

In the meta- and mesothoracic ganglia the G neuron sends out additional side branches, one running toward the lateral boundary of each ganglion; each such branch arborizes within a restricted region of the dorsolateral neuropile at the level of the leg and muscle nerve of that segment. This dorsolateral neuropile region in the mesothoracic and the metathoracic ganglion is an extensive, dense meshwork of fibers, consisting primarily of the dendrites of the motoneurons to the flight and walking muscles (Tyrer and Altmann, 1974). The terminal arborizations of the lateral branches of the G neuron are thus

closely interwoven in space with the input-receiving structures of thoracic motoneurons (see Section IV.B).

The ascending axon trunk of the G neuron runs in the same dorsolateral fiber bundle of the connective as the B neurons described above. The descending part of the axon, by contrast, runs through the central part of the meso-metathoracic connective and in the third thoracic ganglion joins the ventral connective bundle (Fig. 4d), a tract which also contains the ascending tympanal nerve fibers and the axon of the "thoracic low-frequency neuron" (cf. Section C, below) of this ganglion. The caudal trunk of the G neuron has previously been described as a separate neuron type (the I neuron) on the basis of physiological experiments (Kalmring et al., 1972b; Rehbein et al., 1974a). Iontophoretic marking via the pro-mesothoracic connective has demonstrated that this trunk and the remainder of the G neuron are a morphological unit.

In the supraesophageal ganglion the G neuron has at least two projection areas (Fig. 5): a few thin side branches run toward the center of the ganglion at the level of the deutocerebrum, and there is extensive branching in a lateral part of the protocerebrum that is identical to the lateral boundary region found by Adam and Schwartzkopff (1967). This auditory center can be localized histologically as a clearly defined lateral neuropile region of the protocerebral lobe.

There has so far been no case in which ascending neurons in the ventral cord of the migratory locust could be shown to make direct connection with the "association centers" of the protocerebral neuropile (the mushroom bodies, central body, protocerebral bridge) or with the region of the optic lobe.

All the end branches in the head ganglia display periodic "string-of-beads" swellings like those of the medial and lateral side branches in the thoracic region. This and other morphological characteristics can be taken as indications that all these axon branches are presynaptic (output) structures of the auditory ventral-cord neurons (see Section IV.B).

By transecting connectives and cutting off the input from the tympanal organs while recording at different levels of the ventral cord it can be shown that the input (dendritic) structures of the G neuron lie in the mesothoracic ganglion. After transection of the pro-mesothoracic connective the response pattern of the G neuron recorded from the rostral region of the mesothoracic ganglion is unchanged. Attempts to record neuronal responses at the base of the connective in the third thoracic ganglion after transection of the meso-metathoracic connective have not succeeded. Since histological examination (see above) also reveals no connection between the side branches of the G-neuron axon and the auditory neuropile regions of the metathoracic ganglion (Neuropile a and b), the richly arborized branches leaving the axon on the right and left of the midline of the mesothoracic ganglion must be viewed as the only site of information reception. It is only the terminal arborizations of these branches (and, in fact, very few of those) which make contact with receptor fibers in the auditory neuropile complex of the second thoracic ganglion (Neuropile c).

When the response pattern of the G neuron is recorded from the axon trunk and the contralateral meso-metathoracic connective is then cut, the original

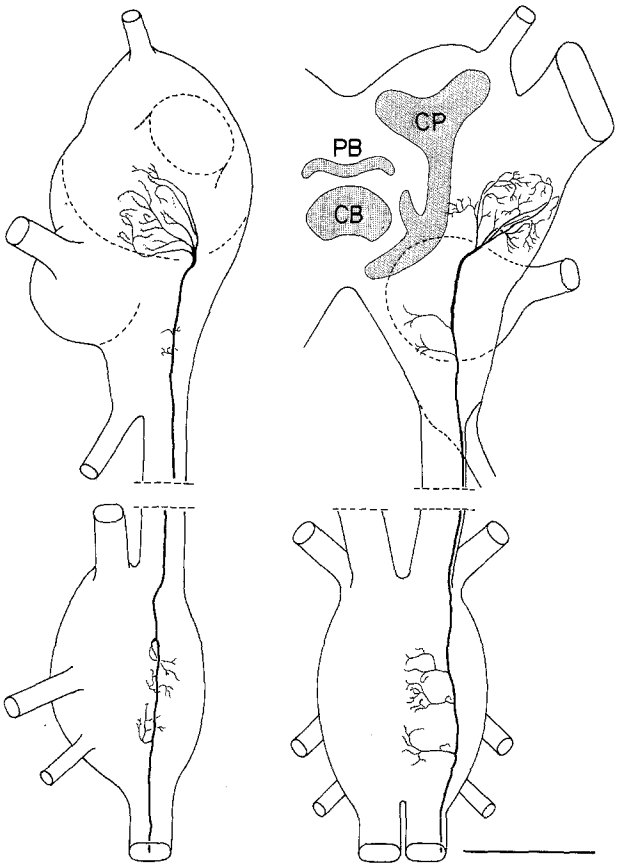

Fig. 5. Drawing of the terminal structures of the G neuron in the head ganglia (supra- and subesophageal ganglia); *right*, view from above; *left*, from the side. *CP*, corpora pedunculata; *CB*, central body; *PB*, protocerebral bridge. The scale line represents 500 µm

pattern of tonic response in the preferred range of intensities is converted to a phasic "residual pattern". Subsequent interruption of the connection to the contralateral tympanal organ has no effect on this residual response pattern. If the input from the contralateral tympanal organ is removed with the connective intact, there is only a slight change in the discharge pattern. Thus the inputs that form the pattern come from receptor neurons of the ipsilateral organ. Transection of the ipsilateral meso-metathoracic connective during recording from the ascending axon trunk again reduces the tonic response pattern obtained in the preferred intensity range to a phasic response. Accordingly, about the same tympanal afferents pass through the meso-metathoracic connectives of the two sides before reaching the dendritic structures of the G neuron.

These physiological and histological experiments are informative with respect to the basic connectivity of the G neuron. Within the two halves of the auditory neuropile of the mesothoracic ganglion (Neuropile c) the neuron makes direct synaptic connection with both ipsilateral and contralateral receptor neurons.

From the frontal auditory neuropile on the ipsilateral side of the metathoracic ganglion (Neuropile b) segmental interneurons cross within the ganglion to the opposite side, thence passing through the contralateral connective to the input structures of the G neuron.

C. Thoracic Ventral-Cord Neurons

The ventral cord of the migratory locust has been shown to contain several auditory neurons limited to the thoracic region. Morphological and physiological studies suggest that these thoracic neurons are interposed between receptor cells and ascending ventral-cord neurons – elements which transmit auditory information to the contralateral ventral cord and play a special role in processing this information by virtue of their excitatory and inhibitory effects.

One of these thoracic interneurons in the locust ventral cord has been the object of a thorough morphological and physiological study. Because it responds preferentially in the lower range of frequencies (with a best frequency of ca. 4 kHz) and is limited to the thoracic part of the ventral cord it has been called the "thoracic low-frequency neuron" (not to be confused with the receptor fibers with best frequency in the 3–5 kHz range which have been referred to as low-frequency receptors or tympanal low-frequency neurons). The basic structural characteristics of this neuron can be seen in Figure 1. The neuron sends processes to the contralateral ventral cord at several points, providing a connection between the auditory neuropiles on the two sides in each ganglion (for a more detailed description see Rehbein et al., 1974a).

The most likely site of the synaptic connection between receptor neurons and the thoracic low-frequency neuron is thought to be the ipsilateral caudal auditory neuropile of the metathoracic ganglion (Fig. 1, Neuropile a). This synaptic region is predominantly (perhaps exclusively) formed by the collaterals of the a-cells (tympanal low-frequency receptors in the classification of Michelsen, 1971, and Römer, 1975), and the responses of the thoracic low-frequency neuron resemble closely those of this receptor group (e.g., the threshold curves are nearly identical: Römer, 1975). These facts imply a direct (one might say, "relay-type") transmission of information from the group-a receptor cells and the thoracic low-frequency neuron.

The morphological and physiological properties of the thoracic low-frequency neuron would qualify it for the role of a segmental interneuron connecting receptor neurons and those ventral-cord neurons ascending to the brain, as follows:

A number of ascending auditory ventral-cord neurons which carry information to the supraesophageal ganglion respond preferentially to sound stimuli at frequencies of 12–40 kHz. In the frequency range below 10 kHz the response is weaker (for example, only phasic "on-responses" remain) or disappears altogether. This characteristic can be explained only by the interposition of inhibitory interneurons (Popov, 1967; Kalmring et al., 1972a; Kalmring, 1975). Experiments using two tones, presented simultaneously or with some delay, have shown that several large neurons ascending to the brain give reduced responses

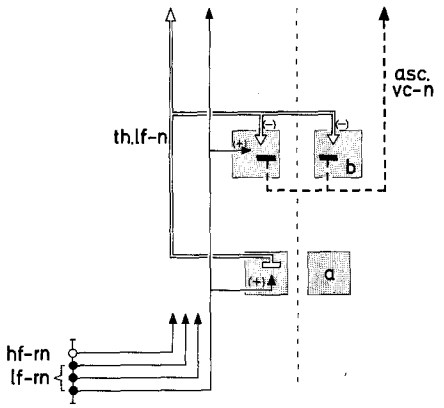

Fig. 6. Model of the function of the thoracic low-frequency neuron. In the caudal auditory neuropile of the third thoracic ganglion (Neuropile *a*) the thoracic low-frequency neuron receives information from tympanal low-frequency receptors; in the frontal auditory neuropile of the same ganglion (Neuropile *b*) it exerts an inhibitory effect on response-pattern formation in ascending ventral-cord neurons. *hf-rn*, high-frequency receptor neurons; *lf-rn*, low-frequency receptor neurons; *th.lf-n*, thoracic low-frequency neuron; *asc.vc-n*, ascending ventral-cord neuron. Each neuron is shown on only one side of the cord. To make the drawing clearer, the synaptic connections by which the ascending ventral-cord neuron receives input from high-frequency receptors and other low-frequency receptors have been omitted

to frequencies below 10 kHz, not because they lack synaptic connections to the appropriate receptor neurons but because of the inhibitory activity of interneurons (Kalmring, 1975).

The side branches from the axon of the thoracic low-frequency neuron cross to the contralateral half of each thoracic ganglion, always at the level of the auditory neuropiles, so that their fine terminal arborizations penetrate the auditory synaptic region on either side. The preferred response region of this neuron covers exactly that part of the frequency spectrum (2–10 kHz) in which many of the auditory ventral-cord neurons ascending to the brain give reduced or no responses. A simplified scheme (Fig. 6) has been designed to illustrate one plausible function of the thoracic low-frequency neuron in connection with ascending or T-shaped neurons. The figure shows an ascending ventral-cord unit; in the metathoracic ganglion it receives excitatory input from low-frequency and high frequency receptors[2]. A group of tympanal low-frequency receptors (a-cells) simultaneously excites the thoracic low-frequency neuron in the region of the caudal neuropile (Neuropile a); this interneuron in turn exerts an inhibitory influence on the ascending neuron, in the region of the frontal neuropile (Neuropile b), with a small delay (1–2 ms). In this way the transformation of a tonic response pattern into a phasic pattern in the low-frequency range can be explained.

In addition to the thoracic low-frequency neuron *Locusta migratoria* must have a number of other segmental ventral-cord neurons, for the occasionally quite complex response patterns of the auditory neurons ascending to the supra-

[2] For clarity, Figure 6 shows the connection with only one group of low-frequency receptors

esophageal ganglion can in many cases be explained only by the existence of a network of interneurons (Kalmring, 1975). However, the number of segmental neurons required need not be very large. The physiological experiments indicate that although the systems of connectivity underlying the responses of most neurons ascending to the brain are complex, the fundamental characteristics of all of them are similar (Kalmring, 1975). Probably, therefore, a limited number of segmental interneurons participate in the connectivity patterns of several ascending neurons. It remains to be seen whether the ascending ventral-cord neurons influence each other – the morphology suggests such a possibility (see Section IV).

IV. Discussion

In all cases in which the structure of locust auditory ventral-cord neurons is known, the neurons conform to a standard basic plan, with different regions clearly distinguishable as to morphology and function (Fig. 7). The following discussion is concerned with the possible significance of the various morphological sections of the neurons in the processing of auditory information and its transmission to other neuronal systems.

A. The Integration Segment with Its Dendritic Arborization

The axons of the auditory ventral-cord neurons are much dilated in the region near the dendritic branchings (cf. Figs. 2, 3, 4a, c). When dendrites are present on both sides of the midline, the dilatation of the axon lies within a commissure (e.g., Neurons B2 and G); when the postsynaptic structures are unilateral, the axon is swollen at the place where dendrites fuse together (e.g., Neuron B1). This morphological characteristic has been noted in several motoneurons and a number of sensory interneurons of orthopterans; the thickened region has been called the "integration segment" (Sandeman, 1969c; Murphey, 1973).

The branches arising from the integration segment can in many cases be distinguished from the side branches of the ventral-cord axons by certain aspects of morphology discernible even with the light microscope. The dendritic branches are characterized by irregular constrictions and dilatations, but lack the vesicular swellings typical of the terminal structures and side branches of axons in head and thorax. A conspicuous property of most ventral-cord neurons (with the exception of the thoracic neurons) is the extensive arborization of the dendrites, which spread through large regions of the neuropile. In view of the fact that only a few of the dendrites penetrate the auditory neuropile region formed by the terminal branches of auditory receptor cells (cf. Figs. 2 and 3), it is interesting to consider what the functional significance of the remaining dendrites may be.

The complex response patterns of the ascending ventral-cord neurons and of the T-shaped neurons cannot be explained on the basis of direct (monosynap-

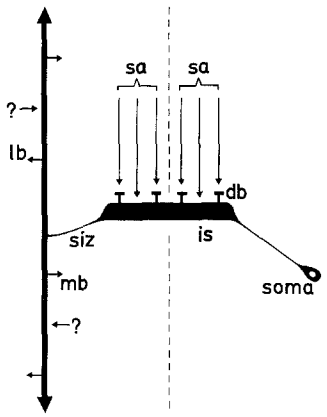

Fig. 7. Schematic diagram of the functional and morphological regions of auditory ventral-cord neurons. *sa*, sensory afferents (excitatory and inhibitory inputs from the tympanum and non-tympanal sources); *lb* and *mb*, lateral and medial branches of the axon; *siz*, spike initiating zone; *is*, integration segment; *db*, dendritic branches

tic) connections with tympanal receptor cells alone (cf. Section III.C). It can be shown that facilitatory and inhibitory afferents provide input to the ascending neurons by way of thoracic interneurons, some of which cross the cord while others are entirely ipsilateral (Popov, 1967; Kalmring et al., 1972a; Rehbein et al., 1974a; Kalmring, 1975). The results of the morphological studies presented here suggest that some of the synaptic inputs to the dendrites of ascending and T-shaped neurons in regions of the ganglion apart from the auditory neuropiles come from the thoracic interneurons.

Within the thoracic ganglia, the medial side-branches of the axons of certain ascending neurons (and of T neurons) project to regions apart from the auditory neuropiles, where the dendritic arborizations of other auditory ventral-cord neurons are located. From a morphological standpoint, then, it is conceivable that the ascending and T-shaped ventral-cord neurons interact so as to produce a reciprocal modification of response pattern; thus the thoracic ventral cord would contain a complex network of auditory elements connected in "loops" (cf. Fig. 1).

The findings of Yanagisawa et al. (1967) indicate that there are afferents from the abdomen, as well as thoracic afferents from non-tympanal sources, which have an inhibitory effect upon the response pattern of auditory ventral-cord neurons. These experiments provide additional clues to the possible function of the extensive dendritic branching.

"Processing" of the information received by the dendrites, by analogy with the situation in a crustacean motoneuron described by Sandeman (1969c), would be expected to occur in the integration segment. The membrane of the integration segment is not electrically excitable; the excitatory potentials are summated and propagate electrotonically to the spike-initiating zone, situated at the point where the integration segment is reduced to a small diameter and gives rise

to the main axon (Fig. 7). According to Sandeman, the inhibitory inputs arrive at the membrane of the integration segment itself, where they reduce the amplitude of depolarization and thus can, under certain circumstances, prevent spike initiation at the main axon. In this regard it is interesting that the integration segments of auditory ventral-cord neurons bear fine processes, only a few μm long, in the region where the dilatation is greatest (cf. Fig. 4b).

These studies by Sandeman, then, may also provide an explanation of certain observations which at first appeared contradictory: The response patterns of most of the auditory ventral-cord neurons so far examined are affected primarily by the tympanal organ on the side ipsilateral to the main axon, even though morphological investigation shows that the neurons involved are connected to receptor fibers on both sides of the ventral cord. The structure of the integration segments of auditory ventral-cord neurons justifies the inference that in the migratory locust, as in the crustaceans studied, the length constants of the integration segment are such that the greatest influence upon amplitude and time-course of the postsynaptic potential (and thus upon the response pattern) is exerted by those facilitatory and inhibitory inputs that arrive in the vicinity of the spike-initiating zone. There is another well-studied aspect of the orthopteran nervous system which deserves mention in this context – the processing of sensory information in the integration segments of abdominal giant fibers. These neurons also have bilateral dendritic arborizations in the last abdominal ganglion, so that they make direct synaptic contact with cercal-nerve fibers on both sides of the cord (Potente, 1975). The response pattern of the giant fibers, however, is determined entirely by the ipsilateral cercus; stimulation of the contralateral cercus elicits at most a weak response and in some cases only a subthreshold EPSP (Callec et al., 1971; Murphey, 1973).

B. The Axon with Its Side Branches and Terminal Arborization

The uniform morphology of all the side branches from an axon in the thorax and the end branches in the supraesophageal ganglion – together with the above-mentioned light-microscopically evident differences from the dendritic structures near the soma – indicate that all the side and end branches of the axon should be regarded as presynaptic (output) components of the auditory ventral-cord neurons. But this view is opposed by recent findings in tettigoniids which confirm that the side branches of the axons of certain ventral-cord neurons can also function as dendritic, information-receiving structures. The following discussion presents an attempt to interpret the available morphological and physiological data with respect to possible functional properties of the side branches.

Some of the auditory neurons in the ventral cord send out *lateral* side branches which project to the dorsolateral boundaries of the thoracic ganglia (cf. Figs. 3 and 4d). These processes split up into terminal arborizations which lie in an extensive dorsolateral meshwork of fibers consisting primarily of the dendrites of motoneurons to the leg and flight musculature (Tyrer and Altman, 1974). There is abundant physiological evidence that the activity patterns of many thoracic motoneurons are affected by inhibitory and facilitatory influences

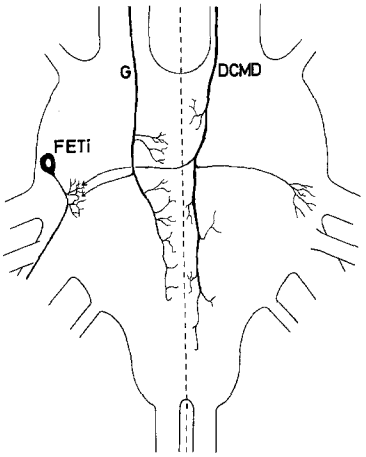

Fig. 8. Information transmission to a motoneuron of the third thoracic ganglion, by way of the lateral side branches of sensory-neuron axons. *FETi*, fast extensor tibialis (motoneuron); *G*, G neuron (T-shaped auditory ventral-cord neuron); *DCMD*, descending contralateral movement detector (visual neuron). Altered from Horridge and Burrows (1974)

from sensory ventral-cord neurons. A direct synaptic connection between descending visual giant fibers (DCMD and DIMD) and motoneurons of the metathoracic ganglion (e.g., the fast extensor tibialis) in the locust *Schistocerca gregaria* has been demonstrated physiologically by Burrows and Rowell (1973). According to the morphological studies of O'Shea et al. (1974), there is a close spatial contact between the dendritic arborizations of metathoracic motoneurons and the postsynaptic structures of one of the two descending visual neurons (DCMD). In the meso- and metathoracic ganglia the DCMD sends out lateral branches comparable to the lateral side-branches of auditory ventral-cord neurons (e.g., the G neuron). The lateral branches of both visual and auditory neurons project to the same part of the dorsolateral motor neuropile (Fig. 8). It is thus probable that auditory ventral-cord neurons also make direct monosynaptic connection with thoracic motoneurons via the lateral branches—particularly in view of Burrows' (pers. comm.) recent finding of a 1:1 correlation between the response patterns of auditory ventral-cord neurons and the postsynaptic potentials of the motoneuron to the fast extensor tibialis.

Every auditory neuron of the ventral cord sends *medial* branches from the axon into all ganglia except that in which the dendrites of that neuron are located (cf. Figs. 2, 3, 4d and 5). Various possible interactions are suggested by the close spatial contact between these and certain neuronal structures.

Many thoracic motoneurons have extensive dendritic arborizations, in some cases spreading as far as the midline of the ganglion. Tyrer and Altman (1974) found that numerous contact points between sensory afferents from the wing and flight motoneurons were located near the midline of the mesothoracic ganglion of the locust *Chortoicetes terminifera*. The morphological findings presented here imply the existence of corresponding connections between the

medial branches of auditory ventral-cord neurons and certain thoracic motoneurons.

It has been pointed out above that the side branches sent toward the midline from the axons of certain ventral-cord neurons make close spatial contact with the dendritic structures of other ventral-cord neurons, and that it is conceivable that these elements in the auditory pathway may interact directly via such connections.

Kalmring and Rehbein (in preparation) have shown that in at least one case – an auditory neuron with T structure and corresponding function in the tettigoniid ventral cord – the medial (and perhaps also lateral) side branches from the axon must be regarded as dendrites, since they receive auditory information from the tympanal afferents.

In conclusion, it remains difficult to formulate a consistent functional interpretation of the available morphological data. A continued program combining histological, ultrastructural and physiological studies of its elements is required if the principles underlying this auditory system are to be understood.

I would like to thank Prof. Dr. J. Schwartzkopff for his unstinted support and helpful criticism of this work. I am also most grateful for the collaboration of my colleagues Drs. K. Kalmring, H. Römer and J. Rheinlaender. Mrs. B. Ewersmeyer gave much-appreciated assistance in the staining experiments.

References

Adam, L.-J., Schwartzkopff, J.: Getrennte nervöse Repräsentation für verschiedene Tonbereiche im Protocerebrum von *Locusta migratoria*. Z. vergl. Physiol. **54**, 246–255 (1967)

Burrows, M., Rowell, C.H.F.: Connections between descending visual interneurons and metathoracic motoneurons in the locust. J. comp. Physiol. **85**, 221–234 (1973)

Callec, J.J., Guillet, J.C., Pichon, Y., Boistel, J.: Further studies on synaptic transmission in insects. II. Relations between sensory information and its synaptic integration at the level of a single giant axon in the cockroach. J. exp. Biol. **55**, 123–149 (1971)

Elsner, N., Huber, F.: Neurale Grundlagen artspezifischer Kommunikation bei Orthopteren. Fortschr. Zool. **22**, 1–48 (1973)

Fink, R.P., Heimer, L.: Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. **4**, 369–374 (1967)

Horridge, G.A., Burrows, M.: Synapses upon motoneurons of locusts during retrograde degeneration. Phil. Trans. B **269**, 95–108 (1974)

Iles, J.F., Mulloney, B.: Procion yellow staining of cockroach motoneurons without the use of microelectrodes. Brain Res. **30**, 397–400 (1971)

Kalmring, K.: Akustische Neuronen im Unterschlundganglion der Wanderheuschrecke *Locusta migratoria*. Z. vergl. Physiol. **72**, 95–110 (1971)

Kalmring, K.: The afferent auditory pathway in the ventral cord of *Locusta migratoria* (Acrididae). I. Synaptic connectivity and information processing among the auditory neurons of the ventral cord. J. comp. Physiol. **104**, 103–141 (1975)

Kalmring, K., Rheinlaender, J., Rehbein, H.G.: Akustische Neuronen im Bauchmark der Wanderheuschrecke *Locusta migratoria*. Z. vergl. Physiol. **76**, 314–332 (1972a)

Kalmring, K., Rheinlaender, J., Römer, H.: Akustische Neuronen im Bauchmark von *Locusta migratoria*. Der Einfluß der Schallrichtung auf die Antwortmuster. J. comp. Physiol. **80**, 325–352 (1972b)

Michelsen, A.: The physiology of the locust ear. I. Frequency sensitivity of single cells in the isolated ear. Z. vergl. Physiol. **71**, 49–62 (1971)

Murphey, R.K.: Characterization of an insect neuron which cannot be visualized in situ. In: Intracellular staining in neurobiology (S.B. Kater, C. Nicholson, eds.), pp. 135–150. Berlin-Heidelberg-New York: Springer 1973

O'Shea, M., Rowell, C.H.F., Williams, J.L.D.: The anatomy of a locust visual interneurone; the descending contralateral movement detector. J. exp. Biol. **60**, 1–12 (1974)

Popov, A.V.: Synaptic transmission at the level of the first synapses of the auditory system in *Locusta migratoria*. [in Russian.] In: Evolutionary neurophysiology and neurochemistry (E.M. Kreps, ed.), pp. 54–67. Leningrad: Nauka 1967

Potente, A.: Untersuchungen zur Morphologie der cercalen Riesenfasern im Bauchmark von *Locusta migratoria*. Staatsexamensarbeit Bochum, 1975

Rehbein, H.G.: Experimentell-anatomische Untersuchungen über den Verlauf der Tympanalnervenfasern im Bauchmark von Feldheuschrecken, Laubheuschrecken und Grillen. Verh. dtsch. zool. Ges. **66**, 184–189 (1973)

Rehbein, H.G., Kalmring, K., Römer, H.: Structure and function of acoustic neurons in the thoracic ventral nerve cord of *Locusta migratoria* (Acrididae). J. comp. Physiol. **95**, 263–280 (1974a)

Rehbein, H.G., Kalmring, K., Schwartzkopff, J.: Verschaltung eines akustischen Neurons im Bauchmark von *Locusta migratoria*. Naturwissenschaften **61**, 689 (1974b)

Römer, H.: Die Informationsverarbeitung tympanaler Rezeptorelemente von *Locusta migratoria* (Acrididae, Orthoptera). J. comp. Physiol. **109**, 101–122 (1976)

Sandeman, D.C.: Integrative properties of a reflex motoneuron in the brain of the crab *Carcinus maenas*. Z. vergl. Physiol. **64**, 450–464 (1969c)

Schwartzkopff, J.: Mechanoreception. In: The physiology of insecta (M. Rockstein, ed.), pp. 273–352. San Francisco, New York: Acad. Press 1974

Tyrer, N.M., Altman, J.S.: Motor and sensory flight neurones in a locust demonstrated using cobalt chloride. J. comp. Neurol. **157**, 117–138 (1974)

Tyrer, N.M., Bell, E.M.: The intensification of cobalt-filled neurone profiles using a modification of Timm's sulphide-silver method. Brain Res. **73**, 151–155 (1974)

Yanagisawa, K., Hashimoto, T., Katsuki, Y.: Frequency discrimination in the central nerve cords of locusts. J. Insect Physiol. **13**, 635–643 (1967)