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Auditory Interneurons in the Cricket *Teleogryllus oceanicus*: Physiological and Anatomical Properties

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Summary. 1. The morphology and physiology of two acoustic interneurons in the prothoracic ganglion have been studied by the use of extracellular microelectrodes filled with cobalt chloride.

2. Interneuron 1 is inhibited by 5 kHz tones (Fig. 1) and does not reliably code the temporal pattern of the calling song (Fig. 2). It is unique in each half of the prothoracic ganglion, sends dendrites unilaterally into the acoustic neuropile, and sends its axon to the brain (Figs. 3, 4).

3. Interneuron 2 is excited tonically by 5 kHz tones (Figs. 5, 6) and accurately codes the temporal structure of song (Fig. 7). One pair of these neurons is present in the prothoracic ganglion. Each interneuron 2 projects to both left and right acoustic neuropiles, but has no process leaving the ganglion (Figs. 8, 9); it is an intra-ganglionic interneuron.

4. The reliability of the relatively new extracellular cobalt staining procedure is discussed.

Introduction

Acoustic behavior in crickets is attractive to neurophysiologists and behaviorists because of its simplicity, stereotypy, and species specificity. The neurophysiological mechanisms of song production by males have been studied down to the level of individually identified cells (Bentley, 1969; Huber, 1974; Kutsch and Huber, 1970). However, in the auditory system, the neurons central to the primary afferents have not been identified. Earlier studies have defined classes of auditory interneurons on the basis of their responses to pure tones of various frequencies and to conspecific and heterospecific calling songs.

This has been done by making extracellular recordings from the cervical connectives with suction electrodes (Zaretsky, 1971; Hill, 1974; Stout and Huber, 1972), or with sharp tungsten electrodes inserted into the prothoracic ganglion or cervical connective (Katsuki and Suga, 1960; Popov, 1973). If the number of auditory interneurons is small (Popov and Shuvalov, 1974 estimated that

there are 12) and they can be individually identified, it is probable that the entire prothoracic auditory system can be analyzed cell-by-cell, as has been done successfully in numerous motor systems of invertebrates (Fentress, 1976).

Although several studies, mentioned above, have resulted in the classification of auditory neurons on the basis of their responses to auditory stimuli, only two such cells in crickets have been uniquely identified in terms of both physiological responses and neuronal morphology (Rheinlaender et al., 1976) using staining techniques capable of resolving single neurons. Only by carrying out this sort of investigation, coupling physiology with cellular anatomy, will it be possible to describe the auditory pathways of the cricket in terms of identifiable interneurons comprising the network. Such a description is a prerequisite for understanding the neurophysiological mechanisms of acoustic behavior. The encouraging results of similar studies undertaken in other orthopteran auditory systems such as those of acridid grasshoppers and tettigoniids (Rheinlaender and Kalmring, 1973; Rheinlaender, 1975; Rehbein et al., 1974) give rise to the hope that a rather complete and detailed description of the cricket auditory system may be possible.

Acoustic behavior in the Australian crickets of the genus *Teleogryllus* has been investigated from several viewpoints including ontogeny, behavior genetics, and neurophysiology (Bentley and Hoy, 1974; Hoy, 1974), and the findings reported here are the first results of a systematic effort to map the cricket auditory system. These results have been briefly reported in abstract form (Hoy and Casaday, 1976).

Materials and Methods

Adult female crickets (*Teleogryllus oceanicus*) were isolated as late instar nymphs and reared apart from males until they were used in the experiment, two to eight weeks after the adult molt. The animals were pinned ventral side up, the meso- and metathoracic legs were removed, the exoskeleton above the prothoracic ganglion was dissected away, and the ganglion itself was carefully stripped of obscuring fatty tissue and supported on a metal platform. Care was taken to leave major tracheal branches (which may have auditory function) and the ganglion sheath intact, and to avoid stretching the leg nerves, in which the auditory fibers pass from the tympanal organ in the tibiae to the prothoracic ganglion.

The recording/staining microelectrodes were glass micropipettes filled with 3 M $CoCl_2$; resistances ranged from 8 to 20 MOhms. The metal platform used to support the ganglion also served as the indifferent electrode. The electrode was advanced normal to the surface of the ganglion with a micromanipulator (Narishige MO-10 hydraulic microdrive) until the acoustic neuropile was encountered 200 μm beneath the ventral surface. This was recognizable by low amplitude multiunit activity which was heard as a distinctive stimulus locked hiss in the audio monitor. After reaching the periphery of the acoustic neurophile, the microelectrode was advanced very slowly until a stimulus related spike discharge due to a putative single unit was detected. Our staining procedure was based on the extracellular cobalt technique reported by Rehbein et al. (1974). The reliability of this method is considered in the Discussion. Our method differed from that of Rehbein et al. in that a backing current (2×10^{-9}A, electrode negative) was applied to retain cobalt in the electrode during recording, and an outward current (1 ms pulses of 5×10^{-7}A at 1/s, electrode positive) was used to eject cobalt from the electrode during staining. The ganglion was excised to a histological preparation dish, and ammonium sulfide was added to precipitate the cellular cobalt as cobalt sulfide, which is visible in favorable whole mount preparations. A great improvement in resolution of the staining was achieved by treating the preparation by the

Timm's modification, as described by Tyrer and Bell (1974). Examination of 30 μm sections of the ganglion revealed fine branches and processes that could not be resolved in the whole mount preparations; details of our modification of the technique of Rehbein et al. will appear elsewhere (Hoy and Casaday, in preparation).

Acoustic stimuli were presented by playing a taped stimulus program of sound pulses and species song on a tape deck (Sony TC-353D), through an audio amplifier (Realistic SA-101), and finally through a loudspeaker (Realistic 12-1844), situated 1 m from the preparation. The cricket was placed such that the speaker was aimed directly at the large posterior tympanic membrane (thus the cricket faced "away" from the speaker). The stimulus tape included pure tone bursts, generated by a microcomputer with an audio section, with stimulus energy concentrated at 5 kHz, the dominant frequency of the natural call of *Teleogryllus oceanicus* (Leroy, 1964; Loftus-Hills et al., 1971). The tone bursts were graded in steps of duration (50 ms to 500 ms) and intensity (40 dB SPL to 90 dB SPL); sound level measurements were made with a Brüel and Kjaer 2209 sound level meter. The stimulus type also included a section of either natural calling song or an electronic model of calling song (all pulses at 5 kHz).

The results are based on 10 recordings of interneuron 1 and 22 of interneuron 2. It was easier to make a successful physiological characterization of an auditory interneuron than to produce a successful anatomical one. Thus we have a smaller number of identifications in which both physiological and anatomical characterization was made in the same neuron: 4 in the case of interneuron 1 and 7 of interneuron 2.

Results

A. Interneuron 1

Physiology

Interneuron 1 (int-1). The typical physiological response of int-1 is shown in Figure 1. It is obvious from the figure that the unit is not excited but rather suppressed by acoustic stimuli. In our experiments we observed that in the absence of acoustic stimulation the unit shows "spontaneous" activity which

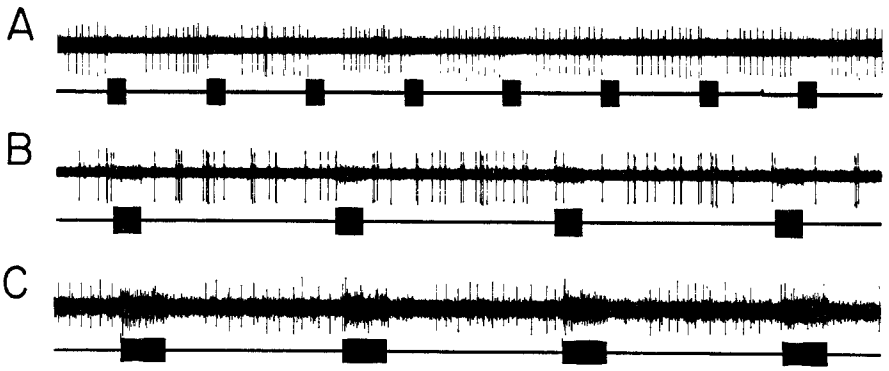

Fig. 1A–C. Physiological response of int-1 to long duration sound pulses (5 kHz tones, played at 80 dB SPL). Recordings from these units show a spontaneous discharge that is suppressed by acoustic stimulation. **A**, **B**, and **C** from 3 different preparations; upon subsequent staining with cobalt their neuroanatomy was revealed and can be seen in A, B, and C of Figure 4. Time scale = 500 ms

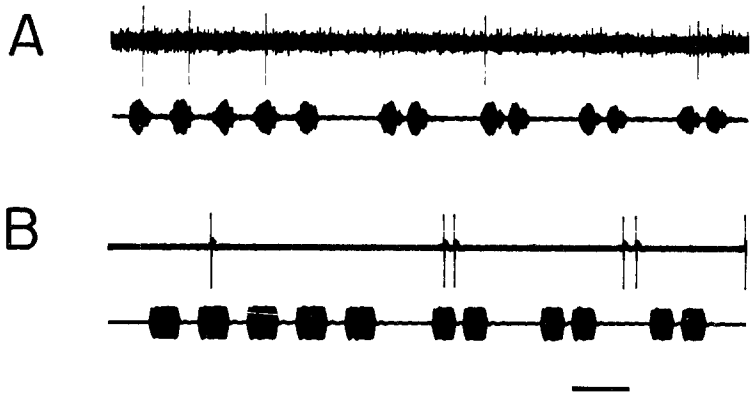

Fig. 2A and B. Physiological response of int-1 to calling song. The response in **A** is to natural calling song and in **B** to electronically generated song; both were played at 80 dB SPL. In neither of these cases does the unit show systematic responsiveness to temporal pattern in the call. **A** and **B** are from different preparations; they correspond to the neurons shown in A and B of Figure 4. Time scale = 100 ms

is suppressed during stimulation. The suppressive effect of sound is not absolute; for tones as long as 500 ms, the unit breaks through with a few impulses, but never with a train of impulses as occurs without stimulation.

This unit responds variably when stimulated by species calling song. Figure 2 shows the response of two different units when stimulated by song; no reliable coding of the song's temporal parameters is apparent in these examples. The occurrence of spikes is at most weakly correlated with any feature of the song. In a few recordings from the cervical connective, a unit with response properties similar to those of int-1, fired between pulses of the *T. oceanicus* calling song (Moiseff, unpublished). Therefore, the possibility remains that under some circumstances int-1 may code the temporal pattern of song.

Anatomy

Three cobalt stained preparations of int-1 are shown in Figures 3 and 4. These figures indicate the anatomical relationship between int-1 and the acoustic neuropile, the region of terminal arborizations of the primary acoustic afferents. The primary afferents from the tympanal organ arborize into a restricted region of the neuropile of the prothoracic ganglion (Rehbein, 1973; Huber, 1975). This area of neuropile appears to correspond to the posterolateral limb of the "ring tract" described by Gregory (1974) in the mesothoracic ganglion of a roach, a region easily recognized in our Timm's intensified preparations. The acoustic neuropile is indicated in outline as a landmark in the anatomical diagrams. The photograph in Figure 3 shows int-1 in a whole mount preparation of the prothoracic ganglion. The main features of the cell are shown, particularly of the part of the neuron that lies in the acoustic neuropile. However the cell body and primary axon are out of the plane of focus. Hence a more

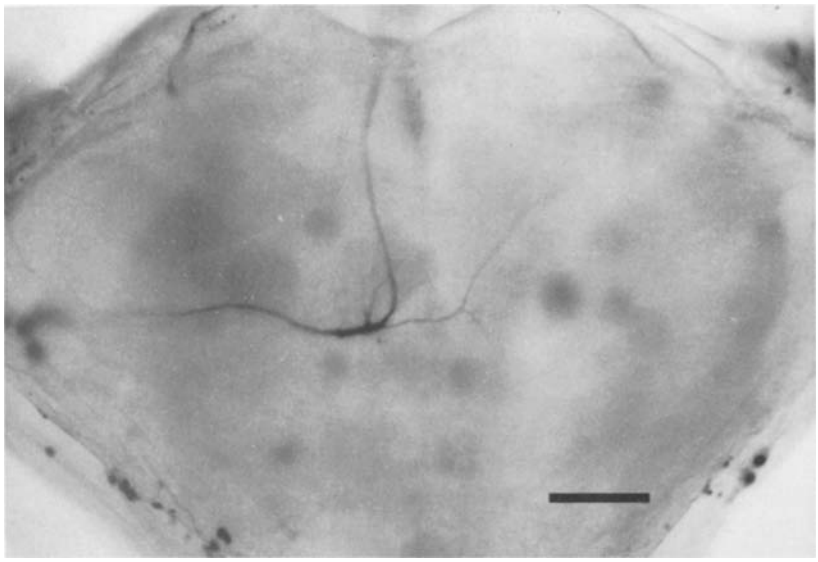

Fig. 3. Interneuron 1. This photograph of a prothoracic ganglion prepared in whole mount shows selective staining of int-1 by the extracellular cobalt method. It is a ventral view of the ganglion. In this and in all subsequent diagrams of neuroanatomy, the anterior direction is at the top of the picture. The main features shown here are the unilateral projection to the acoustic neuropile, the ascending primary axon that terminates in the brain, and the long neurite that connects the visible portion of the neuron to its contralaterally located cell body (which is present in the whole mount but out of the plane of focus in this picture, and hence not seen). Size scale = 100 μm

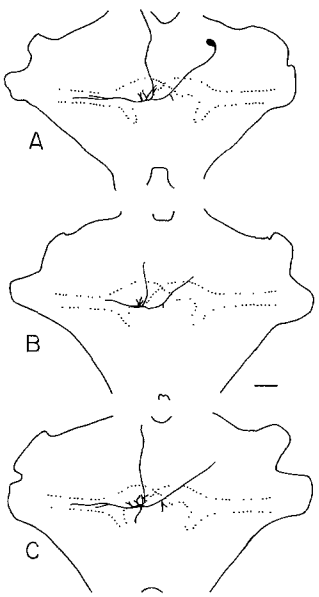

Fig. 4A–C. Camera lucida reconstruction of three different int-1 preparations stained by the extracellular cobalt technique. Outlined by the dotted lines are both acoustic neuropiles. Anterior direction is at the top of each figure. The neuron soma was stained only in preparation **A**.
Scale = 100 μm

complete picture of the cell is obtained from the camera lucida reconstructions of sectioned preparations shown in Figure 4. The major branches of the cell lie in the acoustic neuropile; one branch runs laterally, parallel to the track of the incoming primary auditory afferents, and other branches are more centrally located. The 30 μm diameter cell body, which is connected to the rest of the cell by a thin process, is located on the contralateral side of the ganglion. Thus, the cell body is contralateral to the apparent primary sites of synaptic action as well as to the ascending axon. A single primary axon leaves the ganglion anteriorly through the ventral intermediate tract (Gregory, 1974), and terminates in the brain. To show this termination, we used an independent method of staining int-1 with cobalt. We used the "retrograde-fill" method (Kater and Nicholson, 1973) in which the circumesophageal connective is immersed in a cobalt pool. Presumably the cobalt diffuses into the axon and fills the remainder of the neuron. When we used the retrograde technique on the circumesophageal connective, int-1 invariably became stained; thus the presumption that int-1 sends an axon into the brain is confirmed. We note in passing that in no case did int-2 (see below) ever stain in these preparations; we would not expect it to stain since this interneuron has no axon in the connective.

Int-1 is unique in each hemiganglion as shown by several anatomical techniques. Its distinctive geometry is revealed in unstained paraffin sections examined by phase contrast microscopy or stained by silver, Masson's trichrome, or toluidine blue. These four independent histological techniques and the two different cobalt staining techniques discussed above always show one neuron of type 1 in each side of the ganglion. Thus int-1 is a unique cell and not a representative of a class.

B. Interneuron 2

Physiology

The physiological responses of three examples of int-2 stimulated by 500 ms tones (dominant frequency same as in song 5 kHz) are shown in Figure 5. For one case (Fig. 5A) the stimulus is presented in increasing 10 dB increments of sound pressure level. The unit responds tonically, even though the 500 ms tone is 10 times longer than sound pulses in calling song (50 ms). The effects of increasing the intensity of the stimulus are to shorten the latency of the response (Fig. 5A), and to increase the firing frequency within the prolonged spike burst. The relationship between stimulus intensity and firing rate is shown in the graph of Figure 6. The firing rate falls monotonically with stimulation time, but declines only slowly after 200 ms. So, for sustained stimulation, the firing rate reflects the stimulus intensity.

Figure 7 demonstrates the ability of these units to respond to the species calling song. Recordings from the same three units displayed in Figure 5 are shown to demonstrate that it is a general property of int-2 to faithfully preserve, in its discharge pattern, the temporal pattern of the pulses in the species calling

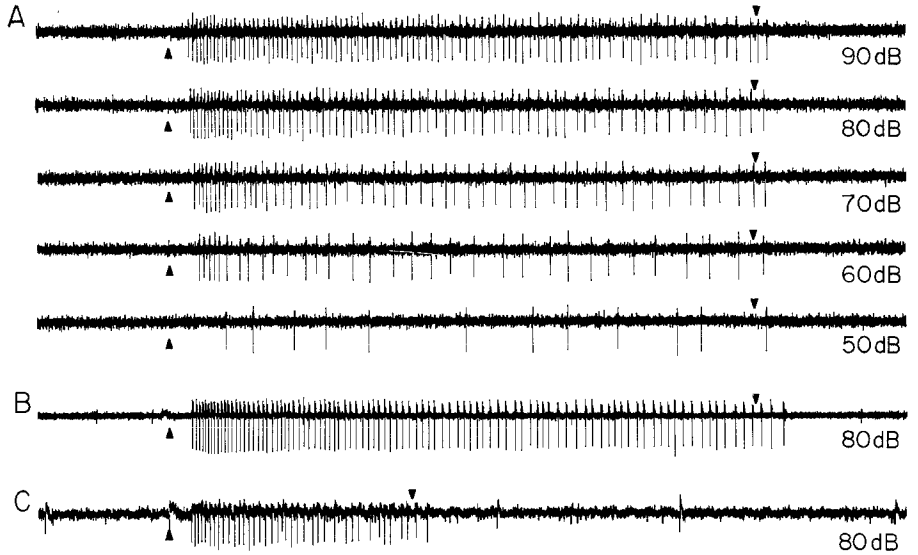

Fig. 5A–C. Physiological response of int-2 to long tone pulses (electronically generated pure tones of 5 kHz; 500 ms duration: **A** and **B**; 200 ms: **C**). The response is tonic and excitatory throughout the tone pulse. **A**, **B**, and **C** are different units whose anatomies are shown in Figure 9. The response to a series of increasing intensities is shown for unit **A**. The onset of tone is indicated by arrow and termination of tone by the inverted arrows. Scale-distance between arrows in **A**: 500 ms; in **B**: 200 ms

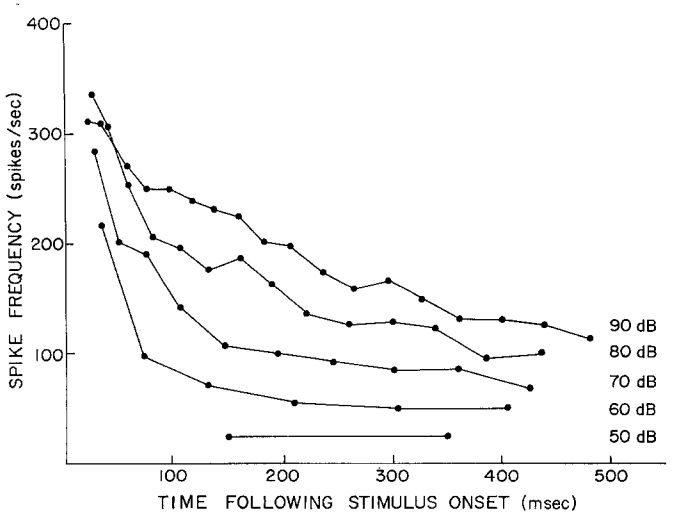

Fig. 6. Intensity coding of the acoustic stimulus by int-2. Spike frequency is plotted as a function of stimulus intensity during a 500 ms duration tone (5 kHz pure tone). Each point represents the average frequency for 5 successive spikes. After the first 100 ms the discharge rate systematically varies with the intensity of acoustic stimulation. Only two points are plotted at an intensity of 50 dB SPL because this was near threshold and the unit was not very active

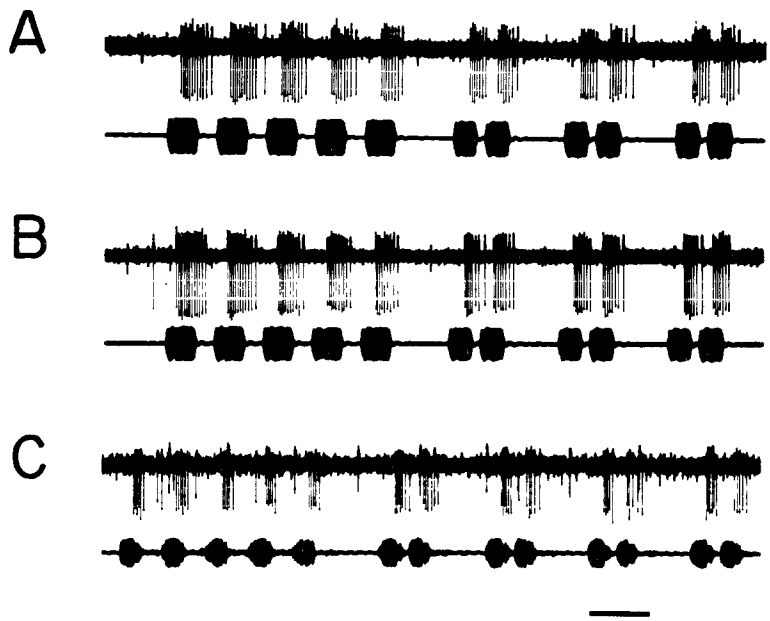

Fig. 7A–C. Response of three different int-2 units to species song. The neuroanatomy of each unit corresponds to A, B, and C of Figure 9. **A** and **B** show responses to artificial song (5 kHz pure tones temporally modulated to correspond to the temporal pattern of natural call) and **C** shows the response to a tape recording from an actual call. Note that in **A** and **B** the five-pulse introductory "chirp" features longer pulse durations than in the pulses of the doublets that comprise the "trill" portion of the call; this is normally typical of natural call. The discharge of the unit approximately follows the duration of the sound pulse. In all three cases the calls were played at 80 dB SPL. Time scale = 100 ms

song. In fact, two (Fig. 7A and B) are responses to electronically generated models of calling song and one (Fig. 7C) is a response to a tape recording of actual calling song; no differences are apparent. We stress that int-2 units closely follow trains of 50 ms sound pulses delivered at pulse repetition rates of up to 16/s. It should be noted that the length of each spike burst closely approximates the duration of each song pulse; thus, we see (Fig. 7) that the first five spike bursts are slightly longer than the succeeding spike bursts in the "doublet" portion of the remaining discharges. This reflects the fact that the sound pulses in the introductory 5 notes of the song phase are slightly longer than the pulses in the 8 doublets of the trill portion of the phrase. It should also be noted that there is only moderate habituation of int-2 to repetitive stimulation; we have never observed a failure to respond to a sound pulse in a train of pulses.

Anatomy

The neuronal geometry of int-2 is shown in Figures 8 and 9. Figure 8 is a photographic composite of a unit from which one of the physiological recordings

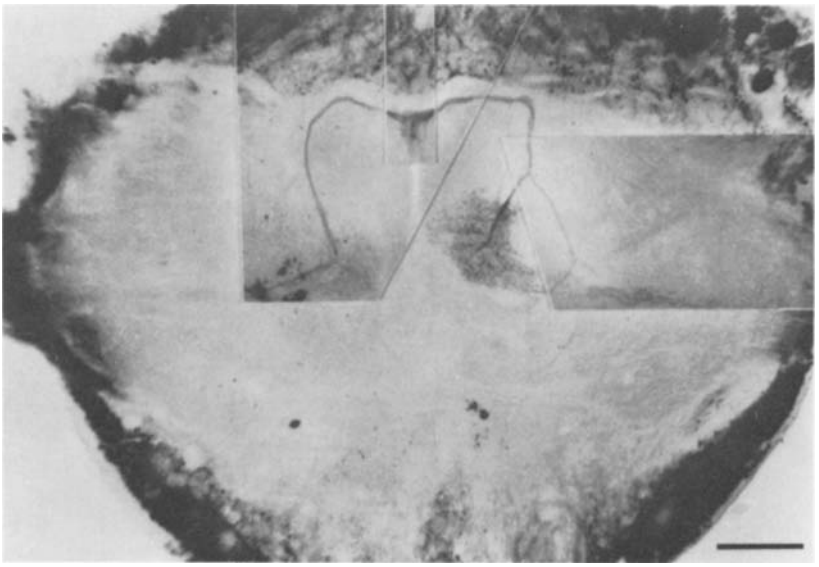

Fig. 8. Interneuron 2. This is a photomontage reconstructed from 30 µm histological cross sections. The neuron was stained by the extracellular cobalt method and the stain enhanced in histological cross section by the Timm's modification of Tyrer and Bell. The main features of neuronal geometry shown here are the extensive arborization to both acoustic neuropiles and the asymmetric branches of the right and left "arms" of the neuron; the right branch bifurcates to form two projections whereas the left branch forms but a single projection. The cell body is not shown in this photograph. The electrode was positioned in the medial bifurcation of the right "arm". Size scale = 100 µm

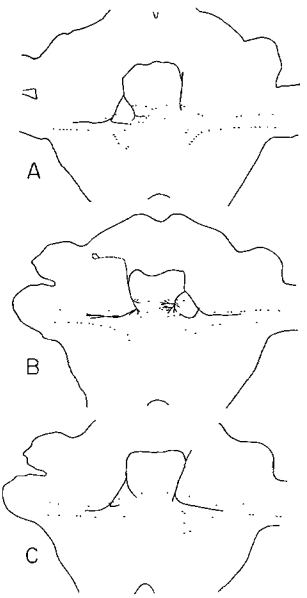

Fig. 9A–C. Camera lucida reconstruction of three different int-2 preparations that were stained by the extracellular cobalt technique. The outlines of both acoustic neuropiles are shown (dotted in) for reference. Anterior direction is at the top of the figure. Of the three, only the preparation **B** revealed a complete staining of the cell, including the soma. Note the asymmetrical branching between right and left major branches of the neuron. Scale = 100 µm

discussed above was made. The photographs were made from 30 μm sections of the Timm's stained ganglion; the ganglion was sectioned along the horizontal plane. The photograph illustrates the main anatomical features of the cell. The cell has an omega shape, with bilateral side branches that apparently penetrate *both* right and left acoustic neuropiles; the connecting "arch" between these branches crosses the midline in Ventral Commissure I (Gregory, 1974) and does not seem to give off further branches. The cell body is attached to the "arch" by a thin process, which is also free from branches. The most interesting feature of the cell is that it lacks a primary axon, i.e., it appears to be an intraganglionic neuron. Hence its physiological activities are confined to the prothoracic ganglion, and possibly to the region of the bilateral acoustic neuropiles.

It should be noted that this neuron is asymmetrical in its branching pattern to the bilateral acoustic neuropiles. With respect to the cell body, the contralateral branch bifurcates just anterior to the acoustic neuropile, whereas the ipsilateral branch does not. On both sides, the branches terminate in the acoustic neuropile, forming a dense, diffuse network of the fine terminals.

In four successful recording/marking experiments of int-2, the cobalt stain was restricted to one member of the pair. In three instances however, the second member of the pair stained lightly. The anatomical picture drawn here from cobalt stained preparations is confirmed by examination of silver stained sections. Because we have occasionally observed two interneurons of type-2, each a mirror image of the other, but never more than two such cells we conclude that, as is the case for int-1, there are two interneurons of type 2 in the ganglion. Thus, int-2, like int-1, is a unique cell rather than a member of a class.

Discussion

A. Extracellular Staining

We chose the extracellular staining technique expecting that it would allow study of neurons too small to impale. This may be an important advantage. However, great care is necessary in interpreting anatomical data from the technique, and we assign a particular response to acoustic stimuli to a particular anatomical type only when two criteria are satisfied. First, staining must be unambiguous. Thus, we have reported cell morphology only when a single neuron is stained or when one neuron is heavily stained and others appear only lightly stained in Timm's intensified sections. When more than one neuron is heavily stained, interpretation is difficult, and no such cases are included here.

The second criterion requires that a particular anatomy must be consistently associated with a particular response to acoustic stimuli. In repeated examples of an unambiguously stained morphological type, the physiological responses must be closely similar. For example, in all cases in which a neuron of type 1 stained, the neuron's firing was suppressed by sound. Furthermore, whenever a neuron inhibited by sound was stained, it proved to have the morphology

of int-1. Whenever a neuron of type 2 stained, its firing response to 5 kHz tones fit the curves of Figure 6, although we have tentatively identified other morphological types with similar response patterns.

In summary, the extracellular cobalt staining technique sometimes produces results which are difficult to interpret. However, we believe that a careful examination of numerous unambiguously stained preparations allows an accurate correlation of cellular morphology and physiology. Carefully applied, this technique can be a powerful tool for defining physiological pathways that underlie behavior. In particular, it can be used for neurons whose small sizes minimize the likelihood of intracellular penetration.

B. Interneuron 2: A Spiking Intraganglionic Interneuron

It is generally accepted that the temporal pattern of pulses in a cricket's call contains important species-identifying information. Thus one would expect that temporal parameters such as pulse duration, interpulse interval, pulse onset, and perhaps pulse termination, might be faithfully encoded in some auditory interneurons. Indeed, int-2 codes pulse onset, duration, and intensity with remarkable fidelity. However, unlike other auditory interneurons which code temporal information and send it to the brain (Zaretsky, 1971; Stout and Huber, 1972; Hill, 1974; Rheinlaender and Kalmring, 1973; Rheinlaender, 1975) int-2 is wholly intrasegmental. It is possible that int-2 drives higher order interneurons that do send axons to the brain and hence serves as a link in the system coding temporal pattern of the song.

We note that int-2 is capable of tonically coding the duration of a 5 kHz tone pulse for duration of 500 ms and longer. If the only role of this interneuron were to code the pulses in calling song, it is "overdesigned," since no song pulse lasts for more than 50 ms. We have found other interneurons that faithfully encode temporal pattern of song and that show strong habituation to long duration tone pulses; such neurons seem to be more "critically designed" for song pulse coding. From Figure 6 we observe that int-2's tonic discharge rate is definitely related to the intensity of the tone pulse. It has been suggested that an important stimulus cue for extracting directional information from a stimulus is the difference in intensity between the two ears (Katsuki and Suga, 1960; Murphey and Zaretsky, 1972; Hill, 1976), and we speculate that since int-2 projects to both acoustic neuropiles, it might be concerned with the detection of directionality of sound. The fact that int-2 occurs as a mirror-image bilateral pair of neurons, both of which connect the bilateral acoustic neuropiles suggests the possibility of physiological interaction between these neurons and the primary auditory afferent terminals themselves, between each other, or between higher order interneurons.

C. Interneuron 1. A Neuron Inhibited by 5 kHz Sound

The relationship between auditory behavior and the physiological properties of int-1 is intriguing. The neuron is spontaneously active, but during acoustic

stimulation by 5 kHz tone pulses, its firing rate is strongly suppressed. In the absence of acoustic stimulation it discharges at a low to moderate rate. Because the response of int-1 to playback of the species call is variable it is not yet possible to relate its activity to the call. The unit is relatively unresponsive to the pulse structure of the call (even when played at high sound pressure levels), or its spike pattern is only statistically correlated to the occurrence of interpulse intervals. Occasionally it fires during the interpulse intervals, possibly coding these parameters, especially when the call is played at 80–90 dB SPL. We do not yet know the reasons for differences in responsiveness.

The axons of both int-1 neurons terminate in the brain. This was demonstrated by staining both neurons from the circumesophageal connective by means of the retrograde backfill technique (Kater and Nicholson, 1973). Thus, whatever information is carried by int-1, it is sent to the brain. We have used the retrograde staining technique in over a dozen animals and invariably only one int-1 stains from each connective (circumesophageal or cervical); hence we are certain that there is only one pair of int-1 type neurons in the prothoracic ganglion. This conclusion is supported by histological sections stained with either silver, toluidine blue, or Masson's trichrome. (We note that our retrograde fills did not stain neurons of type 1 in either the meso- or metathoracic ganglia.)

In sum, we report the mapping of two new auditory interneurons in cricket by an extracellular recording technique that allows precise isolation and localization of a single unit, and at the same time, staining of only that unit with cobalt chloride. This is a first step in mapping the auditory pathways in the prothoracic ganglion; we believe that it is a significant advance. It is our expectation that only by producing a complete "map" of the auditory pathways, in terms of both physiology and neuroanatomy, will we understand acoustic behavior in the cricket, particularly the problems of stimulus localization and species-specific detection.

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References

Bentley, D.: Intracellular activity in cricket neurons during generation of song patterns. Z. vergl. Physiol. **62**, 267–283 (1969)

Bentley, D., Hoy, R.R.: The neurobiology of cricket song. Scient. Amer. 34–44 (August 1974)

Fentress, J.C.: Simpler networks and behavior. Sunderland, Mass.: Sinauer Assoc. Pub. 1976

Gregory, G.E.: Neuroanatomy of the mesothoracic ganglion of the cockroach *Periplaneta americana* (L.). I. The roots of the peripheral nerves. Phil. Trans. roy. Soc. B **267**, 421–465 (1974)

Hill, K.G.: Carrier frequency as a factor in phonotactic behavior of female crickets (*Teleogryllus commodus*). J. comp. Physiol. **93**, 7–18 (1974)

Hill, K.G., Boyan, G.S.: Directional hearing in crickets. Nature **262**, 390–391 (1976)

Hoy, R.R.: Genetic control of acoustic behavior in crickets. Amer. Zool. **14**, 1067–1080 (1974)

Hoy, R.R., Casaday, G.: Physiological and anatomical properties of cricket interneurons. Neurosc. Abstr. **2**, part 1, 347 (1976)

Huber, F.: Sensory and neuronal mechanisms underlying acoustic communication in orthopteran insects. In: Sensory physiology and behavior. (ed. R. Galun, P. Hillman, I. Parnas, R. Werman). New York: Plenum Publ. 1975

Kater, S.B., Nicholson, C.: Intracellular staining in neurobiology. Berlin-Heidelberg-New York: Springer 1973

Katsuki, Y., Suga, N.: Neural mechanisms of hearing in insects. J. exp. Biol. **37**, 279–290 (1960)

Kutsch, W., Huber, F.: Zentrale versus periphere Kontrolle des Gesanges von Grillen (*Gryllus campestris*). Z. vergl. Physiol. **67**, 140–159 (1970)

Leroy, Y.: Transmission du paramètre fréquence dans le signal acoustique des hybrides F1 et Px F1 de deux Grillons: *Teleogryllus oceanicus* et *T. commodus*. C.R. Acad. Sci. **259**, 892–895 (1964)

Loftus-Hill, J.J., Littlejohn, M.J., Hill, K.G.: Auditory sensitivity of the crickets *Teleogryllus commodus* and *T. oceanicus*. Nature New Biol. **233**, 184–185 (1971)

Michelsen, A., Nocke, H.: Biophysical aspects of sound communication in insects. Advanc. Insect Physiol. **10**, 247–296 (1974)

Murphy, R.K., Zaretsky, M.D.: Orientation to calling song by female crickets, *Scapsipedus marginatus* (Gryllidae). J. exp. Biol. **56**, 335–352 (1972)

Paton, J.: Frequency analysis in the auditory system of field crickets. Ph.D. thesis, Cornell University, Ithaca, New York 1975

Popov, A.V.: Frequency selectivity of the response of auditory neurons of first thoracic ganglion of the cricket *Gryllus bimaculatus*. J. Evol. Biochem. Physiol. **9**, 265–267 (1973)

Popov, A.V., Shuvalov, V.F.: Time-characteristics of communicative sounds and their analysis in the auditory system of insects. Acustica **31**, 315–319 (1974)

Rehbein, H.: 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., 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 (1974)

Rheinlaender, J.: Transmission of acoustic information at three levels in the auditory system of *Decticus verrucivorus* (Tettigoniidae, Orthoptera). J. comp. Physiol. **97**, 1–53 (1975)

Rheinlaender, J., Kalmring, K.: Die afferente Höhrbahn im Bereich des Zentralnervensystems von *Decticus verrucivorus* (Tettigoniidae). J. comp. Physiol. **85**, 361–410 (1973)

Rheinlaender, J., Kalmring, K., Popov, A.V., Rehbein, H.: Brain projections and informations processing of biologically significant sounds by two large ventral-cord neurons of *Gryllus bimaculatus* DeGreer (Orthoptera, Gryllidae). J. comp. Physiol. **110**, 251–269 (1976)

Stout, J.F., Huber, F.: Responses of central auditory neurons of female crickets (*Gryllus campestris* L.) to the calling song of the male. Z. vergl. Physiol. **76**, 302–313 (1972)

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

Walker, T.: Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. Ann. ent. Soc. Amer. **50**, 626–636 (1957)

Zaretsky, M.: Patterned response to song in cricket central auditory neurone. Nature **229**, 195–196 (1971)

Zaretsky, M.: Specificity of the calling song and short term changes in the phonotactic response by female crickets *Scapsipedus marginatus* (Gryllidae). J. comp. Physiol. **79**, 153–172 (1972)